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EFFECT OF DOWNREGULATED β-CATENIN ON CELL PROLIFERATIVE ACTIVITY, THE SENSITIVITY TO CHEMOTHERAPY DRUG AND TUMORIGENICITY OF OVARIAN CANCER CELLS

J. WANG[*1], D. ZHOU[*2], X. HE[2], Y. WANG[2], W. HU[2], L. JIANG[2] AND J. DOU[2]

1 Department of Gynecology & Obstetrics; Zhongda Hospital, Medical School, Southeast University, Nanjing, 210009, PR China.
2 Department of Pathogenic biology and Immunology, Medical School, Southeast University, Nanjing, 210009, PR China.

Abstract

The role of Wnt/β-catenin signaling pathway in the etiology and/or progression of ovarian cancer has been well documented. It was domenstrated that ovarian cancer constantly exhibit constitutive activation of canonical Wnt signaling, usually as a result of oncogenic mutations that stabilize and dysregulate the β-catenin protein. In this study, we transfected an expression vector-based small hairpin RNA (shRNA) targeting to β-catenin encoding gene into human A2780 ovarian cancer cells to investigate the effects of β-catenin knockdown on biological characteristics of ovarian cancer cells. The results showed that β-catenin shRNA expression resulted in decreased β-catenin mRNA and protein expression in the transfected A2780 cells, inhibition of cellular proliferation, decreased capability of clonogenicity in the plating and the soft agar, and increased sensitivities to chemotherapy drugs vincristine, paclitaxel and cisplatin compared to untransfected cells. Importantly, we found that shRNA-mediated knockdown of β-catenin strongly decreases tumour growth of human A2780 ovarian cancer cells in xenografts. These results demonstrate that β-catenin might be an effective therapeutic target for human ovarian cancer treatment.

Key words: Ovarian cancer cells, β-catenin, shRNA, tumorgenecity, target therapy.

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Corresponding author

Professor Jun Dou. Department of Pathogenic Biology and Immunology, Medical School, Southeast University, Nanjing 210009, China
Tel: + 86-25-83272454
E-mail: njdoujun@yahoo.com.cn

[*]Co-author, equally contributed

Abbreviations: shRNA: small hairpin RNA; **siRNA**: small interfering RNA; **CSCs**: cancer stem cells; **OD**: optical density; **qRT-PCR**: Quantitative reverse transcriptase-PCR; **MTT**: a 3-(4,5- dimethylthiaxol-2-yl)-2,5- diphenyltetrazolium bromide; **EGFP1**: enhancement green fluorescent protein 1; **β-catenin-shRNAs**: β-catenin- shRNA1, β-catenin-shRNA2 and β-catenin-shRNA3; **s.c.:** subcutaneously

INTRODUCTION

Ovarian cancer is a common female genital cancer and more than 90% of it is an epithelial ovarian cancer originated from ovarian epithelial cells. The incidence rate of ovarian cancer ranks the third in all the gynecological cancer of China, following cervical cancer and uterine cancer (11). Epithelial ovarian cancer still represents the most lethal gynecologic malignancy in the industrialized countries and the indeveloped countries (2,10). Unfortunately, relatively little is known about the molecular events that lead to the development of this highly aggressive disease (7, 10). Currently, therapies for ovarian cancer include operation, chemotherapy, radiotherapy, immunotherapy, and physiotherapy etc. These original approaches are to think ovarian carcinoma as a homogeneous bulk, and the therapeutic goal is to reduce the quantity of cancer cells or to improve the survival rate of patients with this disease. However, the tumor relapse due to chemo/radio-resistance of a part of the tumor cells still is a major obstacle in tumor

therapy. Therefore, exploration of pathogenesis and seeking a novel therapeutic method in ovarian cancer is current major tasks (8,15, 23).

It is known that the Wnt/β-catenin signaling pathway functions as a master switch for precise regulation of cell fate in normal cell development. Activation of the Wnt/β-catenin signaling pathway results in cellular proliferation and self-renew, and inactivation of the pathway induces cell differentiation. However, recent multiple studies involving various types of human cancers have proved that activation of Wnt/β-catenin signaling leading to increased β-catenin nuclear signaling and enhanced cellular invasive activity. The manifestation of cancer by aberrant Wnt signaling results from inappropriate gene activation mediated by stabilized β-catenin (10,17,21). Oncogenic activation of the Wnt signaling pathway also is common in ovarian cancer. The cumulative β-catenin protein have a causal role in tumorigenesis because β-catenin protein is accumulated in the cytoplasm and ultimately translocated to the nucleus where it interacts with lymphoid-enhancer binding factor/T-cell-specific transcriptional factor to induce the expression of the downstream target genes including c-myc and cyclinD1 (3,22,24). In cancer stem cells (CSCs), β-catenin signaling is essential in sustaining the CSC phenotype and deletion of the β-catenin gene results in the loss of CSCs and complete tumour regression (5,18). In view of the fact that the β-catenin are increased in ovarian cancer cells, we wanted to know whether β-catenin could be a new target for ovarian cancer treatment. For this regard, we used small interfering RNA (siRNA) method by vector-based small hairpin RNA (shRNA) targeting to β-catenin encoding gene, CTNNB1, and examined the biological characteristic of human A2780 ovarian cancer cells, such as cell proliferation, the capability of clonogenicity in the plating and the soft agar, the sensitivity to chemotherapy drugs, tumorigenicity after knockdown of β-catenin. Here we show that the our constructed recombinant shRNA β-catenin plasmids effectively down regulated β-catenin mRNA and protein expression in the A2780 cells and obviously changed the biological characteristic of ovarian cancer A2780 cells in *vitro* and in *vivo*.

MATERIALS AND METHODS

Cell lines and animals

A2780 cell line was of ovarian cancer patients' origin, a well-established ovarian cancer model system, purchased from the Cellular Institute in Shanghai, China. The cells were utilized to generate a stable transfection using β-catenin shRNA, and were cultured at 37 ºC in 5% CO_2 in air in complete media consisting of RPMI 1640, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum. Five week old Balb/c nude mice were ordered from the Animal Center of Shanghai of China. Mice were raised in the animal facilities of the Experimental Animal Center, Medical School, under sterile conditions in air-filtered containers. All the experiments were performed in compliance with the guidelines of the Animal Research Ethics Board of Southeast University, China.

Short hairpin RNA sequence design

Short hairpin RNA sequences of human β-catenin were designed according to CTNNB1 DNA sequence (GenBank NO.NM_001904) by the siDESIGN design software of Dharmacon Company (http://www.dharmacon.com/DesignCenter) and Block-iT™ RNAi Designer of Invitrogen Company as well as BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Three target sequence sites for β-catenin shRNA were 1589bp-1607bp,1181bp-1199bp and 661bp-679bp in CTNNB1 cDNA sequence, respectively. In addition, one scrumbe sequence was designed as negative control (1, 22).

Eight shRNA sequences are as follows:

β-catenin-siRNA1: sense, ′ 5 -GATCCCC-**GTGGGTGGTATAGAGGCTC**-TTCAAGAGA-**GAGCCTCTATACCACCCAC**-TTTTTGGAAA-3′; antisense, ′ 5 -AGCTTTT CCAAAA-**GTG GGTGGTATAGAGGCTC**-TCTCTTGAA-**GAGCCTCTATACCACCCAC**-GGG-3′; β-catenin -siRNA 2: sense, ′ 5 -GATCCCC-**GCTTATGGCAACCAAGAAA**-TTCAAGAGA-**TTTCTTGG TTGCCATAAGC**-TTTTTGGAAA-3;antisense, ′ 5 -AGCTTTTCCAAAAA-**GCTTATGGCAAC CAAGAAA**-TCTCTTGAA-**TTTCTTGGTTGCCATAAGC**-GGG-3′;β-catenin-siRNA3: sense, ′ 5 -GATCCCC-**GCTGAAACATGCAGTTGTA**-TTCAAGAGA-**TACAACTGCATGTTTCAG C**-TTTTTGGAAA-3; antisense, ′ 5 -AGCTTTTCCAAAAA-**GCTGAAACATGCAGTTGTA**-T CTCTTGAA-**TACAACTGCATGTTTCAGC**-GGG-3′; Scrambled sequence : sense, 5'-GATC CCCTTCTCCGAACGTGTCACGTTTCAAGAGAACGTG ACACGTTCGGAGAATTTTTGGAAA-3'; antisense, 5'-AGCTTTTCCAAAAATTCTCCGAACGTGTCACGTTCT CTTGAAACG TGACACGTTCGGAGAAGGG-3'. The bold nucleotide sequences serve as sense/antisense sequences, and the nucleotide sequences in pane represent spacer sequences, which assist in forming shRNA links. The nucleotide sequences of underline contain restriction endonuclease *Bgl* II and *Hin*d III sites, respectively. All the primers were synthesized by Company of Gene and Technology of China in Shanghai.

Construction of vectors containing shRNAs targeting to β-catenin encoding gene

A pSUPER-EGFP1(enhancement green fluorescent protein 1) vector was used to construct recombinants. The recombinants pSUPER-EGFP1-β-catenin-shRNAs (β-catenin-shRNA1, β-catenin-shRNA2 and β-catenin-shRNA3) were developed according to previous reports (9,22, 25). A pSUPER-EGFP1-scrambled shRNA was used as a negative control. These recombinants were identified by the analysis of an endonuclease digestion.

Transfection and production of stable cell lines

A2780 cells that were transfected with the different pSUPER-EGFP1-β-catenin-shRNA constructs using Lipofectamine^{TM} 2000 reagent (Invitrogen, USA) according to the manufacturer's protocol. Following antibiotic selection with 800 μg/ml G418 (Clontech, CA), several pSUPER-EGFP1-β-catenin-shRNA-expressing clones were isolated and expanded into cell lines. Three clones transfected with pSUPER-EGFP1-β-catenin-shRNA-expressing vectors and one clone transfected with pSUPER-EGFP1-scrambled shRNA vector were selected and then screened for β-catenin expression by Fluorescence microscope, Quantitative reverse transcriptase- PCR (qRT-PCR) and Western blotting, respectively (2,9). We named three clones stably transfected with pSUPER-EGFP1-β-catenin-shRNA-expressing vectors for the β-catenin-shRNA1 cells, β-catenin-shRNA 2 cells and β-catenin-shRNA3 cells down regulated β-catenin expression, respectively, and the clone stably transfected with pSUPER-EGFP1-scrambled shRNA vector for the scrambled shRNA cells expressing β-catenin no change, and the clone stably transfected with pSUPER-EGFP1 plasmid for the pSUPER-EGFP1 cells no expressing β-catenin.

qRT-PCR

qRT-PCR analysis was performed on an ABI 7300 real-time system (PE Applied Biosys tems). RNA was isolated from each sample by using a Qiagen RNeasy Kit (Qiagen, Valencia, CA). An additional DNase I digestion procedure (Qiagen, Hilden, Germany) was included in the isolation of RNA to remove contaminating DNA and performed according to the manufacturer's protocol. One microgram of total RNA from each sample was subject to cDNA synthesis using Superscript III reverse transcriptase and random hexamer (Invitrogen). cDNA was then amplified by PCR with primers specific for β-catenin (sense, 5′-CGAATGTCTGAGGACAAGCCAC-3′; antisense, 5′-CCATGAGGTCCTGGGCATG-3′) and the β-actin gene of internal contfol (sense, 5 - GGACTTCGAGCAAGAGATGG-3′; antisense, 5′-AGCACTGTGTTGGCGTACAG-3′), using a PCR Master Mix Reagents Kit (PE Applied Biosystems). Relative gene expression was determined based on the threshold cycles of the genes of interest and the internal control gene. The mRNA levels of the genes of interest are expressed as the ratio of each gene of interest to β-actin mRNA for each sample (20,22).

Western blotting analysis

1×10^6 A2780 cells that were stablely transfected with the pSUPER-EGFP1-β-catenin- shRNAs or the pSUPER-EGFP1-scrambled shRNA were collected and lyzed in protein extraction buffer (Novagen, WI, USA) according to the manufacturer's protocol. 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and proteins (15μg/lane) were electrotransferred onto a nitrocellulose membrane that was first blocked with 4% dry milk in Tris-buffered saline with Tween-20 for 1 h at 20 ºC and then incubated with the mouse antibody specific to human β-catenin (BD company, USA) for 1 h at 20 ºC. The membrane was rinsed for 5 min with antibody wash solution 3 times before adding rat anti-mouse secondary antibody conjugated to horseradish peroxidase for 1 h at 20 ºC. Immunoreactive bands were detected by the enhanced chemiluminescence reaction (Amersham) (1, 25).

Cellular proliferation assay

Cellular proliferation was measured with the cells transfected with the pSUPER-EGFP1- β-catenin-shRNAs, the pSUPER-EGFP1 plasmid or with the scrambled control siRNA. Relative numbers of viable cells, from Day 3 to Day 10, were respectively assessed by a 3-(4,5- dimethylthiaxol-2-yl)-2,5-diphenyltetra- zolium bromide (MTT) assay. For colorimetric analysis, the absorbance at 490 nm was recorded using a microplate reader. Each condition was repeated at least 3 times (13,22).

Resistance to chemotherapeutic agents in the transfected cells

1×10^4 A2780 cells that were stablely transfected with the pSUPER-EGFP1-β-catenin- shRNAs, the pSUPER-EGFP1 plasmid or with the pSUPER-EGFP1-scrambled shRNA were seeded into a 96-well plate with 0.5μg/ml vincristine or 0.3μg /ml paclitaxel or 0.2μg/ml cisplatin in each well for 72 h, respectively. Three drugs were all ordered from the Lukang Drug Corporation, Shandong, China. Cellular resistance to chemotherapeutic agents were calculated according to references (6,11, 12).

Colony formation assay

The colony formation capability of A2780 cells transfected with the pSUPER-EGFP1- β-catenin-shRNAs, the pSUPER-EGFP1 plasmid or with the scrambled control siRNA in the the plating and soft agar media were respectivly investigated. Colony diameters larger than 75μm or colony cells more than 50 cells were then counted as 1 positive colony according to our previous reports (12, 20, 22).

Tumor formation in nude mice

The flanks of nude mice were subcutaneously (s.c.) injected with 5×10^6 A2780 cells stably transfected with the pSUPER-EGFP1-β-catenin-shRNAs (n=4) or the scrambled control siRNA (n=3) or the mock plasmids (n=3), respectively. Tumor formation in these groups were monitored diebus tertius by 2-dimensional measurements of individual tumors from each animal (1, 9).

Statistical analysis

Each experiment has been repeated at least 3 times. Values were presented as the mean plus or minus standard deviation. Statistical comparisons were performed using the Student's t-test method and $p<0.05$ was considered significant statistically.

RESULTS

Isolation of stably transfected pSUPER-EGFP1-EGFP1-β-catenin-shRNAs cell clones and identification of β-catenin expression

The A2780 ovarian cancer cells endogenously expressed β-catenin was identified by RT-PCR (data not shown), and then the cells

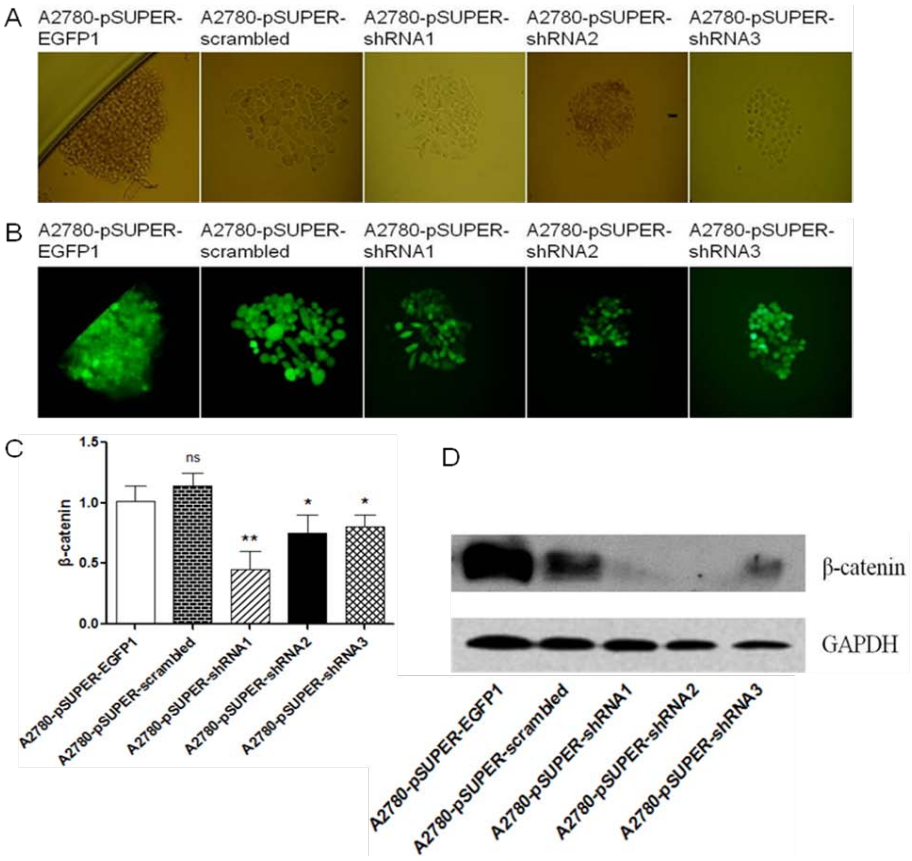

Figure 1. Stable transfected cell clones and β-catenin expression detection. Photos of top-panel observed under a light microscope (A) and bottom-panel observed under a fluorescent microscope (B) in Fig. 1 show the A2780 ovarian cancer cells stable transfected with the different constructs. Fig.1C represents the changes in target genes of the β-catenin expression after downregulation of β-catenin in ovarian cancer cell lines detected by quantitative reverse transcriptase-PCR. Data are shown as fold changes of mRNA expression relative to cells with β-catenin stable transfectants and EGFP1 stable transfectants, respectively. The values are expressed as the mean ± SEM from 3 independent experiments. Significant differences are indicated by asterisk (*) for $p < 0.05$. Fig.1D indicates the results of Western blotting in the A2780 ovarian cancer cells transfected with the different recombinants.

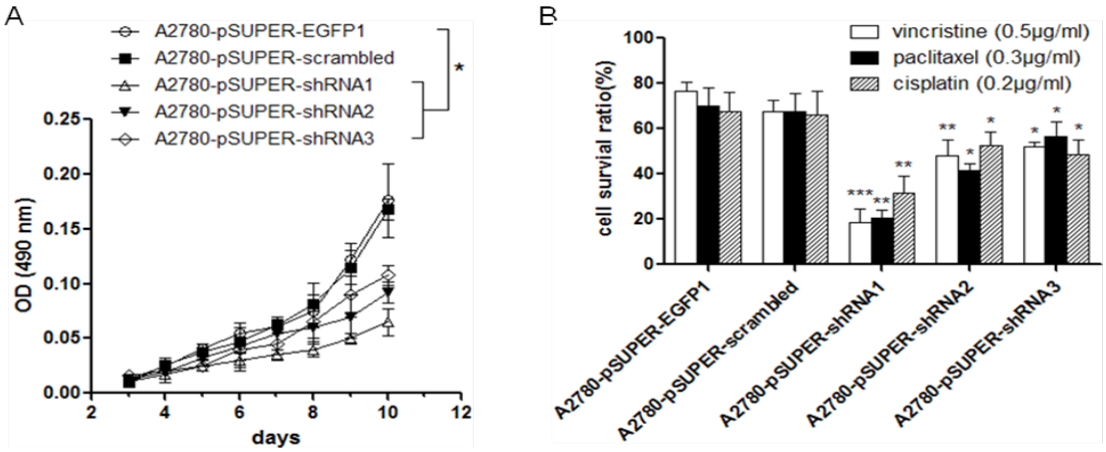

Figure 2. Cellular proliferation and chemoresistance detected by a MTT assay. A cellular proliferative assay was performed in triple wells, and a mean value of the optical density (OD) was detected in triple wells every day, as is shown in Fig. 2A. Relative cell survival ratio were respectively assessed by a MTT assay. For colorimetric analysis, the absorbance at 490 nm was recorded using a microplate reader. Each condition was repeated at least 3 times. Fig. 2B indicates the chemoresistances to vincristine, paclitaxel and cisplatin were reduced after these drugs were respectively incubated with the A2780 cells transfected with the different recombinants for 72 h. Significant differences are indicated by asterisk (*) for $P<0.05$, (**)$P<0.01$, (***)$P<0.005$.

that were stably transfected with the different pSUPER- EGFP1-β-catenin-shRNA constructs were successfully selected after antibiotic selection with 800 μg/ml G418 for 10 days. The clones transfected with pSUPER-EGFP1-β-catenin- shRNA1, pSUPER-EGFP1-β-catenin-shRNA2, pSUPER-GFP1-β-catenin-shRNA3 expressing vectors and the clones transfected with mock plasmids or pSUPER-GFP1-scrambled shRNA vector were respectively isolated through single-clone isolation assay and expanded into cell lines. These clones expressing EGFP1-β-catenin were visible by Fluorescence microscope, as is shown in Fig.1B, respectively. It was also shown that the β-catenin mRNA expression (Fig. 1C) and the β-catenin protein expression (Fig. 1D) were markedly decreased in the transfectant A2780 cells, especially in the β-catenin-shRNA1 cells as well as the β-catenin mRNA expression in the β-catenin-shRNA2 cells, but not in the EGFP1-shRNA cells and the scrambled shRNA cells. The data indicated that our constructed recombinants containing β-catenin-shRNAs were developed appropriately, which assists in further study of the effects of β-catenin downregulation on biological characteristics of ovarian cancer A2780 cell line.

Cellular proliferation and chemosensitivity to drugs in vitro

Cellular proliferation and cellular chemosensitivity to drugs *in vitro* were measured with the A2780 cells transfected with the pSUPER-EGFP1-β-catenin-shRNAs or the scrambled control siRNA. Fig. 2A gives the proliferative activities dynamically every 24 h after 1×10^4 different transfected cells had been seeded into a 96-well plate in complete media. After 1 week of incubation, the value of optical density in the pSUPER-EGFP1cells or the scrambled shRNA cells reached 0.175 or 0.168, whereas the β-catenin-shRNA1 cells was only 0.6, and the difference was statistically significant ($p<0.05$).

The association of β-catenin downregulation with chemosensitivity to vincristine, paclitaxel and cisplatin was determined in the A2780 ovarian cancer cells. In β-catenin-shRNA1 cells, downregulation of β-catenin significantly decreased the chemoresistance to vincristine, paclitaxel and cisplatin in contrast to the pSUPER-EGFP1 cells and the scrambled shRNA cells. As shown in Fig. 2B, after vincristine, paclitaxel and cisplatin was respectively incubated with the β-catenin- shRNA1 cells for 72 h, the cell survival ratio were significantly lower than that of the scrambled shRNA cells or the pSUPER-EGFP1 cells (19% compared with 76% or 68%, $p<0.01$), (19% compared with 70% or 68%, $p<0.01$) and (47% compared with 68% or 67%, $p<0.05$) in order. In the β-catenin-shRNA2 cells and the β-catenin-shRNA3 cells, downregulation of β-catenin significantly decreased the chemoresistance to vincristine, paclitaxel and cisplatin as well, but neither the β-catenin- shRNA2 cells nor the β-catenin-shRNA3 cells decreased as the chemoresistance to vincristine, paclitaxel and cisplatin as the β-catenin- shRNA1 cells did.

Effect of knockdown of β-catenin in A2780 cells on colony formation capability

In the present study, the β-catenin-shRNAs, or the scrambled shRNA were transfected stably into the A2780 cells, respectively, colony formation assays were performed with the stable expression clones. As shown in Fig. 3, the colony formation rates were more than 40% in the plating or near 30% in the soft agar media for the EGFP1-shRNA cells or the the scrambled shRNA cells, whereas thses rates were only less than 20% in the plating or less than 5% in the soft agar media for the β-catenin-shRNAs cells, respectively. The colony formation rates in the the plating and soft agar media were decreased in the β-catenin-shRNAs cells compared with the EGFP1-shRNA cells or the scrambled shRNA cells (statistical significance $P<0.01$, or $P<0.005$).

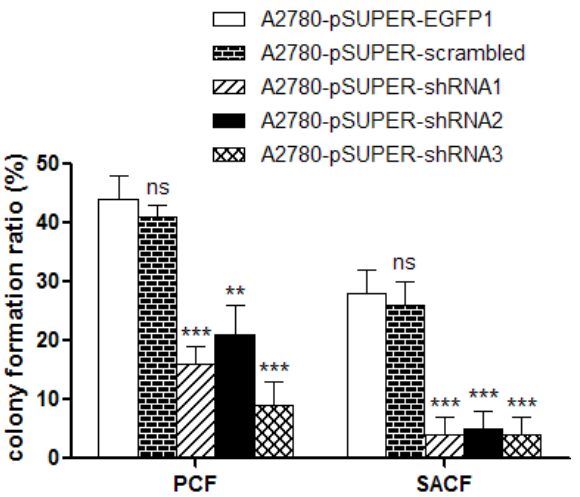

Figure 3. Knockdown of β-catenin suppress colony formation *in vitro*. Compared with the EGFP1-shRNA cells or the scrambled shRNA cells, the colony formation ratio of the transfected A2780 cells in the the plating and the soft agar media were decreased in the β-catenin-shRNA1 cells, the β-catenin-shRNA2 cells and the β-catenin-shRNA3 cells. Significant differences are indicated by asterisk (**) for $P<0.01$. PCF: plating colony formation rate; SACF: soft agar colony formation rate.

Knockdown of β-catenin in A2780 cells inhibits tumor growth in a xenograft mouse model

The effect of knockdown of β-catenin in A2780 cells on tumorigenicity was investigated in Balb/c nude mice. Photos in Fig 4 show that 3 of the 3 mice developed tumors at 14 days or 16 days or 17 days after implantation of 5×10^6 the transfectant scrambled shRNA cells or the EGFP1-shRNA cells. In contrast, 2 of the 4 mice injected with 5×10^6 the transfectant β-catenin-shRNA3 cells developed tumor at 19 days or 23 days, and the remanent 2 mice or another 4 mice injected with 5×10^6 the transfectant β-catenin-shRNA1 cells or injected with 5×10^6 the transfectant β-catenin-shRNA2 cells did not generate tumors until 90 days into the observation. The tumor growth curve in Fig.4 indicates the therapeutic experiment that 1×10^7 A2780 cells were inoculated into mice and 15 days after inoculation, the different β-catenin-shRNAs recombinant 100μg or scrambled shRNA construct or mock plamids 100μg were respectively injected into the sites of inoculated tumor. It is thus evident that the tumor growth were inhibited in the β-catenin-shRNAs transfectant cells, especially in the β-catenin-shRNA1 cells and the β-catenin-shRNA2cells, and that the β-catenin-shRNA1 recombinant could also obviously suppress tumor growth in tumour bearing nude mice.

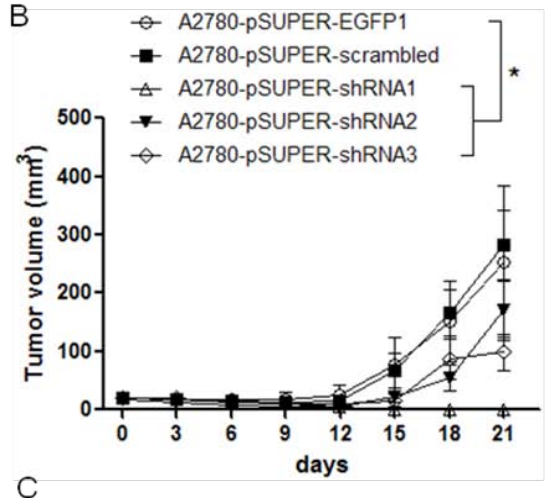

Figure 4. Effect of knockdown of β-catenin in A2780 cells on tumorigenicity. 5×10^6 A2780 cells stably transfected with the different β-catenin-shRNA constructs or β-catenin-scrambled shRNA construct or mock plamids were injected into flank of nude mice, respectively. Photos were pictured at 40 days after the different cells injected. It was found that there are no tumour growth in the β-catenin-shRNA1 group and the β-catenin-shRNA2 group and that 2 of the 4 mice developed in the β-catenin-shRNA3 group, and that all mice generated tumours in the β-catenin-scrambled group and the mock plamids group. The tumor growth curve in Fig. 4C shows the therapeutic results. In this assay, 1×10^7 A2780 cells were inoculated into mice and after 15 days, the 100μg different β-catenin-shRNAs recombinants or the β-catenin-scrambled shRNA construct or the mock plamids were respectively injected into the sites of inoculated tumor. Points represent the mean tumor volumes from 5 independent experiments that showed that the β-catenin-shRNAs recombinant could obviously suppress tumor growth in tumour bearing nude mice, especially in the β-catenin-shRNA1 recombinant and the β-catenin-shRNA2 recombinant. Significant differences are indicated by asterisk (*) for $p < 0.05$.

DISCUSSION

In this investigation, we selected human ovarian cancer cell line A2780 as our study target cells to explore the effects of β-catenin downregulation on biological characteristics of ovarian cancer cells and to find a new treatment target for ovarian cancer therapy.

Our results demonstrated that three eukaryotic vectors expressing shRNA targeting human β-catenin encoding gene CTNNB1 and one eukaryotic vectors expressing scrambled shRNA were respectively constructed in accordance with the CTNNB1 DNA sequence and the feature of pSUPER-EGFP1 plasmid. Further, we transfected the constructed four recombinant plasmids and pSUPER-EGFP1 mock plasmid into A2780 ovarian cancer cells, respectively and the stably transfected cells were selected according to the characteristics of resistance to G418 and expression of EGFP1, as shown in Figures 1 A-B. The results of qRT-PCR and Western blotting suggested that three recombinant shRNAs plasmid targeting human β-catenin could down regulate β-catenin mRNA and protein expression levels, respectively, of which the knockdown effect were obvirously found in β-catenin-shRNA1 cells and β-catenin-shRNA2 cells, as shown in Figures 1 C-D.

To address the functional significance of the downregulation of β-catenin gene expression, we first evaluated the cell proliferation and their chemosensitivity to drugs *in vitro*. Fig. 2A shows the stably transfected β-catenin-shRNAs cells gradually decreased proliferative activity in contrast to the mock plamids or scrambled shRNA cells 3 days after culture, which may be good evidence that the the reduded cell proliferative activity is due to knockdown of β-catenin expression. Since some drugs enable the pharmacological regulation of β-catenin, decreased β-catenin expression results in increased cancer cell apoptosis (4, 16, 17). Therefore, we evaluated the effect of knockdown of the oncogenic protein β-catenin on increase of chemotherapy drugs vincristine, paclitaxel and cisplatin against A2780 ovarian cancer cells. Fig. 2B indicates that the A2780 cells downregulated β-catenin by shRNA were more sensitive to growth inhibition in the β-catenin-shRNAs cells than the scrambled shRNA cells, of which β-catenin-shRNA1 cells appeared to be more sensitive to drug-cytotoxicity than β-catenin-shRNA2 cells and β-catenin- shRNA3 cells. In accordance with the decreased β-catenin expression, the capability of clonogenicity in the plating and the soft agar were markedly reduced in the β-catenin shRNA transfected cells compared to untransfected cells. Studies on the biological characteristic of tumor cells have demonstrated that the clonal capability *in vitro* was used to measure the capability of tumor cells to cross tissue barriers and cell invasion, and that the clonal efficiency correlated positively with the disease stage of carcinoma (6,19,20). Accordingly, our data that the knockdown of β-catenin expression suppress colony formation capability *in vitro* implied that the A2780 cells knocked down β-catenin may lead to reduced their tumorigenesis in nude mice models. The hypothesis has been validated by subsequence tumor growth experiments in a xenograft mouse model. The tumorigenic capacity of the A2780 cells knocked down β-catenin was compared with that of mock plamids transfected A2780 cells *in vivo*. As shown in Fig. 4, shRNA-mediated knockdown of β-catenin in A2780 cells strongly inhibited tumour growth, and using β-catenin-shRNAs recombinant significantly decreased tumour volume in xenografts ($P<0.05$, n=4). This emphasizes that the β-catenin is essential for tumorigenesis in transplantation experiments in xenografts although the molecule mechanism of β-catenin remains to be further validated.

Conclusions, these preclinical results show β-catenin, a molecule associated with drug resistance, clonal capability *in vitro* and tumorigenesis *in vivo*, may be a target of for ovarian cancer therapy. The downregulation of β-catenin is associated with decreased cellular proliferative activity and increased multiple drug cytotoxicity in A2780 ovarian cancer cells and is also associated with attenuated tumorigenesis of A2780 ovarian cancer cells in nude mice. Further investigation of β-catenin targeting genes still is a necessary for the strategy targeting treatment ovarian cancer.

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