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Original Article

SGO1 induces proliferation and metastasis of prostate cancer through AKT-mediated signaling pathway

Qi Chen*, Xiang Wan*, Yanbo Chen*, Chong Liu, Meng Gu, Zhong Wang

*Department of Urology, Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. *Equal contributors.*

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Abstract: Although studies have revealed some of the pathological causes associated with prostate cancer progression, further studies are still needed. Shugoshin 1 (SGO1) is a protein essential for precise chromosome segregation during mitosis and meiosis. However, the role and mechanism of SGO1 in tumors and even prostate cancer is not completely clear. In this study, expression of SGO1 in human prostate tumors were higher than that of adjacent normal tissues and were positively correlated with the poor prognosis of prostate cancer patients. SGO1 expression levels are also higher in several prostate cancer cell lines. In cell experiments, knockdown of SGO1 reduced cell proliferation, migration, and invasion in vitro and in vivo, and also inhibited cell cycle progression of prostate cancer cells. In contrast, ectopic expression of SGO1 has the opposite effects. In addition, knockdown of SGO1 induces apoptosis in prostate cancer cells by promoting cleaved caspase-3, caspase-9, and PARP. Importantly SGO1 function is dependent on AKT. Inhibition of AKT activity by AKT inhibitor abolished the role of SGO1 overexpression in promoting cell proliferation and metastasis. Therefore, SGO1 promotes the proliferation and metastasis of prostate cancer through the AKT pathway, and can be considered as an effective candidate for developing an effective prostate cancer treatment strategy.

Keywords: SGO1, prostate cancer, metastasis, AKT

Introduction

At present the diagnostic accuracy of prostate cancer is relatively low, which leads to the loss of optimal treatment time for many patients, and the survival rate is not improved [1]. The prognosis indicators for the diagnosis and evaluation of prostate cancer are mainly based on clinical features, pathological results, and detection of dynamic changes of blood prostate specific antigen (PCA) [2-4]. However, these methods cannot fully adapt to clinical diagnosis and timely treatment. Therefore, it is necessary to find new prostate cancer-specific biomarkers for diagnosis and treatment.

In the mitosis of cells, the precise separation of the sister chromatid is important for maintaining the stability of the genome and the survival of the cells [5, 6]. If it's isolated abnormally, it will lead to the formation of aneuploidy, which in turn will promote the occurrence of tumors. Genetic instability caused by human chromosome abnormalities could lead to tumorigenesis [7]. The centromere related protein Shugoshin1 (SGO1) has been shown to ensure the correct and orderly conduct of mitosis by protecting and maintaining centripetal adhesions during meiosis and mitosis [8]. In addition, it ensures that cohesin is stably attached to chromosomes before the onset of mitotic terminal stages, senses sister chromatid tension and regulates the stability of microtubules, thus playing an important role in ensuring the stability of DNA [9]. Several studies have shown that the loss of SGO1 leads to premature dissociation of sister chromatids, which in turn leads to mitotic arrest [10, 11]. Wang et al. found SGO1 was also highly expressed in adriamycin-resistant gastric cancer, and may mediate the resistance of adriamycin to cells [12]. In this experiment, we found that SGO1 was highly expressed in prostate cancer, and SGO1 promoted cell proliferation and inhibits apoptosis. In terms of mechanism, the promotion of prostate cancer metastasis by SGO1 was dependent on the AKT signaling pathway.

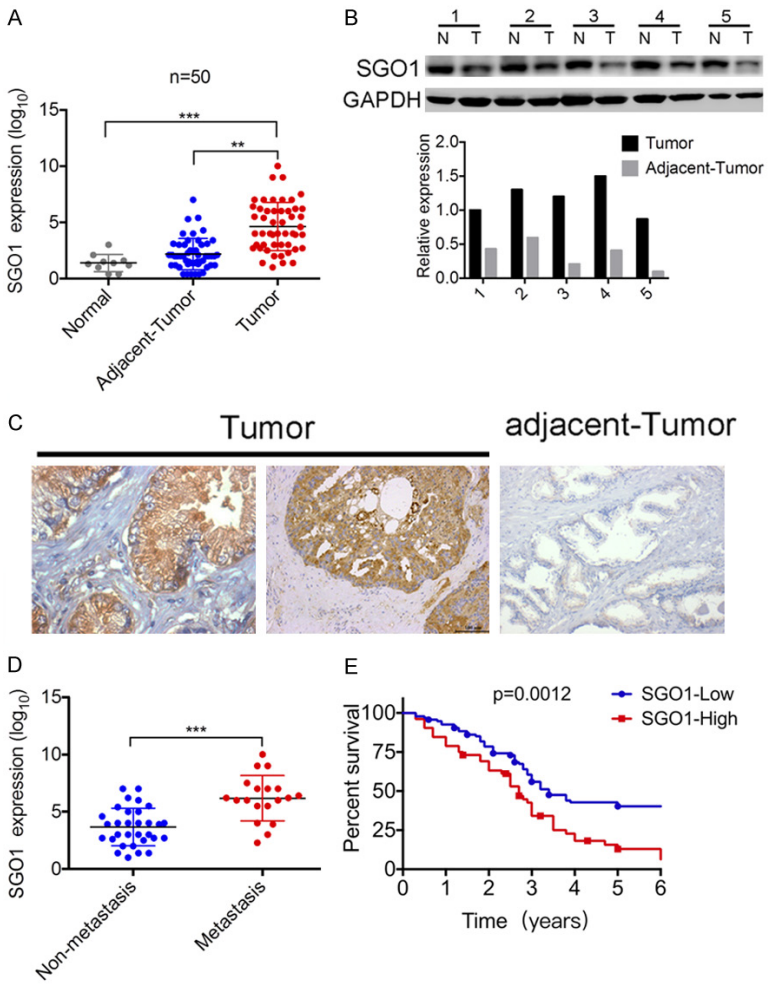


Figure 1. SGO1 is highly expressed in human prostate cancer and represents a poor prognosis. A. Total RNA from 50 pairs of prostate cancer and their paracancerous tissues was extracted, and the expression level of SGO1 mRNA was detected by RT-PCR. B. Five pairs of prostate cancer and its adjacent tissue proteins were extracted and western blot was used to detect SGO1 protein levels. C. Typical IHC schematic shows the expression of SGO1 in prostate cancer and adjacent tissues. D. SGO1 was highly expressed in human prostate cancer. The IHC score was determined by staining intensity and staining density. E. Survival curves of prostate cancer patients expressing SGO1 at high and low levels.

Materials and methods

Cell lines and reagents

Various prostate cancer cell lines are from the American Type Culture Collection (ATCC). The cell line was cultured in DMEM or RPMI 1640 supplemented with 10% fetal bovine serum and grown at 37°C, 5% CO_2. The antibodies purchased include SGO1 (Abcam, ab58023), GAPDH (Cell Signaling Technology, 2118), cyclin A2 (Cell Signaling Technology, 4656), CDK2 (Cell Signaling Technology, 2546), cyclin D1 (Cell Signaling Technology, 4978), AKT (Cell Signaling Technology, 4685), pAKT Ser473 (Cell Signaling Technology, 4060), cleaved caspase-3 (Abcam, ab2302), cleaved caspase-9 (Abcam, ab2324), caspase-3 (Abcam, ab138-47), caspase-9 (Abcam, ab-32539), cleaved PARP1 (Abeam, ab32064), PARP (Abeam, ab74290), pBad Ser136 (Cell Signaling Technology, 4336).

Patient and tumor samples

All clinical sample experiments were approved by the ethics committee. A total of 148 primary prostate cancer specimens surgically resected were selected, including the corresponding paracancerous tissue. All prostate cancer tissue samples were fixed in 4% paraformaldehyde for immunohistochemical preparation and a small portion of tumor tissue samples were frozen in liquid nitrogen for RNA extraction and protein identification.

Immunohistochemistry

All paraformaldehyde-fixed prostate cancer tissue samples were paraffin-embedded and sectioned. Immunohistochemistry of tissue sections was performed according to manufacturer's instructions (Beyotime Biotechnology). The staining results were assessed by semi-quantitative methods including staining intensity (0-negative, 1-low, 2-moderate, 3-strong, 4-strong) and the percentage of stained cells (0-0%, 1-1-10%, 2-11-35%, 3-26-50%, 4-51-80%, 5-81-100%). Add the staining intensity fraction and the percentage of stained cells to obtain the final evaluation result. In pathological statistics, 4 points or less are considered low expression,

Table 1. Correlative analysis of SGO1 expression and clinical data in 148 patients with prostate cancer

Characteristic	Patients	SGO1 expression		P
		Low	High	
Age				0.817
< 65	60	35	25	
≥ 65	88	53	35	
Prostate volume (cm^3)				0.586
< 50	70	40	30	
≥ 50	78	48	30	
Histological grade				0.700
G1/G2	86	50	36	
G3	62	38	24	
TNM stage (AJCC)				0.002
I/II	56	44	12	
III/IV	92	44	48	
Gleason score				0.010
1-7	56	61	31	
8-10	92	20	26	
Lymph node metastasis				0.001
Present	46	18	28	
Absent	102	70	32	
Distant metastasis				0.001
Present	38	12	26	
Absent	110	76	34	
Total	148	88	60	

and 5 points or more are considered high expression.

shRNA knockdown

Each shRNA sequence of SGO1 is: SGO1-shRNA-A 5'-CCGGCGGGCTTCACATCCTTAGAAACTCGAGTTTCTAAGGATGTGAAGCCCGTTTTTG-3'; SGO1-shRNA-B 5'-CCGGCCGCAAATTCCTCTTGAAGAACTCGAGTTCTTCAAGAGGAATTTGCGGTTTTTG-3'; SGO1-shRNA-C 5'-CCGGGAAGATCAGATACCTACTATTCTCGAGAATAGTAGGTATCTGATCTTCTTTTTTG-3'. SGO1-shRNA and control shRNA were packaged separately for lentivirus, then infected PC3, DU145 cells to obtain a stable knockdown cell line.

Colony formation assays

500 SGOI-shRNA knockdown cells and control groups were seeded in 12 well plates and cultured in complete medium for two weeks. The medium was then discarded, fixed with 4% paraformaldehyde and stained with 0.05% crystal violet. Take a photo and count the number of colonies in each well.

Transwell assays

Transwell (Corning) was placed in a 24-well culture plate. In the lower chamber, 600 μL of DMEM medium containing 10% FBS was added. In the upper chamber, serum-free DMEM and treated cells were added. After 24 hours of culture, the migrated cells were fixed with 4% paraformaldehyde and stained with 0.05% crystal violet. Finally, the stained cells were counted under a light microscope.

Mice xenograft model

The experimental method for subcutaneous transplantation of tumors was to implant 5 × 10^6 PC3 and DU145 cells subcutaneously into BALB/c nude mice. The length and width of tumors were measured every week to determine the tumor volume. After 4 weeks, the tumor-bearing mice were photographed, tumors were extracted and weighed. The lung metastasis models were constructed via 5 × 10^6 treated PC3 cells by tail vein injecetion into BALB/c mice. Mice were sacrificed 30 days later to observe the number of metastasis of prostate cancer cells in the lung and to perform statistics. All experiments were approved by the Ethics Committee.

Statistical methods

Use *SPSS 19.0* and *Graphpad 6* to complete the required statistical analysis. The T test is used for statistical analysis of the categorical data. *P* values < 0.05 were considered significant differences.

Results

SGO1 is highly expressed in human prostate cancer and predicts poor prognosis

Clinically obtained human prostate cancer samples were firstly studied. 148 patients with prostate cancer and their adjacent tissues were collected for detection. Fifty tumor and adjacent tissue mRNAs were extracted and subjected to RT-PCR analysis. RT-PCR results showed that SGO1 mRNA levels were highly expressed in prostate cancer compared to adjacent tissues (**Figure 1A**). We lysed tissue specimens and examined SGO1 protein levels.

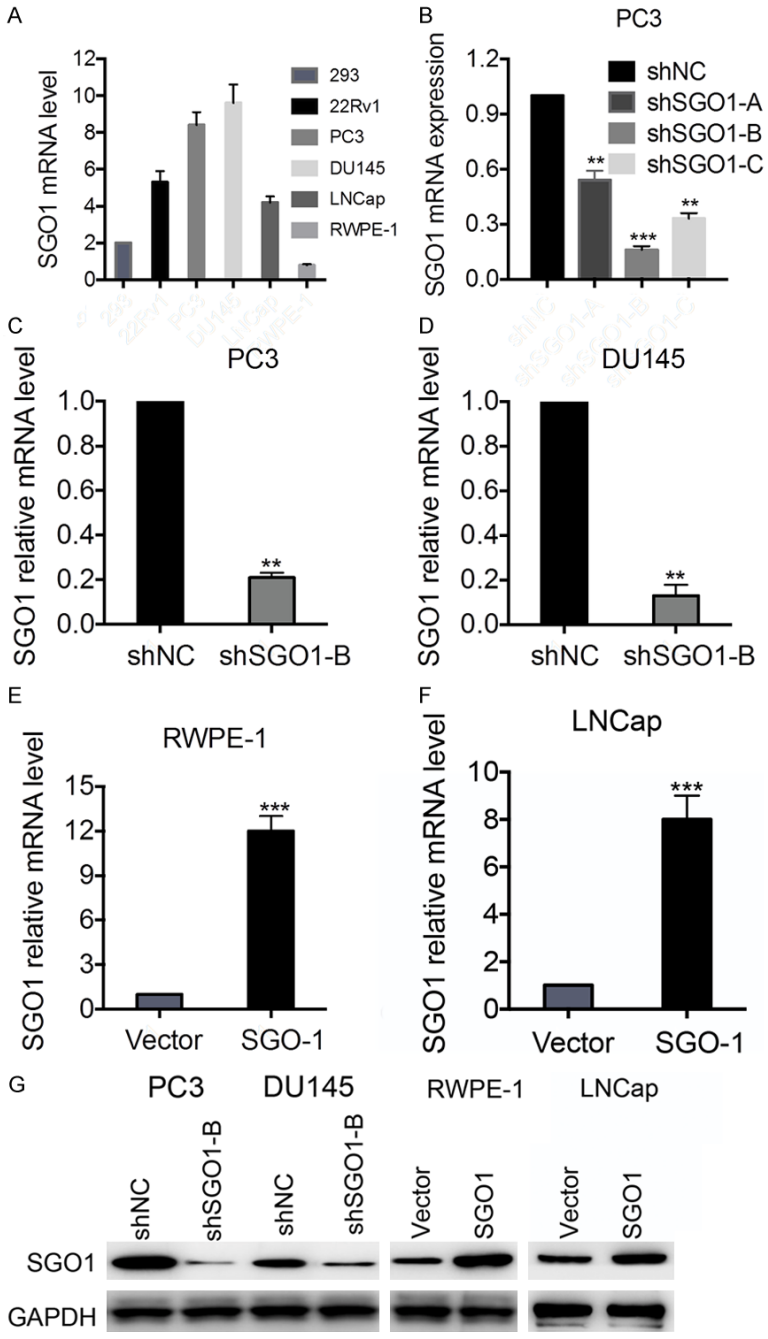


Figure 2. SGO1 is highly expressed in prostate cancer cell lines PC3 and DU145. (A) 293, 22Rvl, PC3, DU145, LNCap and RWPE-1 cells were lysed and SGO1 protein levels were detected by western blot. (B) SGO1-shRNA-A, SGO1-shRNA-B, SGO1-shRNA-C, and NC-shRNA (control) were transfected into PC3 cells and RT-PCR showed knockdown efficiency. The most efficient SGO1-shRNA-B was selected and transfected into PC3 (C) and DU145 (D) cells. RT-PCR showed knockdown efficiency. SGO1 overexpression vector and control were transfected into RWPE-1 (E) and LNCap (F) cells. RT-PCR showed overexpression efficiency of SGO1. (G) The knockdown and overexpression efficiency of SGO1 in PC3, DU145, RWPE-1 and LNCap cells was examined by western blot.

We found that SGO1 proteins were highly expressed in tumor tissues (**Figure 1B**). The expression levels of SGO1 in tissue samples were then detected by immunohistochemistry. SGO1 was significantly higher in prostate cancer than in adjacent tissues (**Figure 1C** and **1D**). According to the IHC score, 148 tumor tissues were divided into 60 high expression groups and 88 low expression groups, and correlation analysis was performed with the corresponding clinical data. The results showed that SGO1 was closely related to the patient's TNM stage (P = 0.002), gleason score (P = 0.010), lymph node metastasis (P = 0.001), and distant metastasis (P = 0.001) (**Table 1**). In addition, the expression of SGO1 was closely related to the prognosis of patients with prostate cancer, that is, the survival rate of patients with high expression of SGO1 was significantly lower than that of patients with low expression (**Figure 1E**). The above results indicate that SGO1 is highly expressed in human prostate cancer tissues and predicts poor prognosis.

High expression of SGO1 in prostate cancer cell lines increases proliferation

Next the function of SGO1 in prostate cancer cell lines was studied. In several prostate cancer cell lines, we detected the expression levels of SGO1 by western blot and found that SGO1 was highly expressed in PC3 and DU145 cells, but low in RWPE-1 and LNCap cells (**Figure 2A**). Therefore, we chose PC3, DU145, RWPE-1 and LNCap cells as research cell lines. In PC3 cells, we tested three shRNAs of the SGO1 gene and found that shSGO1-B knockdown was

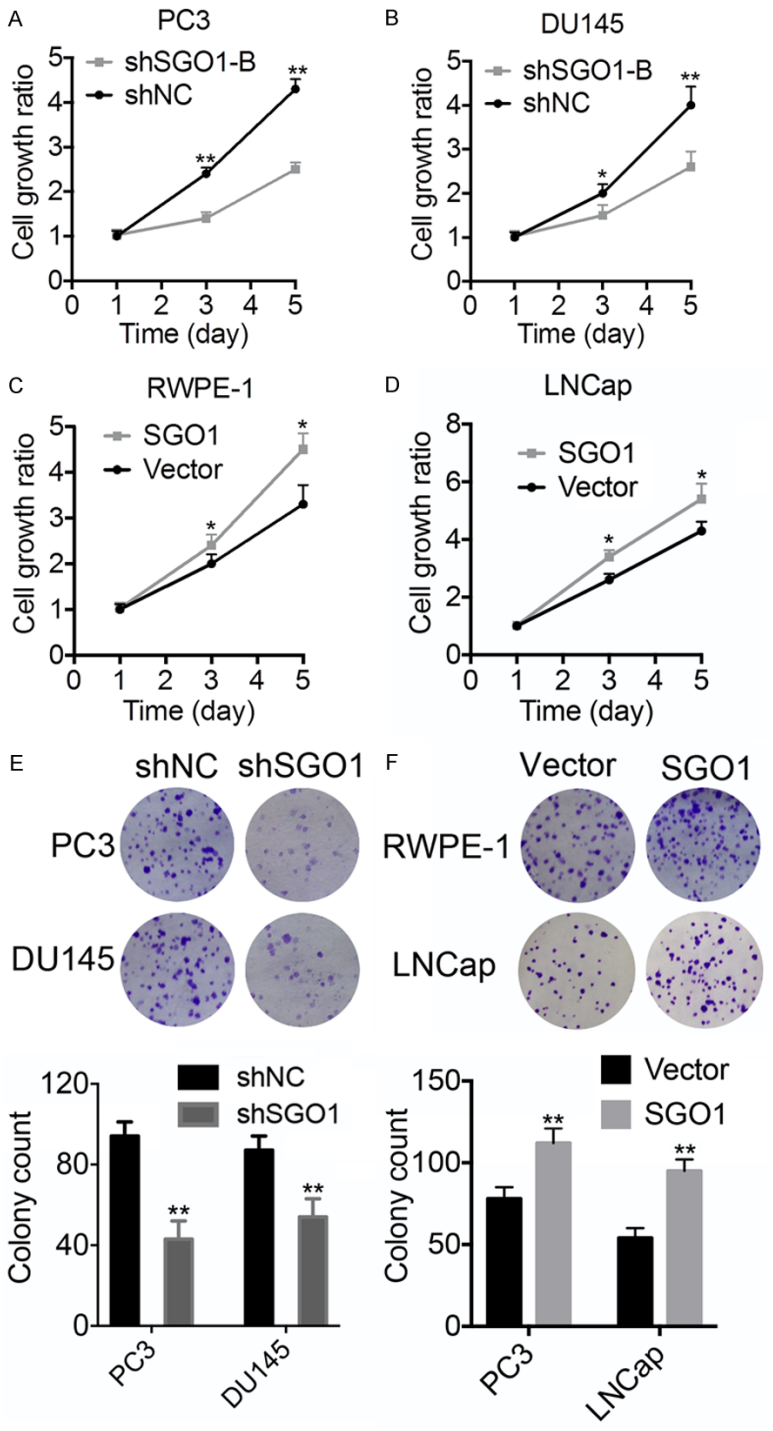
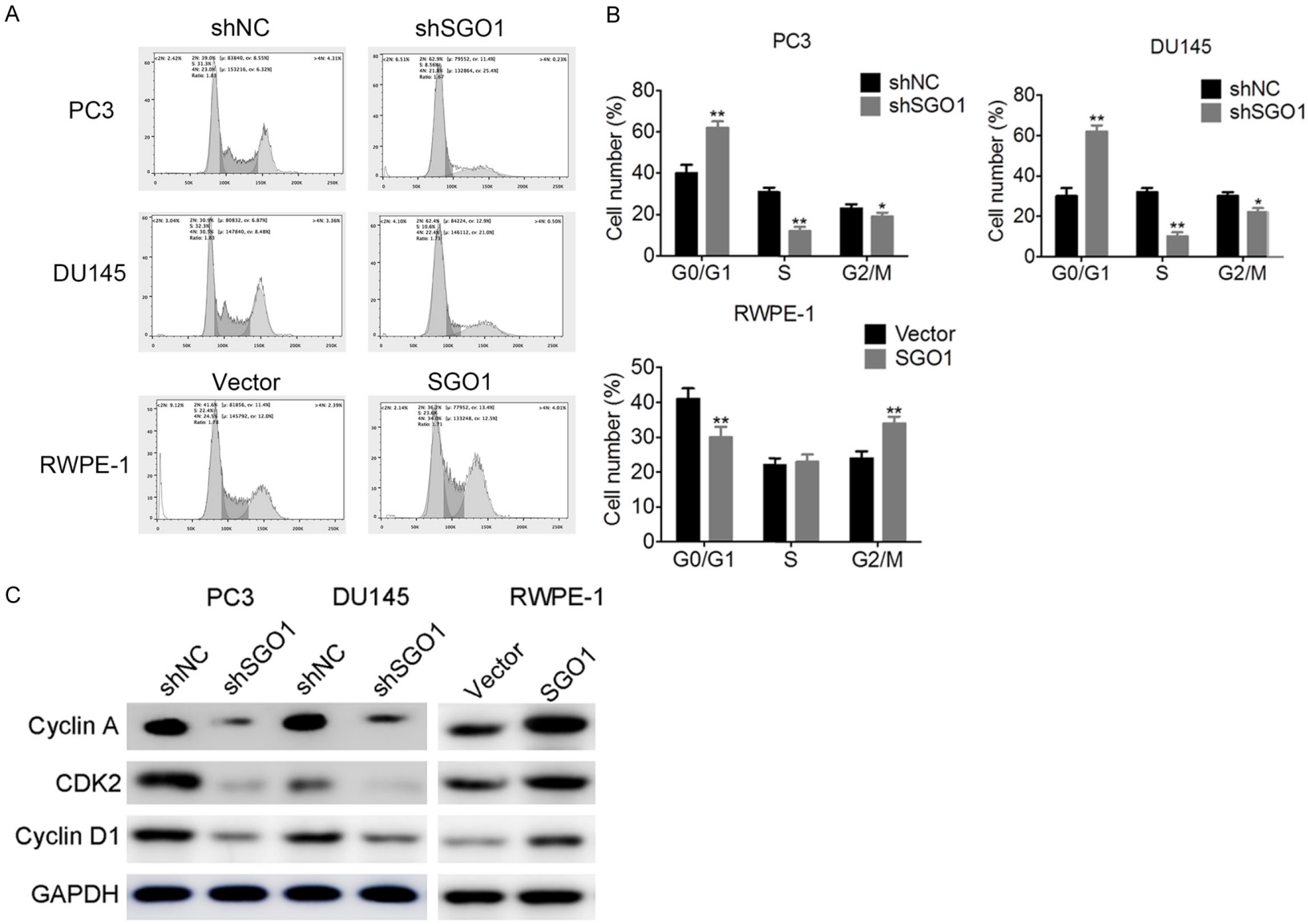


Figure 3. SGO1 increases the proliferation of prostate cancer cell lines. 1000 SGO1-shRNA-B knockdown PC3 (A), DU145 (B) cells, and SGO1 overexpressed RWPE-1 (C) cells and LNCap (D) were cultured in 96-well plates and cell counts were performed after 3 and 5 days culture. (E, F) 500 cells treated as described above were plated in a 12-well plate for colony formation experiments, photographed after 14 days of culture, and the number of clones was counted separately.

the most efficient (**Figure 2B**). The shRNA-B knockdown of SGO1 was then performed in PC3 and DU145 cells (**Figure 2C** and **2D**), respectively, and overexpression of SGO1 was performed in RWPE-1 and LNCap cells (**Figure 2E** and **2F**). Knockdown and overexpression of SGO1 in prostate cancer cells were then verified by western blot (**Figure 2G**). In PC3 and DU145 cells, knockdown of SGO1-shRNA significantly decreased cell proliferation (**Figure 3A** and **3B**). In addition, another one shSGO1 (shSGO1-C) like shSGO1-B could also inhibit the cells proliferation (Supplementary Figure 1). In RWPE-1 and LNCap cells, overexpression of SGO1 increased the rate of cell proliferation (**Figure 3C** and **3D**). Colony formation assays confirmed a reduction in the number of clones in PC3 and DU145 cells following SGO1 knockdown (**Figure 3E**). After SGO1 overexpression, the number of RWPE-1 and LNCapcell clones increased (**Figure 3F**). In summary, high expression of SGO1 in prostate cancer cells promotes cells proliferation.

SGO1 promotes cell cycle progression and inhibits apoptosis in prostate cancer cells

In prostate cancer cells, the proportion of G0/G1 phase in SGO1-shRNA knockdown PC3 and DU145 cells was increased compared with the control group shNC, and the proportion of S phase and G2/M phase was decreased, indicating that SGO1 knockdown inhibited the cell cycle process. However, the ratio of G0/G1 phase in RWPE-1 cells after overexpression of SGO1 was decreased, and the ratio of S and G2/M phases was increased (**Figure 4A** and **4B**). At the same time, the results of the detection of cycle-related proteins showed that the expression of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shNC
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B
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SGO1
Cell number (%)
G0/G1
S
G2/M
C
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Vector
SGO1
Cyclin A
CDK2
Cyclin D1
GAPDH

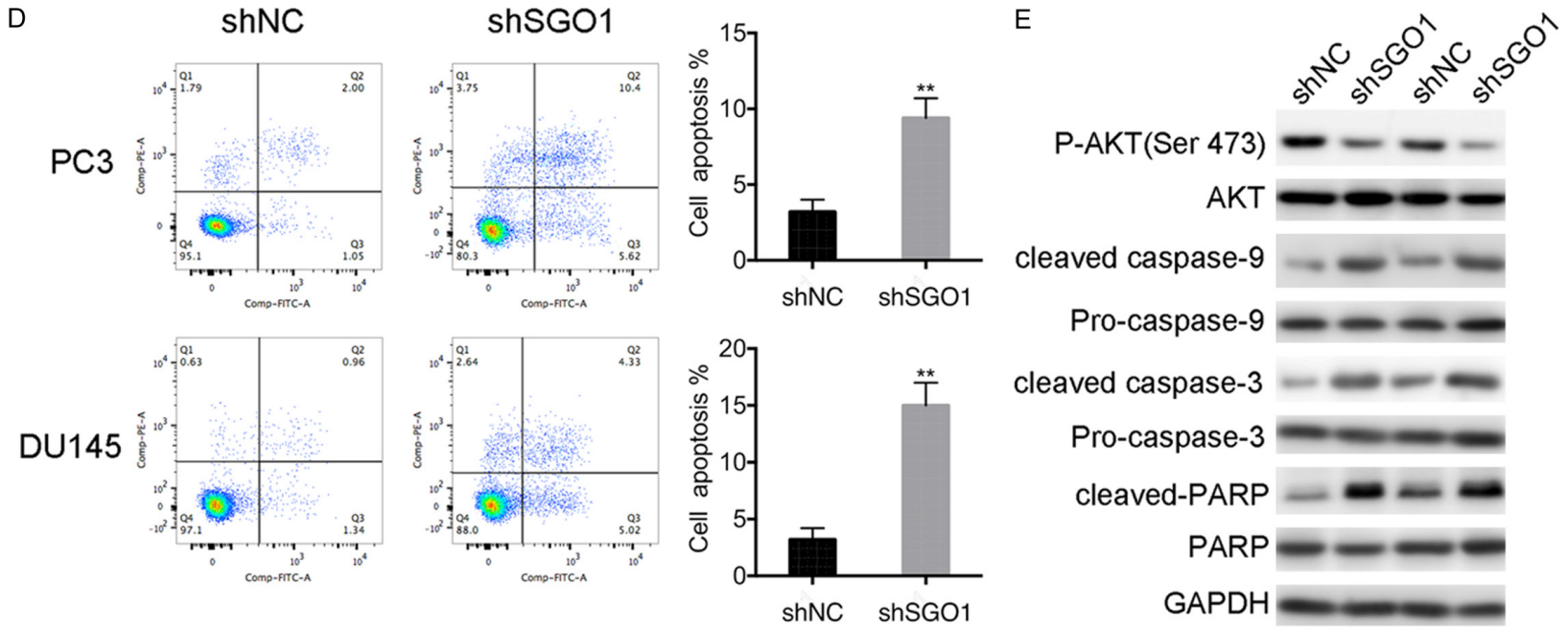

Figure 4. SGO1 accelerates the cell cycle progression of prostate cancer cells and inhibits apoptosis. (A) SGO1-shRNA-B knockdown PC3, DU145 cells and over-expressing SGO1 RWPE-1 cells were collected and cell cycle assays were performed by flow cytometry. The results were analyzed using *Modfit* software and the proportion of each period (B) was counted. (C) Detection of cell cycle-related proteins levels in the above cells by western blot. (D) SGO1-shRNA-B knockdown PC3, DU145 cells were collected and Annexin V and PI staining was detected by flow cytometry. The proportion of apoptotic cells was analyzed using *Flowjo* software. (E) The level of a series of apoptotic markers was detected by western blot in the above treated cells.

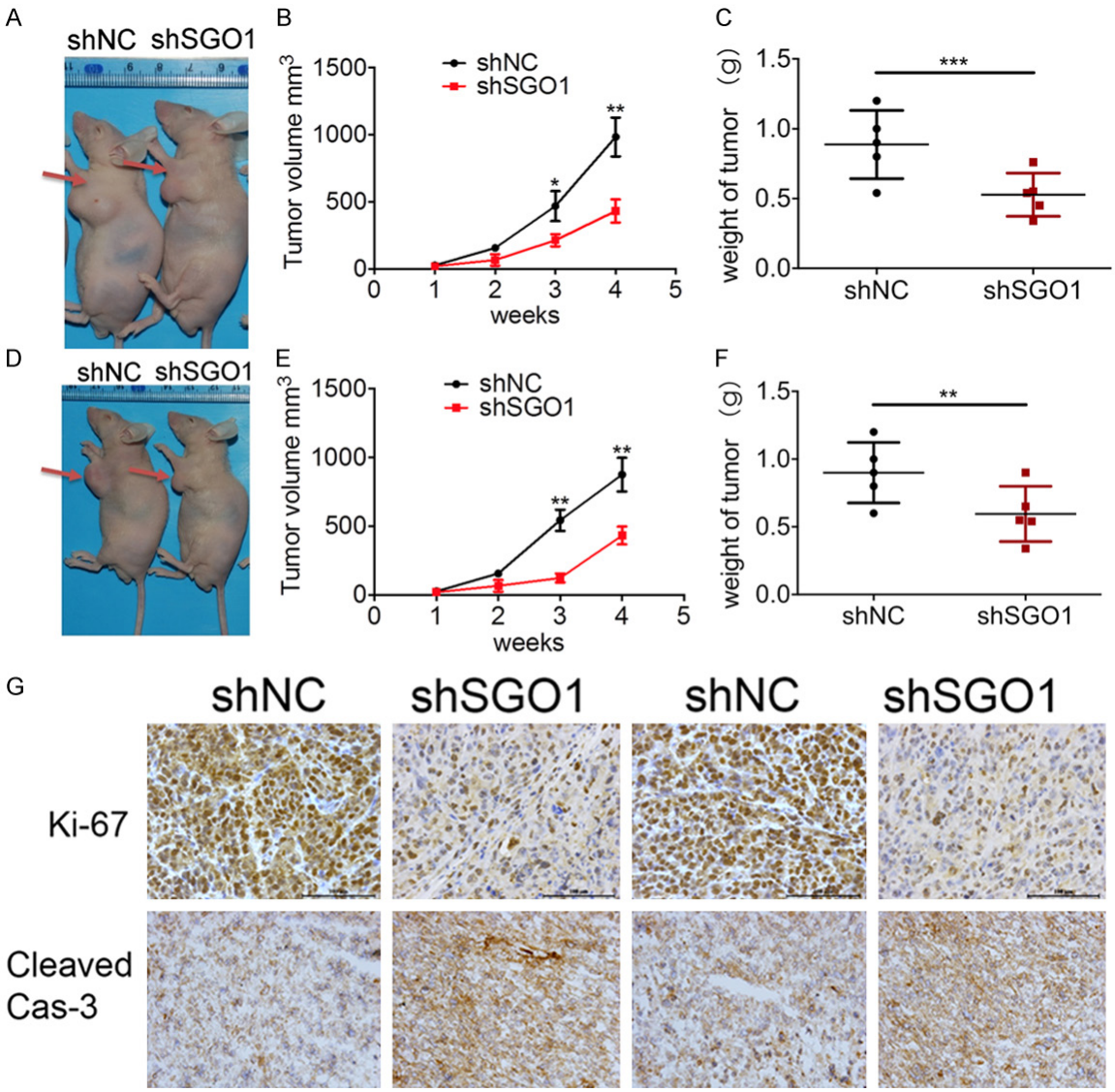

Figure 5. SGO1 promotes tumor formation in nude mice model. PC3 cells (A) and DU145 cells (D) were infected with the lentivirus containing SGO1-shRNA-B respectively, and the cells were implanted subcutaneously in nude mice, and the tumor-bearing nude mice were photographed three weeks later. (B, E) Tumor volume of PC3 cells and DU145 cells were recorded during tumor growth respectively. (C, F) At the end of the experiment, the tumor weight of each group was measured. (G) After embedding the above-described tumor tissues in paraffin, the expression of Ki67 and cleaved caspase 3 was detected by immunohistochemistry.

cyclin A, CDK2, and cyclin D1 was significantly decreased in PC3 and DU145 cells with SGO1 knockdown. The expression of these proteins was increased in RWPE-1 cells after overexpression of SGO1 (**Figure 4C**). Next, we studied the effect of SGO1 on cell apoptosis. SGO1 knockdown in PC3 and DU145 cells resulted in a significant increase in the proportion of apoptotic cells compared to control shNC (**Figure 4D**). SGO1 knockdown significantly increased cleaved caspase-3, cleaved caspase-9, cleaved PARP expression, and decreased pRWPE-1phorylation of AKT in PC3 and DU145 cells (**Figure 4E**). These results show that high expression of SGO1 in prostate cancer cells promotes cell cycle and inhibits apoptosis.

SGO1 promotes tumor formation and development in vivo

The function of SGO1 in vivo was verified by nude mice xenograft model. We implanted PC3 and DU145 cells stably expressing SGO1-shRNA in nude mice for tumorigenesis experiments. The tumorigenicity of SGO1-knockdown PC3 cells (**Figure 5A**) and DU145 cells (**Figure**

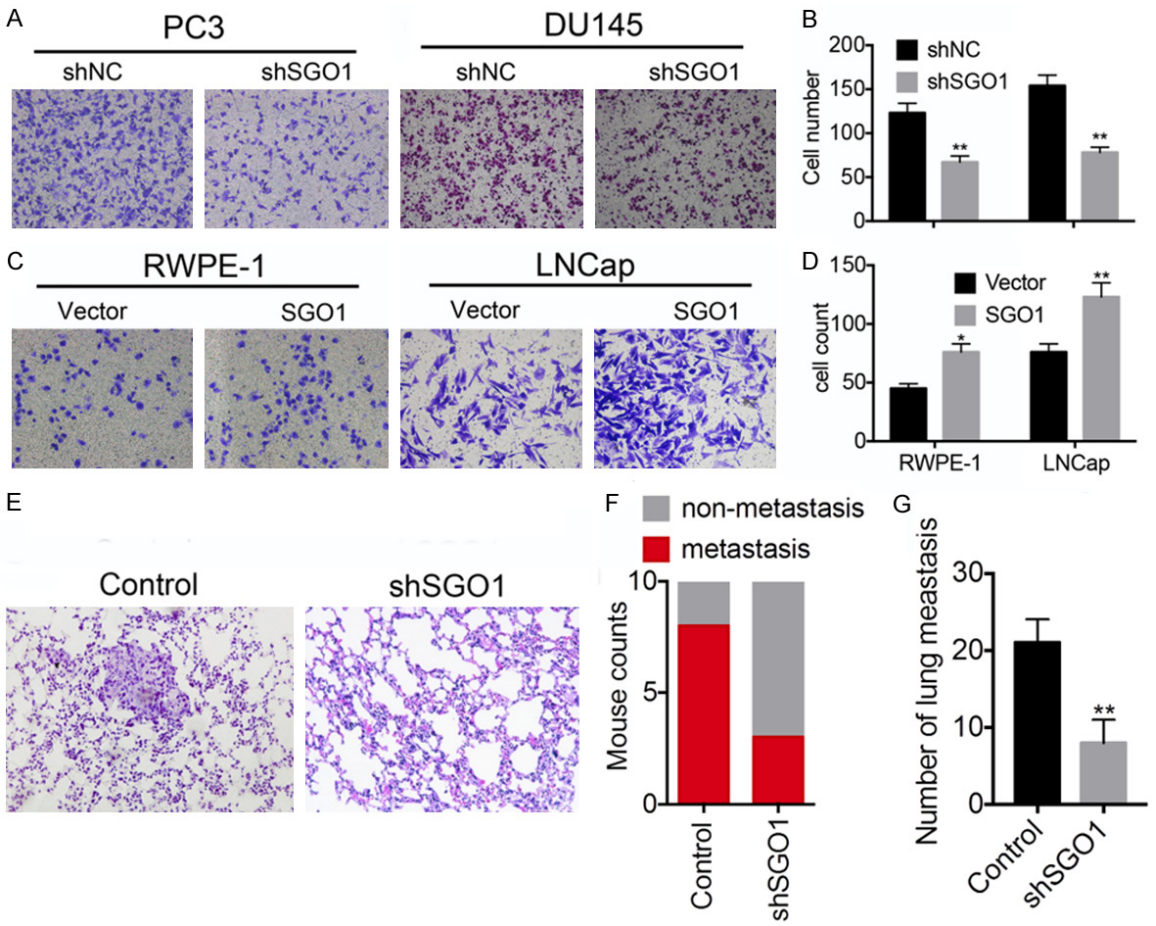

Figure 6. SGO1 promotes the invasion and metastasis of prostate cancer cells. SGO1-shRNA-B knockdown PC3 and DU145 cells (A) and overexpressing SGO1 RWPE-1 and LNCap cells (C) were subjected to cell migration assay by transwell assays, and the migration cells were stained with crystal violet and photographed. (B, D) The number of invading cells was counted under a microscope. (E) Control and SGO1-shRNA-B knockdown PC3 cells were injected into the nude mice via tail vein, and HE staining showed metastatic cells in lungs. (F) The proportion of mice with lung metastasis in both groups were statistically compared. (G) Statistical comparison of the number of lung metastatic cells in the control and SGO1 knockdown groups.

5D) was significantly reduced, and tumor size (**Figure 5B** and **5E**) and tumor weight (**Figure 5C** and **5F**) were significantly smaller than control group shNC. Then, we performed immunohistochemistry on the SGO1 knockdown tumor tissue and found that the expression of the proliferating antigen Ki67 was significantly reduced after SGO1 knockdown, and the level of apoptotic cell marker cleaved caspase-3 was significantly increased (**Figure 5G**). These results indicate that SGO1 promotes tumor formation in nude mice model.

SGO1 promotes invasion and metastasis of prostate cancer cells

In the transwell assays, the migration ability of SGO1-shRNA knockdown PC3 cells and DU145 cells (**Figure 6A** and **6B**) was reduced, and RWPE-1 and LNCap cells after SGO1 overexpression (**Figure 6C** and **6D**) increased. Therefore, we believed that the high expression of SGO1 promoted the metastasis of prostate cancer cells. In mice lung metastasis model of prostate cancer cells in vivo, we injected the SGO1 knockdown PC3 cells into nude mice. HE staining showed that the shNC prostate cancer cells as control group had tumor cell aggregation in the lung, whereas the SGO1 knockdown PC3 cells had less aggregation (**Figure 6E**). Moreover, after SGO1 knockdown, the proportion of lung metastatic mice was significantly reduced (**Figure 6F**). The number of lung metastatic tumor cells was counted and lung metastatic cells after SGO1 knockdown was significantly reduced (**Figure 6G**). In conclusion, SGO1 increases prostate cancer cell invasion and lung metastasis.

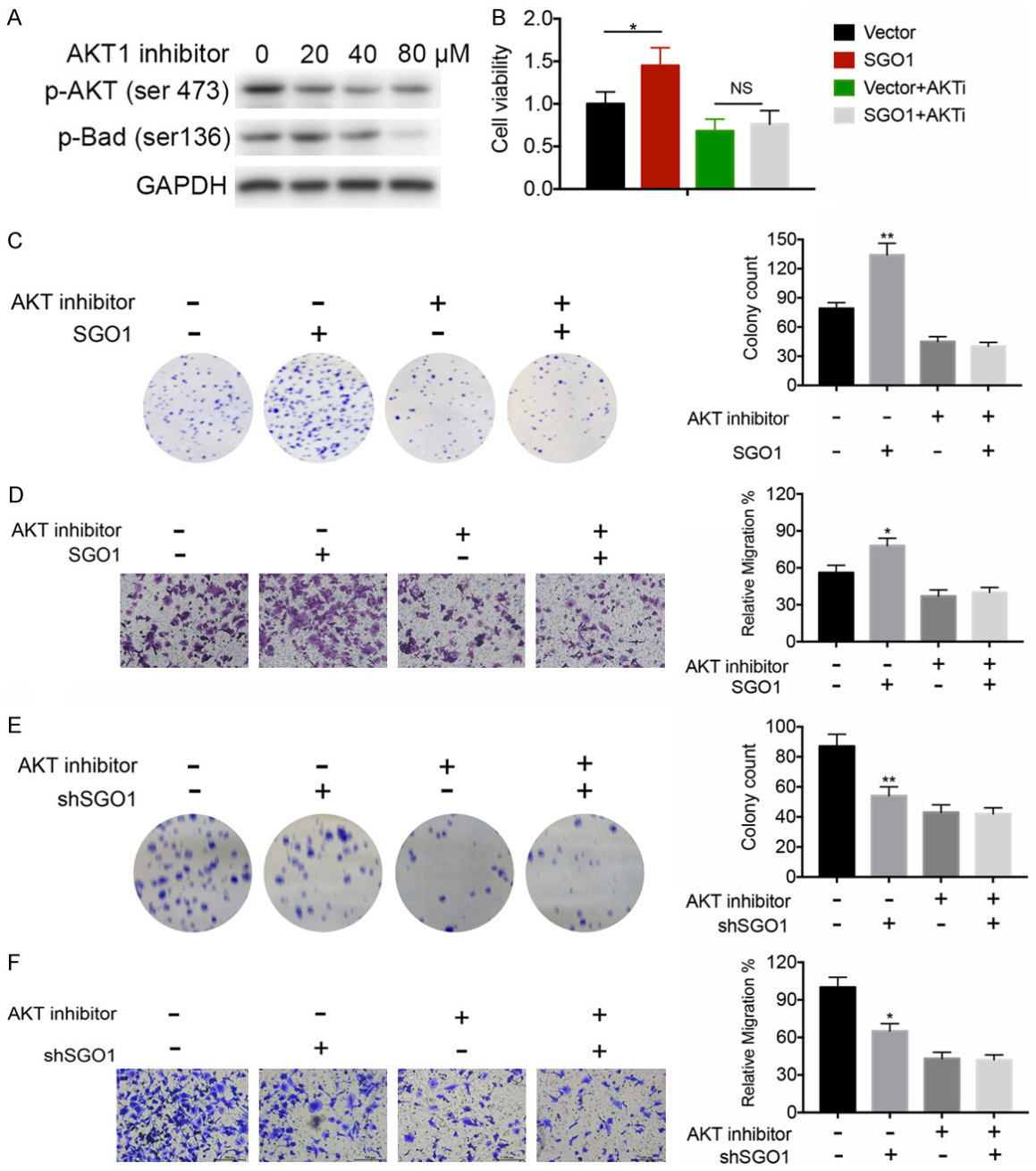

Figure 7. SGO1 promotes AKT signaling pathway mediated proliferation and metastasis of prostate cancer cells. A. PC3 cells were treated with 0, 20, 40, and 80 μM AKT inhibitor MK-2206, respectively, and phosphorylation of AKT (Ser473) and Bad (Ser136) was detected by western blot. B, C. The overexpressing RWPE-1 cells and control cells were treated with 80 μM MK-2206 for proliferation assay and colony formation experiments. D. Transwell experiments were performed after the above cells were processed. E. The shNC and shSGO1 PC3 cells were treated with 80 μM MK-2206 respectively, for colony formation experiments. F. Transwell experiments were performed after the above cells were processed.

SGO1 enhances prostate cancer cell invasion and metastasis dependent on AKT pathway

We use AKT inhibitor MK-2206 to interfere the AKT signaling pathway. High concentrations of AKT inhibitor MK-2206 inhibited PC-3 phosphorylation of AKT and Bad, thereby inhibiting the AKT pathway (**Figure 7A**). In proliferation and colony formation assay, overexpression of SGO1 in RWPE-1 cells promoted cell growth and colony formation, whereas overexpression of SGO1 had little effect on cell growth after

treatment with MK-2206 (**Figure 7B** and **7C**). In transwell experiments, MK-2206 also eliminated the effect of SGO1 on RWPE-1 cells (**Figure 7D**). Knockdown of SGO1 inhibited cell growth and migration ability, whereas AKT inhibitor treatment attenuated the colony forming and migration capacities in SGO1 knockdown PC-3 cells (**Figure 7E** and **7F**). Therefore, we believe that AKT signaling pathway is involved in the function of SGO1 in prostate cancer.

Discussion

Studies have shown inhibition of human SGO1 expression leads to erroneous chromosome division, leading to gene instability and tumor formation, suggesting that decreased or missing SGO1 expression promotes tumor formation [13, 14]. The role of SGO1 in normal tissues and tumor tissues seems to be contradictory, and the different molecular characteristics of the cells at different stages of development and differentiation warrant further investigation [15]. In this experiment, we first studied the effect of SGO1 on proliferation of prostate cell lines and found that SGO1 was overexpressed in prostate cancer and cell lines. After down-regulating SGO1 expression, cell proliferation slowed down and apoptosis increased significantly. We constructed a lentivirus-mediated vector shSGO1, and transfected into prostate cancer cells, which specifically downregulates cell proliferation, suggesting that shSGO1 can be used as a tumor therapy.

By mid-mitosis, a series of monitoring mechanisms including spindle detection sites inhibit the activation of the APC/C complex by inhibiting separase activation [16]. Once the APC/C complex is activated, the end of mitosis begins, separase is activated, and centromeric fibronectin is dissociated [17]. SGO1 is a substrate for APC/C, and proteomics studies have revealed that SGO1 is a component of the mitotic spindle [18]. Loss of SGO1 function activates the spindle checkpoint and activates APC/C separase [16]. As a result, fibronectin dissociates from the centromere and mitosis stops. We also confirmed that prostate cancer cell cycle was arrested after downregulating SGO1.

The AKT/mTOR pathway in prostate cancer is associated with the expression of matrix metalloproteinases (MMPs) and can upregulate the expression of MMP-9 [19]. MMP-9 degrades the extracellular matrix and participates in prostate cancer metastasis by EMT [20-22]. As one of the downstream proteins of AKT-mTOR, p70S6K promotes filament remodeling of actin and promotes tumor cell movement [23]. AKT phosphorylation in prostate cancer down-regulates E-cadherin expression, reduces cell-to-cell adhesion, and increases prostate cancer cell motility and invasiveness [24-26]. At present, AKT inhibitors have been used clinically for the treatment of prostate cancer. Paclitaxel can induce cell cycle arrest in PC-3 and PTEN-deficient prostate cancer cells LNCaP [27]. Although paclitaxel showed better safety in early clinical trials, no changes in radiographic examination and serum PSA levels were seen in castration-resistant prostate cancer patients [28]. It has been found that Akt-selective inhibitors that compete with ATP, such as GDC-0068, show better growth inhibition in prostate cancer xenografts [29]. In summary, we found downregulating the expression of SGO1 can inhibit the proliferation of prostate cancer. At the same time, SGO1 promotes lung metastasis of prostate cancer by regulating AKT signaling. SGO1 is expected to be an effective molecule and combined with AKT inhibitors for prostate cancer targeted therapy.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Meng Gu and Zhong Wang, Department of Urology, Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. E-mail: gmgumeng@126.com (MG); zhongwang2000@sina.com (ZW)

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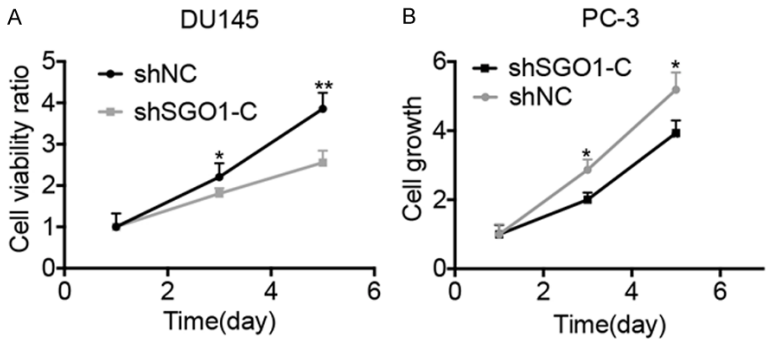

Supplementary Figure 1. SGO1 knockdown inhibited the proliferation of prostate cancer cell lines. 1000 SGO1-shRNA-C knockdown DU145 (A) and PC3 (B) cells, were cultured in 96-well plates and cell counts were performed after1, 3 and 5 days culture.