

# Secreted frizzled-related protein 5 suppresses aggressive phenotype and reverses docetaxel resistance in prostate cancer

Qiang Xu,<sup>1</sup> Zhong Lü,<sup>2</sup> Xugang Wang,<sup>2</sup> Qingxian Zhu,<sup>2</sup> Haoran Wu<sup>2</sup>

<sup>1</sup>Department of Urology, Affiliated Hospital of Jiangsu University, Zhenjiang, China  
<sup>2</sup>Department of Urology, Wujin Hospital Affiliated to Jiangsu University, Changzhou, China

**Correspondence to**  
Dr Haoran Wu, Wujin Hospital Affiliated to Jiangsu University, Changzhou 213003, China; fdwhr2009@163.com

Accepted 14 January 2019

## ABSTRACT

Secreted frizzled-related protein 5 (SFRP5) has been reported to be downregulated in prostate cancer. However, its biological role in this malignancy has not been clarified yet. In the present study, we performed SFRP5 overexpression experiments to determine its function in prostate cancer cell growth, invasion, tumorigenesis, and docetaxel sensitivity. Our results showed that overexpression of SFRP5 significantly suppressed the proliferation and colony formation of PC-3 and DU-145 cells, compared with vector-transfected control cells. SFRP5 overexpression arrested PC-3 and DU-145 cells at G0/G1 phase and induced apoptosis. Transwell invasion assay revealed that ectopic expression of SFRP5 inhibited the invasion of PC-3 cells. Overexpression of SFRP5 resensitized docetaxel-resistant PC-3 and DU-145 cells to docetaxel, which was coupled with increased apoptosis. Mechanistically, SFRP5 overexpression blocked  $\beta$ -catenin nuclear translocation and transcriptional activity. In vivo studies confirmed that overexpression of SFRP5 significantly suppressed the growth of PC-3 xenograft tumors. SFRP5-transfected xenograft tumors showed a reduction in the percentage of Ki-67-positive proliferating cells and an increase in terminal deoxynucleotidyl transferase/biotin-dUTP nick end labeling-positive cells. These data suggest that SFRP5 overexpression suppresses the aggressive phenotype of prostate cancer cells and overcomes docetaxel resistance through inactivation of  $\beta$ -catenin signaling. Therefore, delivery of SFRP5 may offer therapeutic benefits in the treatment of prostate cancer.

## INTRODUCTION

Wnt/ $\beta$ -catenin signaling plays a pivotal role in both physiological and pathological processes.<sup>1,2</sup> This pathway coordinates numerous cellular behaviors such as differentiation, proliferation, migration, and invasion.<sup>3,4</sup> Canonical Wnt/ $\beta$ -catenin signaling is activated by Wnt binding to the frizzled receptors and co-receptor LRP5 or LRP6, which causes the dissociation of the  $\beta$ -catenin degradation complex and subsequent  $\beta$ -catenin nuclear translocation.<sup>4</sup> In the nucleus,  $\beta$ -catenin dimerizes with TCF/LEF transcription factors and transactivates a number of target genes such as cyclin D1, c-myc, and survivin.<sup>5</sup> Aberrant activation of Wnt/ $\beta$ -catenin signaling

## Significance of this study

### What is already known about this subject?

- ▶ Aberrant activation of Wnt/ $\beta$ -catenin signaling is linked to disease progression in multiple cancers including prostate cancer.
- ▶ Secreted frizzled-related proteins (SFRPs) are known to antagonize the Wnt/ $\beta$ -catenin pathway.
- ▶ SFRP5 is downregulated in prostate cancer.

### What are the new findings?

- ▶ Overexpression of SFRP5 suppresses the proliferation and colony formation of prostate cancer cells.
- ▶ SFRP5 overexpression inhibits the invasion of PC-3 prostate cancer cells.
- ▶ Overexpression of SFRP5 resensitizes docetaxel-resistant prostate cancer cells to docetaxel.

### How might these results change the focus of research or clinical practice?

- ▶ Delivery of SFRP5 may offer therapeutic benefits in the treatment of prostate cancer.

is linked to disease progression in multiple cancers including prostate cancer.<sup>6,7</sup> Hence, it is important to dissect the mechanism for regulation of the Wnt/ $\beta$ -catenin pathway.

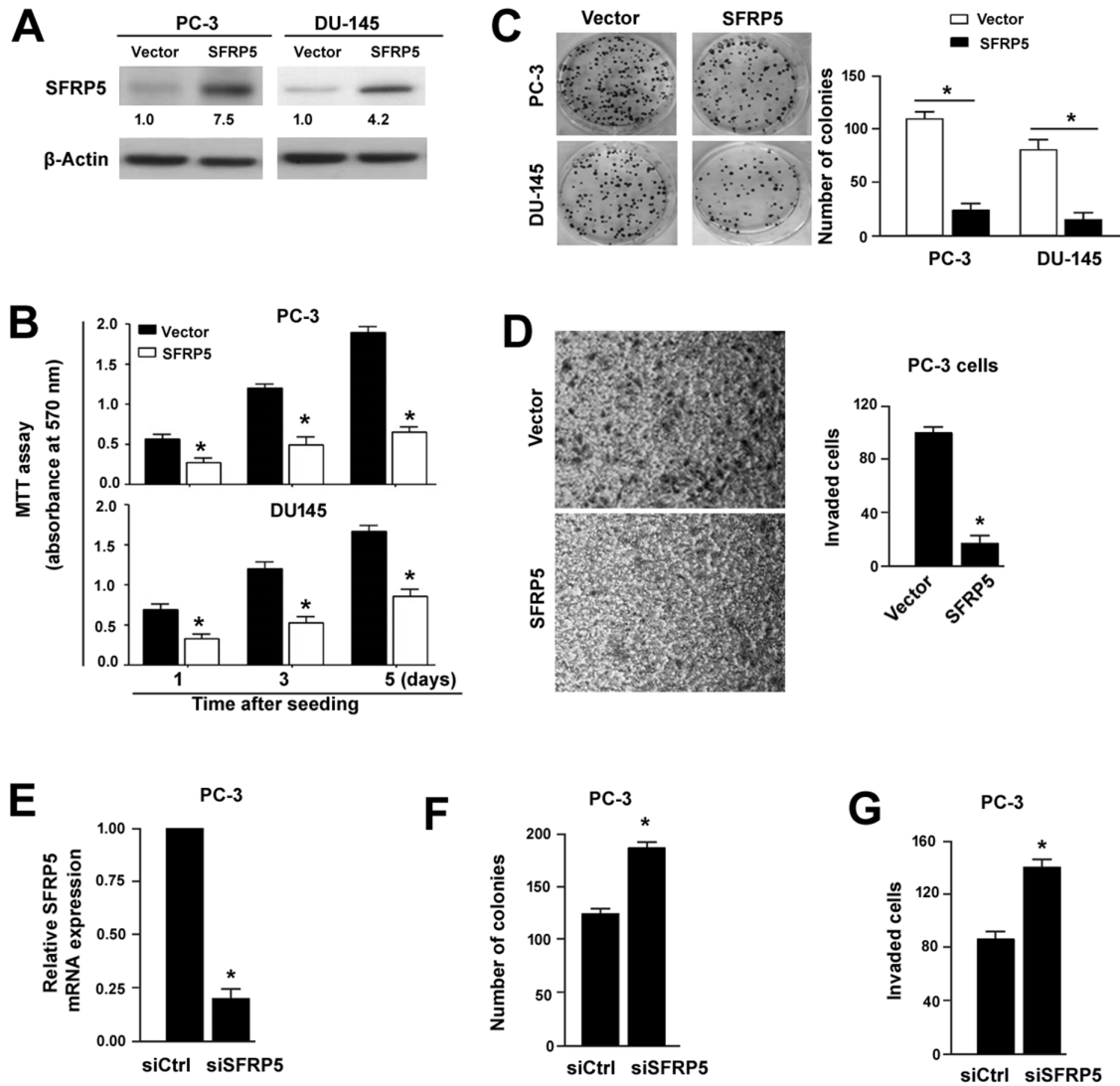
Secreted frizzled-related proteins (SFRPs) are a family of secreted glycoproteins that have been identified to antagonize the Wnt/ $\beta$ -catenin pathway.<sup>3</sup> They have the capacity to bind Wnt ligands through a frizzled-type cysteine-rich domain (CRD) and interfere with the ligation of frizzled receptors by Wnts.<sup>8</sup> In addition, SFRPs can inhibit Wnt signal transduction by forming non-functional complexes with frizzled receptors.<sup>8</sup>

The SFRP family comprises five members in humans, that is, SFRP1–5.<sup>9</sup> They are frequently downregulated in cancers as a result of hypermethylation of the promoter regions.<sup>10,11</sup> Downregulation of SFRP1 is associated with shorter survival in patients with prostate cancer.<sup>12</sup> SFRP3 shows the ability to inhibit the aggressive phenotype of prostate cancer cells.<sup>13</sup> A previous study has documented that *SFRP5* hypermethylation is commonly detected in prostate cancer specimens.<sup>14</sup> Another study demonstrated that



© American Federation for Medical Research 2019. No commercial re-use. See rights and permissions. Published by BMJ.

**To cite:** Xu Q, Lü Z, Wang X, et al. *J Investig Med* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jim-2018-000849



**Figure 1** Overexpression of secreted frizzled-related protein 5 (SFRP5) inhibits prostate cancer cell growth and invasion. (A) Western blot analysis of SFRP5 protein levels in PC-3 and DU-145 cells transfected with empty vector or SFRP5-expressing plasmid. Numbers indicate fold change in SFRP5 levels. (B) Cell proliferation measured by 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. (C) Colony-forming assays. PC-3 and DU-145 cells stably transfected with empty vector or SFRP5-expressing plasmid were cultured for 10 days, and colonies were counted. (D) Transwell invasion assay. Cells were allowed to invade the matrigel membrane for 48 hours, and invaded cells were counted. \* $P < 0.05$  vs vector-transfected cells. (E) Depletion of SFRP5 expression in PC-3 cells by small interfering RNA (siRNA) technology. The mRNA level of SFRP5 was quantified by real-time PCR analysis. siCtrl, control siRNA; siSFRP5, SFRP5-targeting siRNA. (F) Colony-forming assay and (G) Transwell invasion assay were performed in PC-3 cells transfected with siCtrl or siSFRP5. \* $P < 0.05$  vs siCtrl-transfected cells.

SFRP5 expression is reduced in prostate cancer compared with normal prostate tissues.<sup>10</sup> However, the biological role of SFRP5 in prostate cancer is largely unknown.

In this work, we performed SFRP5 overexpression experiments to explore the function of SFRP5 in regulating prostate cancer cell growth, survival, invasion, tumorigenesis, and docetaxel sensitivity. The effect of SFRP5 on Wnt/β-catenin signaling was also examined.

## MATERIALS AND METHODS

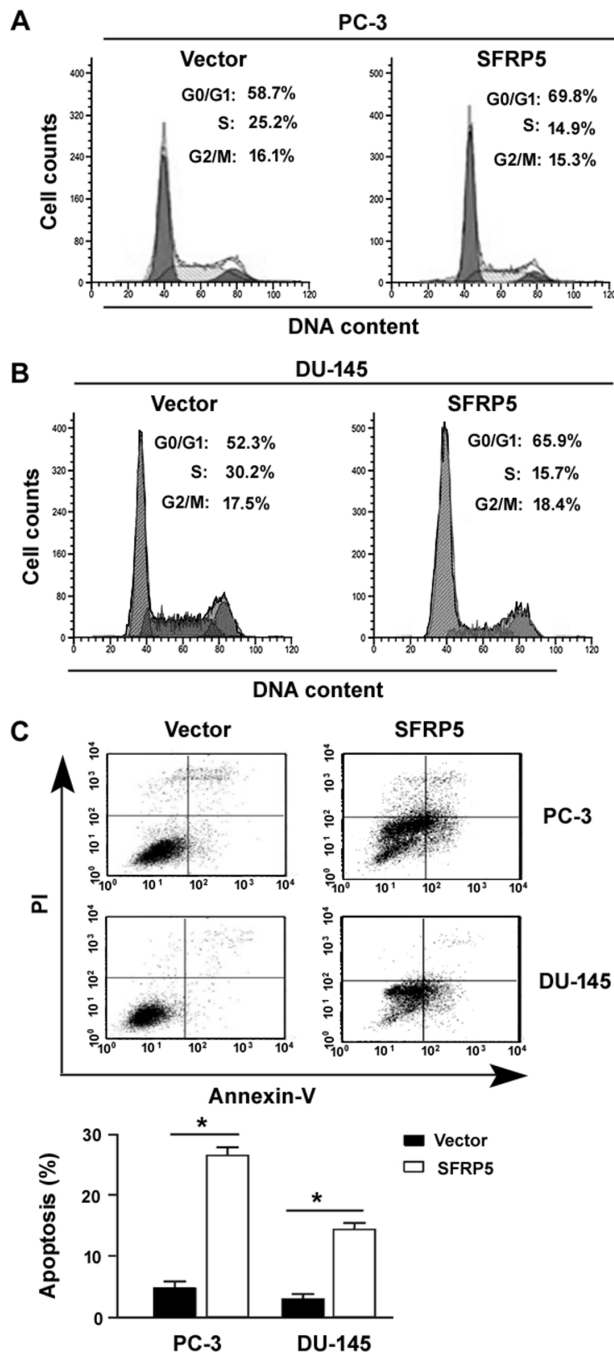
### Cell culture

Two human prostate cancer cell lines PC-3 and DU-145 were purchased from American Type Culture Collection

(Manassas, Virginia, USA). Cells were cultured in RPMI 1640 medium (Life Technologies, Rockville, Maryland, USA) supplemented with 10% fetal bovine serum (FBS; Life Technologies) in a humidified, 5% CO<sub>2</sub> incubator at 37°C. Short tandem repeat analysis was conducted by the Affiliated Hospital of Jiangsu University (Zhenjiang, China) to confirm cell identity.

### Plasmids, small interfering RNA, and transfection

Full-length human *SFRP5* and β-catenin cDNA was obtained from Origene (Rockville) and cloned to pIRES2-EGFP vector. This vector produces a single bicistronic mRNA that can be translated to SFRP5 and enhanced green fluorescent



**Figure 2** Secreted frizzled-related protein 5 (SFRP5) overexpression induces cell cycle arrest at G0/G1 phase and apoptosis. (A and B) Flow cytometric analysis of DNA contents in PC-3 and DU-145 cells transfected with empty vector or SFRP5-expressing plasmid. Numbers inserted show the percentage of cells at each cell-cycle phase. (C) Apoptosis analyzed by flow cytometry after annexin-V/propidium iodide (PI) staining. \* $P < 0.05$  vs vector-transfected cells.

protein (EGFP). SFRP5-targeting small interfering RNA (siRNA) and negative control siRNA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). For stable transfection, PC-3 and DU-145 cells at 70% confluence were transfected with either pIRES2-EGFP vector or pIRES2-EGFP-SFRP5 plasmid using TransFast transfection reagent

(Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. Transfected cells were selected with G418 (800  $\mu\text{g}/\text{mL}$ ; Sigma-Aldrich) 48 hours after transfection. G418-resistant, EGFP-expressing cells were sorted using a fluorescence-activated cell sorter and pooled together for further experiments.

### Western blot analysis

Cells were lysed in ice-cold lysis buffer (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] (pH=7.0), 0.5% NP-40, 2 mM sodium vanadate, and 0.1% sodium dodecyl sulfate (SDS)) supplemented with 1 $\times$  proteinase inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). For preparation of nuclear fractions, a commercially available kit (Pierce Biotechnology, Rockford, Illinois, USA) was used according to the manufacturer's instructions. Protein samples were separated by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes. The membranes were blocked with 5% fat-free milk and probed with anti-SFRP5 (sc-374397), anti- $\beta$ -catenin (sc-7963), anti-Lamin B (sc-365962), and anti- $\beta$ -actin (sc-130656) antibodies (Santa Cruz Biotechnology, Santa Cruz, California, USA). The membranes were then incubated with secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology). Protein bands were developed by enhanced chemiluminescence (Millipore, Billerica, Massachusetts, USA) and quantified using the software ImageJ (National Institutes of Health, Bethesda, Massachusetts, USA).

### Cell proliferation assay

Cells were plated on 96-well plates ( $2 \times 10^3$ /well) and cultured for 1, 3 and 5 days. Cell viability was tested using the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. In brief, MTT solution (0.5 mg/mL; Sigma-Aldrich) was added to each well and incubated at 37°C for 4 hours. Dimethyl sulfoxide (DMSO) was added to dissolve MTT formazan crystals. Absorbance was measured at 570 nm.

### Colony-forming assay

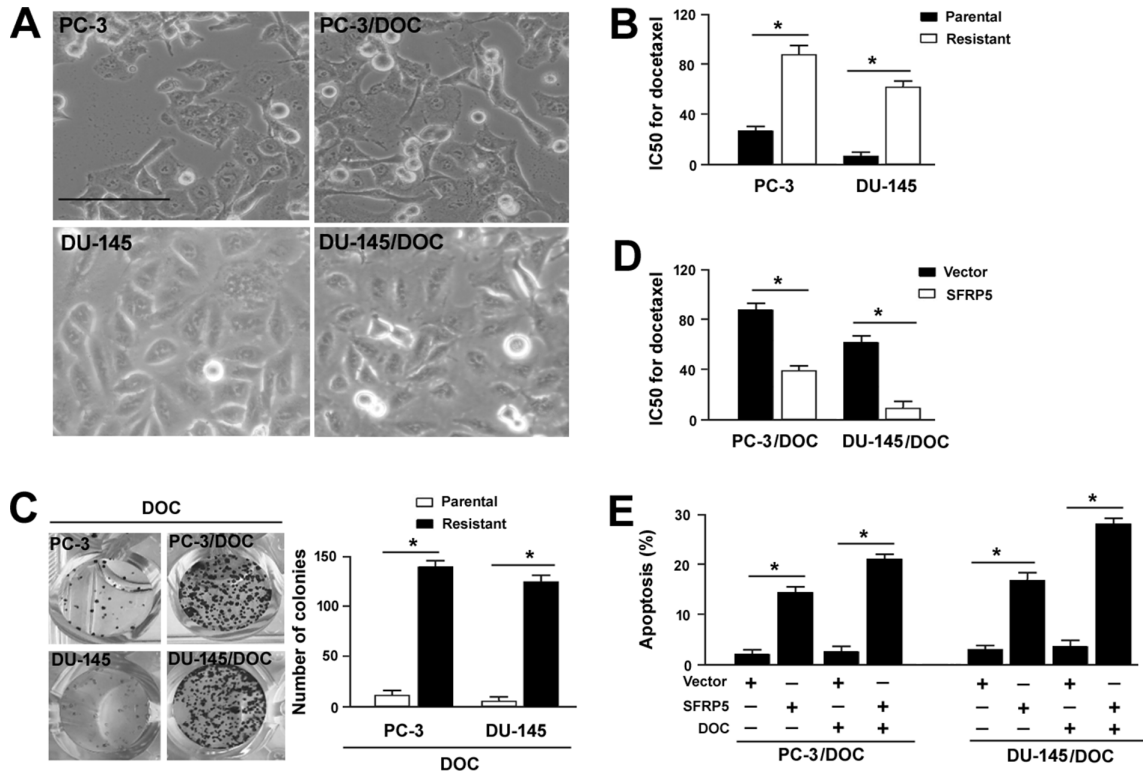
Cells were seeded at a density of 800 cells per well in 6-well plates. After culturing for 10 days, colonies were stained with 0.1% crystal violet and counted.

### Transwell invasion assay

PC-3 cells suspended in serum-free medium were plated over the insert precoated with matrigel (BD Biosciences, San Jose, California, USA) in a 24-well plates ( $1 \times 10^4$  cells/well). Complete media with 10% FBS were added to the lower chamber. After incubation for 48 hours at 37°C, the cells that invaded through the matrigel membrane were stained with 0.1% crystal violet. Stained cells from five randomly selected microscopic fields were counted.

### Flow cytometry

For analysis of cell cycle distribution, cells were trypsinized, washed, and fixed in 70% ethanol. The cells were suspended in propidium iodide (PI) staining solution (25  $\mu\text{g}/\text{mL}$ ) containing RNase (100  $\mu\text{g}/\text{mL}$ ; Sigma-Aldrich) at room temperature for 30 min. Stained cells were analyzed by



**Figure 3** Secreted frizzled-related protein 5 (SFRP5) overexpression reverses docetaxel (DOC) resistance in prostate cancer cells. (A) Examination of morphological features of DOC-resistant and parental PC-3 and DU-145 cells. Scale bar=50  $\mu$ m. (B) Compared with parental cells, DOC-resistant PC-3 and DU-145 cells had a 3.3-fold and 8.7-fold increase in the IC<sub>50</sub> values for DOC, respectively. (C) Colony-forming assays. Cells were cultured in the presence of DOC (80 nM) for 10 days, and colonies were counted. (D) DOC-resistant PC-3/DOC and DU-145/DOC cells were transfected with empty vector or SFRP5-expressing plasmid and exposed to different concentrations of DOC for 48 hours, and the IC<sub>50</sub> values were calculated. (E) Measurement of apoptosis in PC-3/DOC and DU-145/DOC cells transfected with empty vector or SFRP5-expressing plasmid in the presence or absence of DOC (80 nM). \*P<0.05.

flow cytometry (FACSCalibur, BD Bioscience, Heidelberg, Germany). For detection of apoptosis, cells were fixed and stained with annexin-V-fluorescein isothiocyanate (FITC) and PI (Nanjing KeyGen Biotech, Nanjing, China) following the manufacturer's instruction. Cell apoptosis was then analyzed by flow cytometry.

#### Docetaxel cytotoxic assay

Docetaxel-resistant PC-3 and DU-145 cells were generated as described previously.<sup>15</sup> In brief, PC-3 and DU-145 cells were exposed to an initial dose of 2 nM docetaxel. The surviving cells were continuously exposed to increasing doses of docetaxel up to 20 nM. Parental cells were exposed to DMSO with the same procedure.

For docetaxel treatment, cells were plated onto 96-well plates ( $4 \times 10^3$ /well) and cultured in the presence of different concentrations of docetaxel (2, 10, 40, 80, and 120 nM; Sigma-Aldrich). Forty-eight hours later, cell viability was measured using the CCK8 assay, and half maximal inhibitory concentration (IC<sub>50</sub>) was calculated.

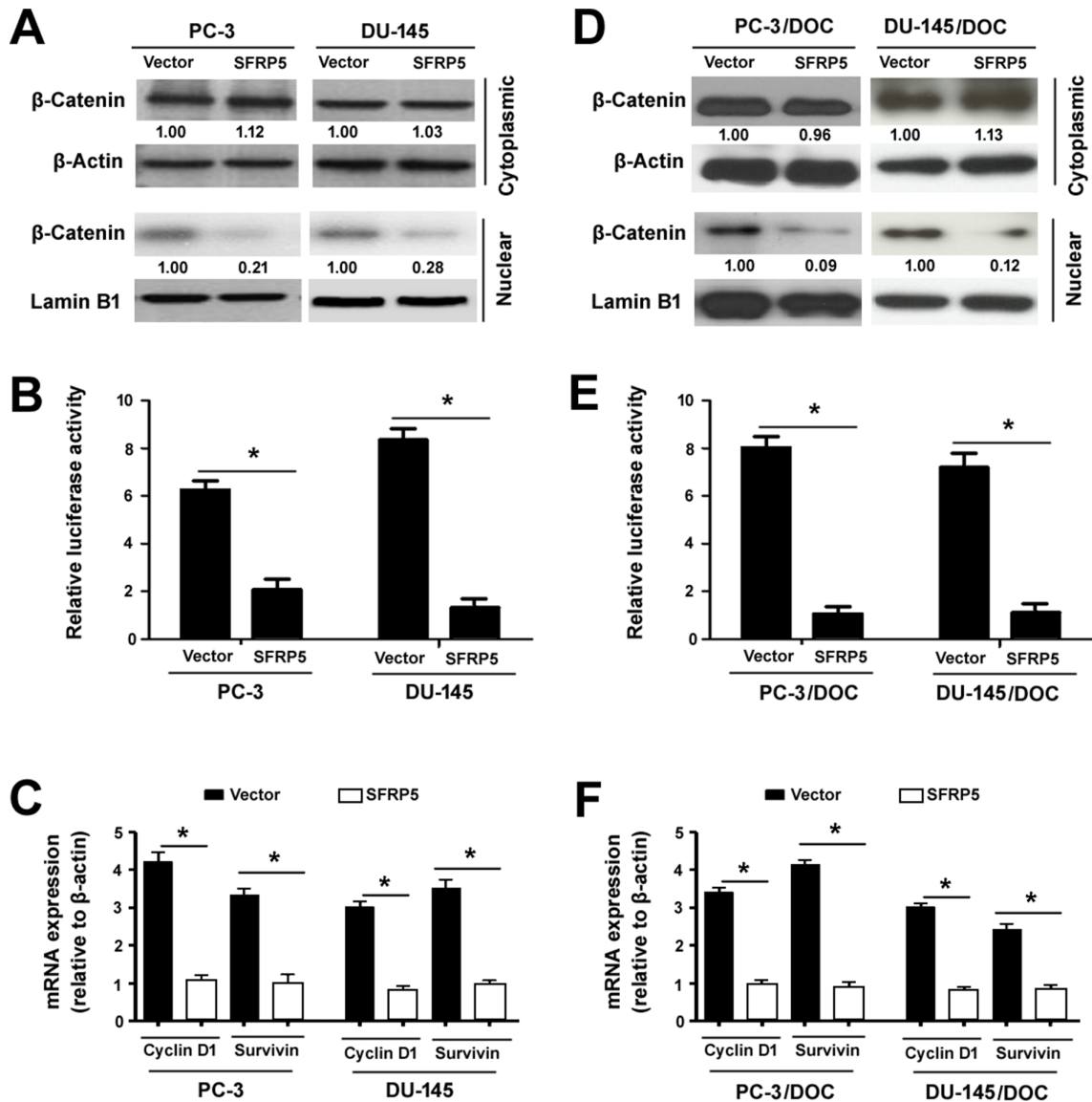
#### Luciferase reporter assay

Cells were plated onto 12-well plates ( $2 \times 10^4$  cells/well) the day before transfection. Cells at 70% confluence were co-transfected with TOP-FLASH or FOP-FLASH reporter (50 ng; Millipore) containing six copies of wild-type

or mutated TCF binding sites, respectively, along with SFRP5-expressing plasmid or empty vector (200 ng). The phRL-TK plasmid (Promega) that expresses *Renilla* luciferase was used as internal control. Cells were lysed 48 hours post-transfection, and luciferase activities were measured using a dual luciferase assay kit (Promega). TOP-FLASH or FOP-FLASH firefly luciferase activity was normalized to *Renilla* activity. Results are expressed as a ratio of normalized TOP-FLASH/FOP-FLASH.

#### Quantitative real-time PCR analysis

Total RNA was isolated from cells using RNeasy Mini Kit according to the manufacturer's protocol (Qiagen, Germantown, Maryland, USA). For cDNA synthesis, total RNA was reverse transcribed using random hexamers and SuperScript III reverse transcriptase (Invitrogen, Carlsbad, California, USA). PCR amplification was performed on an iQ5 iCycler (Bio-Rad, Hercules, California, USA) with the following primers: *Sfrp5* forward, 5'-CGCCTCCAGT-GACCAAGAT-3' and *Sfrp5* reverse, 5'-GATGCGCA TTTTGACCACAAAG-3'; *Birc5* forward, 5'-TGAAC TTC AGGTGGATGAGAGA-3' and *Birc5* reverse, 5'-GTCT AATCACACAGCAGTGGCAA-3'<sup>16</sup>; *Ccnd1* forward, 5'-CCGTCCATGCGGAAGATC-3' and *Ccnd1* reverse, 5'-ATGGCCAGCGGAAGAC-3'<sup>17</sup>; *ACTB* forward, 5'-GTGGCATCCACGAACTACC-3' and *ACTB* reverse,



**Figure 4** Overexpression of secreted frizzled-related protein 5 (SFRP5) inhibits  $\beta$ -catenin signaling. (A) Western blot analysis of  $\beta$ -catenin levels in cytoplasmic and nuclear fractions from PC-3 and DU-145 cells transfected with empty vector or SFRP5-expressing plasmid. Numbers indicate fold change in  $\beta$ -catenin protein levels. (B) Luciferase reporter assay. Ectopic expression of SFRP5 significantly suppressed the  $\beta$ -catenin/TCF transcriptional activity in both PC-3 and DU-145 cells. (C) Quantification of the mRNA levels of cyclin D1 and survivin by real-time PCR analysis. (D) Western blot analysis of  $\beta$ -catenin levels in cytoplasmic and nuclear fractions from docetaxel (DOC)-resistant PC-3 and DU-145 cells. (E) Luciferase reporter assay performed in DOC-resistant PC-3 and DU-145 cells. (F) Quantification of the mRNA levels of cyclin D1 and survivin. \* $P < 0.05$ .

5'-CAGGGCAGTGATCTCCTTCT-3'. *ACTB* was amplified in parallel and used as an internal control.

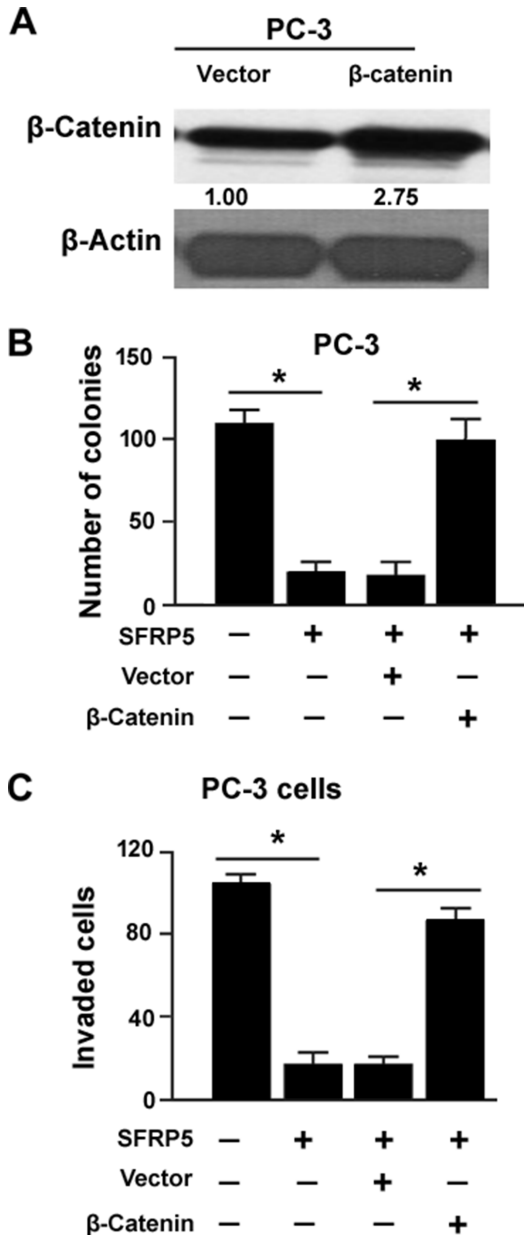
#### Tumorigenic studies in nude mice

Docetaxel-resistant and parental PC-3 cells stably expressing SFRP5 or control cells ( $2 \times 10^6$ ) were subcutaneously injected to the left flank of male BALB/c nu/nu mice aged 5 weeks ( $n=5$ ). Tumor volume was measured weekly from 1 week after cell injection. At 5 weeks, mice were killed and tumors were excised and weighed. Tumor specimens were fixed in formalin, embedded in paraffin, and cut into sections. Sections were immunostained with anti-Ki-67 antibody (Santa Cruz Biotechnology; 1:200 dilution) for assessment

of proliferation. For detection of apoptosis, sections were subjected to terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) staining (Upstate Biotechnology, Lake Placid, New York, USA). The percentages of Ki67-positive and TUNEL-positive cells were determined in 10 random fields at  $\times 100$  magnification.

#### Statistical analysis

Data are expressed as the mean  $\pm$  SD from three independent experiments and analyzed by the Student's *t*-test or one-way analysis of variance. Differences were considered significant when the *p* value was  $< 0.05$ .



**Figure 5** Overexpression of  $\beta$ -catenin rescues the inhibitory effects of secreted frizzled-related protein 5 (SFRP5) on prostate cancer cells. (A) Western blot analysis of  $\beta$ -catenin levels in PC-3 cells transfected with empty vector or  $\beta$ -catenin-expressing plasmid. (B) Colony-forming assays. PC-3 cells transfected with indicated constructs were cultured for 10 days, and colonies were counted. (C) Transwell invasion assay. Cells were allowed to invade the matrigel membrane for 48 hours, and invaded cells were counted. \* $P < 0.05$ .

## RESULTS

### SFRP5 inhibits prostate cancer cell growth and invasion

To explore the biological role of SFRP5 in prostate cancer, we overexpressed SFRP5 in both PC-3 and DU-145 cells. Western blot analysis confirmed that SFRP5 protein levels were increased by 7.5-fold and 4.2-fold in PC-3 and DU-145 cells stably transfected with the SFRP5-expressing plasmid, respectively (figure 1A). Consistent with the western blot results, the levels of secreted SFRP5 were significantly

higher in conditioned media from SFRP5-overexpressing PC-3 and DU-145 cells (data not shown). SFRP5-overexpressing PC-3 and DU-145 cells had a significantly lower proliferation rate than control cells ( $p < 0.05$ ; figure 1B). At the end of the 5-day culture period, cell proliferation was reduced by 70% and 51% in SFRP5-overexpressing PC-3 and DU-145 cells, respectively. Colony-forming assays further demonstrated that SFRP5 overexpression significantly inhibited the ability of PC-3 and DU-145 cells to form colonies ( $p < 0.05$  vs corresponding controls; figure 1C). Moreover, ectopic expression of SFRP5 led to a 42% reduction in PC-3 cell invasion, as determined by Transwell invasion assays ( $p < 0.05$  vs vector-transfected control; figure 1D).

To complement the overexpression experiments, we depleted SFRP5 expression using siRNA technology. We found that silencing of SFRP5 (figure 1E) led to an enhancement in colony formation (figure 1F) and invasion (figure 1G).

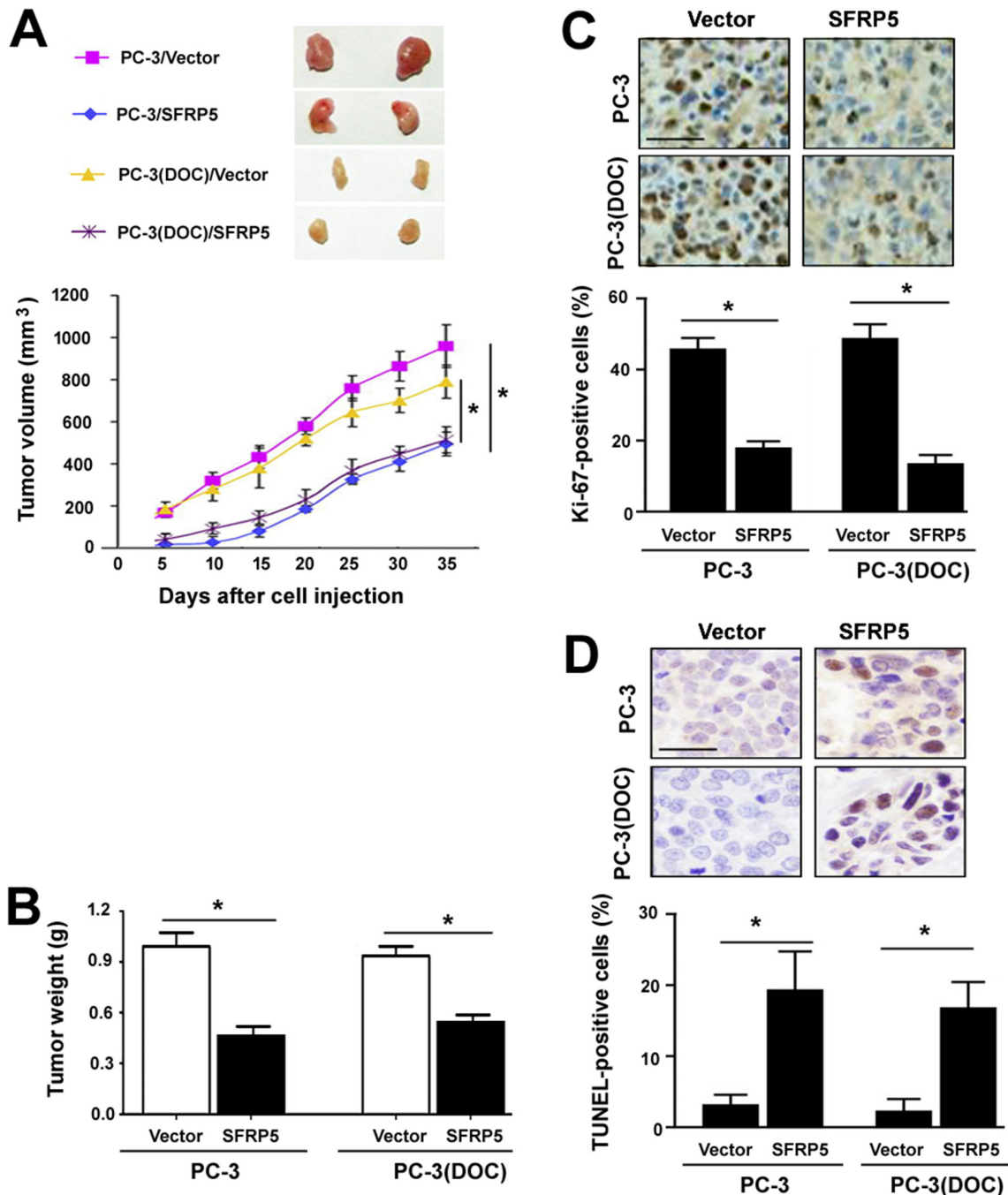
### SFRP5 overexpression induces cell cycle arrest at G0/G1 phase and apoptosis

To determine the mechanism for SFRP5-mediated growth suppression, cell cycle distribution was analyzed. While vector-transfected PC-3 cells had 58.7% of cells at G0/G1 phase and 25.2% of cells at S phase, SFRP5-overexpressing PC-3 cells had 69.8% of cells at G0/G1 phase and 15.3% of cells at S phase (figure 2A). Similarly, SFRP5 overexpression caused an accumulation of cells at G0/G1 phase in DU-145 cells (figure 2B). In addition to cell cycle alterations, there was a 5.2-fold and 3.6-fold induction of apoptosis in SFRP5-overexpressing PC-3 and DU-145 cells compared with corresponding controls (figure 2C).

### SFRP5 overexpression reverses docetaxel resistance in prostate cancer cells

Next, we checked the role of SFRP5 in docetaxel resistance in prostate cancer cells. Docetaxel-resistant and parental PC-3 and DU-145 cells showed similar appearance (figure 3A). Docetaxel-resistant and parental cells were exposed to different concentrations of docetaxel and tested for viability after 48 hours. Compared with parental PC-3 (26.4 vs 86.5 nM) and DU-145 (7.2 vs 62.8 nM) cells, docetaxel-resistant equivalents had a 3.3-fold and 8.7-fold increase in the  $IC_{50}$  values for docetaxel (figure 3B). Colonial survival assay demonstrated that the number of survival colonies was significantly higher in the docetaxel-resistant group than that in the parental control group after docetaxel (80 nM) treatment (figure 3C).

Notably, ectopic expression of SFRP5 significantly reduced the  $IC_{50}$  values for docetaxel in docetaxel-resistant PC-3 and DU-145 cells (figure 3D). Next, we determined whether the effect of SFRP5 overexpression on docetaxel resistance is related to apoptosis. Flow cytometric analysis revealed that SFRP5 overexpression significantly induced apoptosis in docetaxel-resistant PC-3 (13.2% vs 2.4%) and DU-145 (16.5% vs 3.1%) cells, compared with corresponding controls (figure 3E). Moreover, in the presence of docetaxel (80 nM), ectopic expression of SFRP5 caused apoptotic response in 28.6% and 35.8% docetaxel-resistant PC-3 and DU-145 cells, respectively (figure 3E).



**Figure 6** Secreted frizzled-related protein 5 (SFRP5) suppresses tumorigenicity of prostate cancer cells. (A) In vivo tumorigenic experiments. Docetaxel (DOC)-resistant and parental PC-3 cells stably transfected with empty vector or SFRP5-expressing plasmid were injected subcutaneously into nude mice ( $n=5$ ). Tumor growth was monitored for 5 weeks. *Upper*, representative photographs of xenograft tumors. (B) Tumor weight was determined 5 weeks after cell injection. (C) Immunohistochemistry for Ki-67 in tumor sections. Bar graphs indicate the percentage of Ki-67-positive cells. (D) Detection of apoptosis by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) in tumor sections. Scale bar=50  $\mu\text{m}$ . \* $P<0.05$ .

#### Overexpression of SFRP5 inhibits $\beta$ -catenin signaling

To check whether the growth-suppressive activity of SFRP5 is linked to inactivation of  $\beta$ -catenin signaling, we examined the expression levels of  $\beta$ -catenin after SFRP5 overexpression. Western blot analysis revealed a decrease in the level of nuclear  $\beta$ -catenin in SFRP5-overexpressing PC-3 and DU-145 cells (figure 4A). Consistently, docetaxel-resistant

PC-3 and DU-145 cells exhibited reduced accumulation of nuclear  $\beta$ -catenin when SFRP5 was overexpressed (figure 4A). Luciferase reporter assay further demonstrated that ectopic expression of SFRP5 significantly suppressed the  $\beta$ -catenin/TCF transcriptional activity in both PC-3 and DU-145 cells, compared with transfection with empty vector ( $p<0.05$ ; figure 4B). In addition, the mRNA levels

of cyclin D1 and survivin were decreased by threefold to fivefold after overexpression of SFRP5 (figure 4C). Similar findings were detected in docetaxel-resistant PC-3 and DU-145 cells (figure 4D–F). Taken together, these results indicate that SFRP5 suppresses  $\beta$ -catenin signaling in prostate cancer cells.

### Overexpression of $\beta$ -catenin rescues the inhibitory effects of SFRP5 on prostate cancer cells

To validate the involvement of the  $\beta$ -catenin signaling in the action of SFRP5, we performed rescue experiments. It was found that overexpression of  $\beta$ -catenin (figure 5A) significantly restored the colony-formation ability of SFRP5-overexpressing PC-3 cells (figure 5B). Moreover, SFRP5-dependent suppression of cell invasion was remarkably reversed by overexpression of  $\beta$ -catenin (figure 5C). Taken together, these findings suggest that SFRP5-induced anticancer effects are in part mediated through inhibition of  $\beta$ -catenin activity.

### SFRP5 suppresses tumorigenicity of prostate cancer cells in vivo

Finally, we investigated the role of SFRP5 in the growth of prostate cancer cells in vivo. PC-3 cells stably expressing SFRP5 or empty vector were subcutaneously injected to nude mice and tumor was monitored. As shown in figure 6A, SFRP5 overexpression significantly suppressed the growth of PC-3 xenograft tumors, compared with control tumors ( $p < 0.05$ ). Average tumor weight determined 5 weeks after cell injection was significantly lower in the SFRP5 group than that in the control group ( $0.48 \pm 0.09$  vs  $0.99 \pm 0.12$  g,  $p < 0.05$ ; figure 6B). Similarly, we found that overexpression of SFRP5 significantly restrained the growth of tumors derived from docetaxel-resistant PC-3 cells (figure 6A,B). Immunohistochemistry demonstrated that SFRP5 overexpression led to a significant decrease in the percentage of Ki-67-positive proliferating tumor cells (figure 6C). In addition, SFRP5-overexpressing tumors showed an increase in the percentage of TUNEL-positive cells, indicating tumor cell apoptosis, compared with control tumors ( $p < 0.05$ ; figure 6D). These results demonstrate the suppression of prostate cancer cell growth by SFRP5 in vivo.

## DISCUSSION

Growing evidence indicates that SFRP5 plays a suppressive role in multiple cancers such as hepatoma and ovarian cancer.<sup>18 19</sup> In this study, we showed that SFRP5 overexpression suppressed the proliferation, colony formation, and invasion of prostate cancer cells. Consistent with the in vitro observations, in vivo tumorigenic studies demonstrated that SFRP5-overexpressing PC-3 xenograft tumors had a significantly lower growth rate than control tumors, indicating SFRP5 as a tumor suppressor in prostate cancer. These findings provide a biological explanation for clinical data showing that SFRP5 is downregulated in prostate cancer relative to normal prostate tissues.<sup>10</sup> Similar to SFRP5, SFRP3 and SFRP4 exhibit the ability to suppress the aggressive phenotype of prostate cancer, suggesting the importance of SFRPs in prostate cancer progression.<sup>13 20</sup> However, it should be mentioned that the role of SFRPs in tumors is context-dependent, as in some cancer types such

as renal cancer,<sup>21</sup> SFRP3 can enhance tumor cell growth and invasion and prevent apoptotic death.

Our data further demonstrated that ectopic expression of SFRP5 induced an accumulation of G0/G1-phase cells in both PC-3 and DU-145 cells. Moreover, overexpression of SFRP5 significantly promoted apoptotic death in prostate cancer cells. These results suggest that the growth-suppressive activity of SFRP5 is causally linked to induction of G0/G1 cell cycle arrest and apoptosis. Having identified the ability to suppress proliferation and induce apoptosis, we next checked the effect of SFRP5 overexpression on chemosensitivity of prostate cancer cells. Notably, we found that overexpression of SFRP5 significantly reversed the resistance to docetaxel in docetaxel-resistant PC-3 and DU-145 cells. Consistently, SFRP5 overexpression caused significant apoptosis in docetaxel-resistant prostate cancer cells, particularly in the presence of docetaxel. Similarly, SFRP4 has been found to improve chemotherapeutic efficacy against prostate cancer cells.<sup>22</sup> These data suggest that SFRP5 overexpression may represent a promising strategy to overcome docetaxel resistance in prostate cancer cells.

SFRPs are important negative regulators of Wnt/ $\beta$ -catenin signaling.<sup>3 8</sup> It has been documented that SFRP3 overexpression leads to a decline in cytosolic  $\beta$ -catenin levels in PC-3 cells.<sup>13</sup> Another study reported that overexpression of SFRP4 promotes membranous location of  $\beta$ -catenin in prostate cancer cells.<sup>23</sup> Inhibition of  $\beta$ -catenin transcriptional activity is involved in the anticancer activity of SFRP3 and SFRP5.<sup>13 23</sup> In agreement with these studies, we found that enforced expression of SFRP5 restrained nuclear accumulation of  $\beta$ -catenin and thus inhibited  $\beta$ -catenin-dependent TCF transcriptional activity. Rescue experiments showed that overexpression of  $\beta$ -catenin significantly reversed SFRP5-mediated suppression of prostate cancer cell growth and invasion. Both cyclin D1 and survivin, two downstream target genes of  $\beta$ -catenin,<sup>5</sup> were significantly suppressed by SFRP5 overexpression. Cyclin D1 plays a critical role in cell cycle progression and promotes DNA synthesis and G1-S phase transition.<sup>24</sup> It was reported that knockdown of cyclin D1 inhibits tumorigenicity and enhances cancer cells to radiotherapy in prostate cancer.<sup>25</sup> Downregulation of cyclin D1 by WWOX leads to a cell cycle arrest at the G1 phase in prostate cancer cells.<sup>26</sup> Survivin serves as an anti-apoptotic protein belonging to the inhibitor of apoptosis protein family and has the ability to augment cell proliferation and inhibit apoptosis.<sup>27</sup> Inhibition of survivin is responsible for apoptotic death in prostate cancer cells induced by zoledronic acid.<sup>28</sup> These studies, combined with our findings, suggest that SFRP5-mediated tumor suppression is at least partially ascribed to downregulation of cyclin D1 and survivin in prostate cancer.

In conclusion, we demonstrate that SFRP5 overexpression suppresses cell growth and reverses docetaxel resistance in prostate cancer through inhibition of Wnt/ $\beta$ -catenin signaling and downregulation of cyclin D1 and survivin. Re-expression of SFRP5 may confer therapeutic benefits in the treatment of prostate cancer.

**Contributors** QXu and HWu participated in study design, data collection, and drafting of the manuscript; ZLü, XW, and QZ conducted experiments.



**Funding** This work was supported by the Changzhou Science and Technology Program (no. WS201514) and the Wujin Hospital, Affiliated to Jiangsu University, Changzhou, China.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** All animal studies were approved by the Institutional Animal Care and Use Committee of Wujin Hospital Affiliated to Jiangsu University (Changzhou, China).

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

- Murakami T, Mitomi H, Yao T, *et al.* Epigenetic regulation of Wnt/ $\beta$ -catenin signal-associated genes in gastric neoplasia of the fundic gland (chief cell-predominant) type. *Pathol Int* 2017;67:147–55.
- Xiao Q, Chen Z, Jin X, *et al.* The many postures of noncanonical Wnt signaling in development and diseases. *Biomed Pharmacother* 2017;93:359–69.
- Warrier S, Marimuthu R, Sekhar S, *et al.* sFRP-mediated Wnt sequestration as a potential therapeutic target for Alzheimer's disease. *Int J Biochem Cell Biol* 2016;75:104–11.
- Wang S, Zhang D, Han S, *et al.* Fibulin-3 promotes osteosarcoma invasion and metastasis by inducing epithelial to mesenchymal transition and activating the Wnt/ $\beta$ -catenin signaling pathway. *Sci Rep* 2017;7:6215.
- Fiskus W, Sharma S, Saha S, *et al.* Pre-clinical efficacy of combined therapy with novel  $\beta$ -catenin antagonist BC2059 and histone deacetylase inhibitor against AML cells. *Leukemia* 2015;29:1267–78.
- Li Q, Ye L, Zhang X, Xin Z, *et al.* FZD8, a target of p53, promotes bone metastasis in prostate cancer by activating canonical Wnt/ $\beta$ -catenin signaling. *Cancer Lett* 2017;402:166–76.
- Seo WI, Park S, Gwak J, *et al.* Wnt signaling promotes androgen-independent prostate cancer cell proliferation through up-regulation of the hippo pathway effector YAP. *Biochem Biophys Res Commun* 2017;486:1034–9.
- Povelones M, Nusse R. The role of the cysteine-rich domain of Frizzled in Wntless-Armadillo signaling. *Embo J* 2005;24:3493–503.
- Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. *Nat Rev Cancer* 2008;8:387–98.
- García-Tobilla P, Solórzano SR, Salido-Guadarrama I, *et al.* SFRP1 repression in prostate cancer is triggered by two different epigenetic mechanisms. *Gene* 2016;593:292–301.
- Liang J, Kang X, Halifu Y, *et al.* Secreted frizzled-related protein promoters are hypermethylated in cutaneous squamous carcinoma compared with normal epidermis. *BMC Cancer* 2015;15:641.
- Zheng L, Sun D, Fan W, *et al.* Diagnostic value of SFRP1 as a favorable predictive and prognostic biomarker in patients with prostate cancer. *PLoS One* 2015;10:e0118276.
- Zi X, Guo Y, Simoneau AR, *et al.* Expression of Frzb/secreted Frizzled-related protein 3, a secreted Wnt antagonist, in human androgen-independent prostate cancer PC-3 cells suppresses tumor growth and cellular invasiveness. *Cancer Res* 2005;65:9762–70.
- Perry AS, O'Hurley G, Raheem OA, *et al.* Gene expression and epigenetic discovery screen reveal methylation of SFRP2 in prostate cancer. *Int J Cancer* 2013;132:1771–80.
- Marín-Aguilera M, Codony-Servat J, Kalko SG, *et al.* Identification of docetaxel resistance genes in castration-resistant prostate cancer. *Mol Cancer Ther* 2012;11:329–39.
- Fukuda S, Foster RG, Porter SB, *et al.* The antiapoptosis protein survivin is associated with cell cycle entry of normal cord blood CD34(+) cells and modulates cell cycle and proliferation of mouse hematopoietic progenitor cells. *Blood* 2002;100:2463–71.
- Howe JG, Crouch J, Cooper D, *et al.* Real-time quantitative reverse transcription-PCR for cyclin D1 mRNA in blood, marrow, and tissue specimens for diagnosis of mantle cell lymphoma. *Clin Chem* 2004;50:80–7.
- Xie Q, Chen L, Shan X, *et al.* Epigenetic silencing of SFRP1 and SFRP5 by hepatitis B virus X protein enhances hepatoma cell tumorigenicity through Wnt signaling pathway. *Int J Cancer* 2014;135:635–46.
- Su HY, Lai HC, Lin YW, *et al.* Epigenetic silencing of SFRP5 is related to malignant phenotype and chemoresistance of ovarian cancer through Wnt signaling pathway. *Int J Cancer* 2010;127:555–67.
- Horvath LG, Henshall SM, Kench JG, *et al.* Membranous expression of secreted frizzled-related protein 4 predicts for good prognosis in localized prostate cancer and inhibits PC3 cellular proliferation in vitro. *Clin Cancer Res* 2004;10:615–25.
- Hirata H, Hinoda Y, Ueno K, *et al.* Role of secreted frizzled-related protein 3 in human renal cell carcinoma. *Cancer Res* 2010;70:1896–905.
- Deshmukh A, Kumar S, Arfuso F, *et al.* Secreted Frizzled-related protein 4 (sFRP4) chemo-sensitizes cancer stem cells derived from human breast, prostate, and ovary tumor cell lines. *Sci Rep* 2017;7:2256.
- Horvath LG, Lelliott JE, Kench JG, *et al.* Secreted frizzled-related protein 4 inhibits proliferation and metastatic potential in prostate cancer. *Prostate* 2007;67:1081–90.
- Qie S, Diehl JA. Cyclin D1, cancer progression, and opportunities in cancer treatment. *J Mol Med* 2016;94:1313–26.
- Marampon F, Gravina GL, Ju X, *et al.* Cyclin D1 silencing suppresses tumorigenicity, impairs DNA double strand break repair and thus radiosensitizes androgen-independent prostate cancer cells to DNA damage. *Oncotarget* 2016;7:64526.
- Lin JT, Li HY, Chang NS, *et al.* WWOX suppresses prostate cancer cell progression through cyclin D1-mediated cell cycle arrest in the G1 phase. *Cell Cycle* 2015;14:408–16.
- Su C. Survivin in survival of hepatocellular carcinoma. *Cancer Lett* 2016;379:184–90.
- Fragini M, Bonini SA, Stabile A, *et al.* Inhibition of survivin is associated with zoledronic acid-induced apoptosis of prostate cancer cells. *Anticancer Res* 2016;36:913–20.