Stromal antigen 2 functions as a tumor suppressor in bladder cancer cells

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Abstract. Stromal antigen 2 (STAG2) is a subunit of the cohesion complex that plays an important role in the normal segregation of sister chromatids during mitosis or meiosis. However, the effect of STAG2 on the bladder cancer cell proliferation, migration, and invasion has not yet been fully clarified. In this study, we aimed to characterize STAG2 expression and functional significance in BC and adjacent normal tissue. Notably, STAG2 expression was markedly lower in BC cells and tumor tissues than their normal counterparts at the gene and protein levels. Moreover, clinicopathological analysis showed that the low STAG2 expression is associated with TNM stage. Functional analysis demonstrated that STAG2 overexpression attenuated cell proliferation via G_1-phase arrest, invasion, and migration, and promoted apoptosis in BC cell lines, while the opposite was observed with STAG2 knockdown cells. Furthermore, STAG2 overexpression upregulated E-cadherin, caspase-3, and caspase-7 and downregulated vimentin, matrix metalloproteinase (MMP)2, and MMP9. Collectively, these data suggest that STAG2 acts as a tumor suppressor gene in bladder cancer and may be a potential therapeutic target in BC.

Introduction

Bladder cancer is the fourth most common malignant tumor in men and the ninth most common malignant cancer overall. In 2015, 75,000 new cases of bladder cancer were diagnosed in the United States. Of these cases, 56,320 occurred in men and 16,000 succumbed to their disease (1). Bladder cancer is classified as either non-muscle-invasive bladder cancer (NMIBC) or muscle-invasive bladder cancer (MIBC); NMIBC accounts for 70-80% of new cases and is treated by transurethral resection of the bladder tumor (TURBT). However, the recurrence rate in patients with NMIBC is high after treatment (≤70%) and requires lifelong monitoring, often resulting in multiple surgical treatments. Cystoscope and urine cytology are still the gold standard for the diagnosis of bladder cancer and detection of recurrence (2). Approximately 30% of recurrent tumors will develop into MIBC. Partial cystectomy and radical cystectomy are performed routinely for disease management; however, many patients cannot undergo surgery because of early tumor metastasis. Thus, the 5-year survival rate in patients is only 20-40% (3,4). Accordingly, comprehensive treatment of bladder cancer involves the use of radiotherapy, chemotherapy, biological therapy, and other approaches (5,6). Despite therapeutic advancements, no effective treatment options currently exist to prevent the progression from NMIBC to MIBC (7). Accordingly, a clear understanding of the molecular mechanisms mediating the bladder cancer occurrence and progression are urgently needed in order to develop methods for the early diagnosis and treatment of this disease.

We previously reported on a genomic analysis of 149 transitional cell cancer (TCC) samples by whole-genome and whole-exome sequencing. Of these, 16 samples harbored deletions or truncating mutations in stromal antigen 2 (STAG2), including 12 cases of metastatic bladder cancer (8). These mutations abolished STAG2 expression in bladder cancer specimens, whereas STAG2 was still expressed in normal

tissues. STAG2 inactivation requires only a single mutation event because the *STAG2* gene is located on the X chromosome. *STAG2* comprises 39 exons and encodes the SA2 subunit of cohesion (9,10). In 2011, Solomon *et al* demonstrated that targeted inactivation of *STAG2* caused chromatid cohesion defects and aneuploidy (11). However, Balbás-Martínez *et al* (18) and Djos *et al* (12) confirmed that recurrent inactivation of *STAG2* is not associated with aneuploidy in bladder cancer or neuroblastoma tumors. Subsequently, loss of STAG2 expression was demonstrated in gastric cancer, colorectal cancer, and prostate cancer tissues, although no mutations have been found in these cancers. In contrast, *STAG2* somatic mutations have been identified in acute leukemia (13,14), and recurrent mutations and deletions are associated with the development of myeloid neoplasms (15).

STAG2 is a subunit of cohesion - a highly conserved protein complex that contains four core subunits (SMC1A, SMC3, RAD21 and STAG1/2) - and plays an important role in the separation of sister chromatids during mitosis and meiosis, DNA repair, gene expression regulation, genomic imprinting, and the epithelial-to-mesenchymal transition (EMT). Because EMT is a key step in cancer cell metastasis, functional defects in cohesin may be related to both the occurrence and metastasis of cancer. Interestingly, *STAG2* mutations are not related to chromosomal aberrations in tumor cells in NMIBC (10,16-18); however, *STAG2* mutations are known to be associated with chromosomal aneuploidy in MIBC and metastatic bladder cancer (8,11). Moreover, frequent inactivating mutations in STAG2 are associated with low tumor grade and stage and are inversely related to chromosomal copy number changes (10).

In this study, we aimed to explore the role of STAG2 in bladder cancer development and progression. Our findings provide important insights into the role of this molecule in the pathogenesis of bladder cancer.

Materials and methods

Patient tissue specimens. Fifty-eight pairs of bladder cancer and matched adjacent normal tissues were obtained by radical cystectomy from Zhujiang Hospital (Southern Medical University, Guangdong, China). The tissues were fixed in RNAlater reagent (Sigma, St. Louis, MO, USA) and stored in liquid nitrogen. All procedures conducted in studies involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Cell lines and culture. The T24, 5637, J82, UM-UC-3, and SW780 human bladder cancer and SV-HUC-1 normal bladder cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were maintained in a humidified atmosphere of 5% carbon dioxide at 37˚C and cultured in medium supplemented with 10% fetal bovine serum.

Lentivirus packaging and generation of stable cell lines. The *STAG2* coding sequence was amplified by polymerase chain reaction (PCR) using specific primers (forward: 5'-GAGGATC CCCGGGTACCGGTCGCCACCATGATAGCAGCTCCAG AAATACCAAC-3'; reverse: 5'-CACACATTCCACAGGCTA GTTAAAACATTGACACTCCAAGAACTG-3') containing restriction enzyme sites for *Bam*HI and *Nhe*I at the 5' and 3' ends, respectively. STAG2 shRNA was synthesized using the following sequences: shRNA1, 5'-TTATGCTTCCAAGAT CAA-3' and 5'-CTATGTAATCCTTTGGCAA-3'; shRNA2, 5'-AACGTGAATACTACTGTTA-3' and 5'-AACGTGAAT ACTACTGTTA-3'. The STAG2 cDNA and shRNA fragments were ligated into the Ubi-MCS-SV40-EGFP-IRES-puromycin and hU6-MCS-Ubiquitin-EGFP-IRES-puromycin plasmids, respectively, and then transfected into 293-T cells. Lentivirus titers and concentrations were determined using standard procedures. For transfection, $2x10^5$ UMUC-3 or T24 cells were seeded in 6-well culture plates and cultured for 24 h prior to transduction. Cells were divided into the following four groups: UMUC-3 cells transfected with a lentiviral construct expressing STAG2; UMUC-3 cells transfected with a negative control lentiviral construct (UMUC-3-NC); T24 cells transfected with a lentiviral construct expressing STAG2 shRNA (T24-shSTAG2); and T24 cells transfected with a negative control lentiviral construct (T24-shNC). All cell lines were cultured using medium supplemented with polybrene for 12 h, and the medium was then replaced with normal medium. EGFP expression was used to confirm plasmid expression 3 days later. Stable cell lines were screened using medium containing a certain concentration of puromycin.

Total RNA and cDNA synthesis. Total RNA from bladder cancer cells and normal bladder cells was isolated using TRIzol reagent (Invitrogen, Burlington, ON, USA) according to the manufacturer's protocol. Then, cDNA was synthesized using a QuantiTect Reverse Transcription kit (Qiagen GmbH, Hilden, Germany) with gDNA wipeout buffer in a 20-μl reaction containing 1 μg RNA. The reaction mixture was incubated at 37˚C for 5 min, heated at 95˚C for 30 min, and then cooled on ice.

Quantitative real-time reverse transcription PCR (qRT-PCR). qRT-PCR was performed using SYBR Premix Taq II (Takara, Shiga, Japan) with β-actin (*ACTB*) as an internal reference. Primer sequences were designed in Primer5 and are as follows: *STAG2* forward, 5'-ACGGAAAGTGGTTGAGGG-3'; reverse, 5'-GTGGAGGTGAGTTGTGGTGT-3'. *ACTB* forward, 5'-GAAATCGTGCGTGACATTAA-3'; reverse, 5'-AAGGAA GGCTGGAAGAGTG-3'. Each 20-μl reaction mixture contained 0.5 μM of each primer, 10 μl SYBR Premix, 7 μl RNase water, and 2 μl cDNA. Amplification was carried out using a QuantStudio Dx System (Applied Biosystems, Monza, Italy) with an initial denaturation at 95˚C for 30 sec; followed by 40 cycles of 95˚C for 5 sec, 60˚C for 34 sec, and 72˚C for 30 sec. All reactions were performed in duplicate. Gene expression was quantified using the comparative threshold cycle (CT) method.

Western blotting. Cell protein was extracted using RIPA lysis buffer and a protease inhibitor mixture (Thermo Fisher Scientific, Waltham, MA, USA) and the concentration was measured using a Piece BCA Protein assay kit (Thermo Scientific). Protein samples (20 μg) were subjected to SDS-PAGE

Table I. Correlation between STAG2 mRNA expression and clinicopathological characteristics of the patients with bladder cancer.

Parameters	Patients, no. (%)	STAG2 expression high, no. (%)	STAG2 expression, low no. (%)	P-value
All cases	58 (100)	21 (36.2)	37 (63.8)	
Sex				
Male	39 (67.24)	15 (38.46)	24 (61.54)	0.416
Female	19 (32.76)	6 (31.58)	13 (68.42)	
Age				
<65	31 (53.45)	12 (38.71)	19 (61.29)	0.441
≥65	27 (46.55)	9 (34.48)	18 (65.52)	
TNM stage				
0/I	15 (25.86)	10 (66.67)	5 (33.33)	0.006[a]
II/III/IV	43 (74.14)	11 (25.58)	32 (74.42)	
Histological grade				
L	35 (70)	14 (40)	21 (60)	0.324
H	23 (30)	7 (30.43)	16 (69.57)	
Lymph node metastasis				
N0	50 (86.21)	17 (34)	33 (66)	0.569
N1 or above	8 (13.79)	3 (37.5)	5 (62.5)	

[a]Statistically significant.

on 10% gels and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% non-fat skim milk in 50 mM Tris-HCl, 50 mM NaCl, and 0.1% Tween-20 (TBST) for 1 h at 25°C, probed with rat polyclonal anti-STAG2 antibodies (1:4,000; Abcam, Cambridge, UK) at 4°C overnight, and then incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:8,000; Abcam) in TBST for 1 h at room temperature.

Cell proliferation assays. Cell proliferation was measured with a Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) and 5-ethynyl-20-deoxyuridine (Edu) assay kit (Ribobio, Wuhan, China) according to the manufacturers' protocols. For CCK-8 assay, cell lines were seeded at $4x10^3$ cells/well in a 96-well plate. After culturing for 0-4 days, CCK8 reagent was added to plates at different time points and then incubated at 37°C for 2 h. Proliferation was based on absorbance at 450 nm measured with a microplate reader. For Edu assays, cells were seeded at $6x10^3$ cells/well in a 96-well plate for 24 h, and then incubated with 50 μM EdU for 3 h at 37°C, fixed with 4% formaldehyde for 15 min, treated with 0.5% Triton X-100 for 20 min at room temperature, and then washed with PBS three times before incubating with 1X Apollo® reaction cocktail (Ribobio, 100 μl/well) for 30 min. The cells were then stained with Hoechst 33342 (5 μg/ml, 100 μl/well) for 30 min prior to imaging with a fluorescent microscope (Olympus Corp., Tokyo, Japan).

Transwell cell migration and invasion assays. Cell migration and invasion were evaluated using transwell chambers with an 8-μm pore size (Costar, Corning, NY, USA). For migra-

tion assays, $4x10^4$ cells were seeded into the upper chamber in serum-free medium, and 500 μl medium supplemented with 10% FBS was added to the lower chamber. A similar procedure was used for invasion assays, except that the upper chamber was precoated with Matrigel basement membrane matrix (BD Biosciences, Bedford, MA, USA). The cells were then incubated at 37°C for 24 or 48 h for migration and invasion studies, respectively. The wells were then placed in 100% methanol to fix the cells, and cells remaining on the upper side of the chamber were then removed carefully using cotton swabs. Cells that had migrated or invaded through the membrane were then stained using 5% Giemsa staining, and the chambers were washed with PBS. Photographs from five independent fields per well were captured using an Olympus microscope (Hamburg, Germany). Each assay was carried out in triplicate.

Cell cycle analysis. To determine the role of STAG2 in cell cycle progression, transfected cells were harvested with trypsin and centrifuged at 265 x g for 5 min. The cell pellet was washed in pre-chilled Hanks D buffer (pH 7.2-7.4) and fixed with 75% ethanol for at least 1 h before staining with 40X propidium iodide (PI) solution (2 mg/ml), 100X RNase solution (10 mg/ml), and 1X Hanks D buffer at 25:10:1,000 (v/v/v). The stained cells were then analyzed with a Navi 105 flow cytometer (Beckman Coulter, Brea, CA, USA) at a rate of 300-800 cells/sec.

Cell apoptosis assay. Apoptotic cells were identified by staining with an Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection kit (Invitrogen) and PI (Invitrogen).
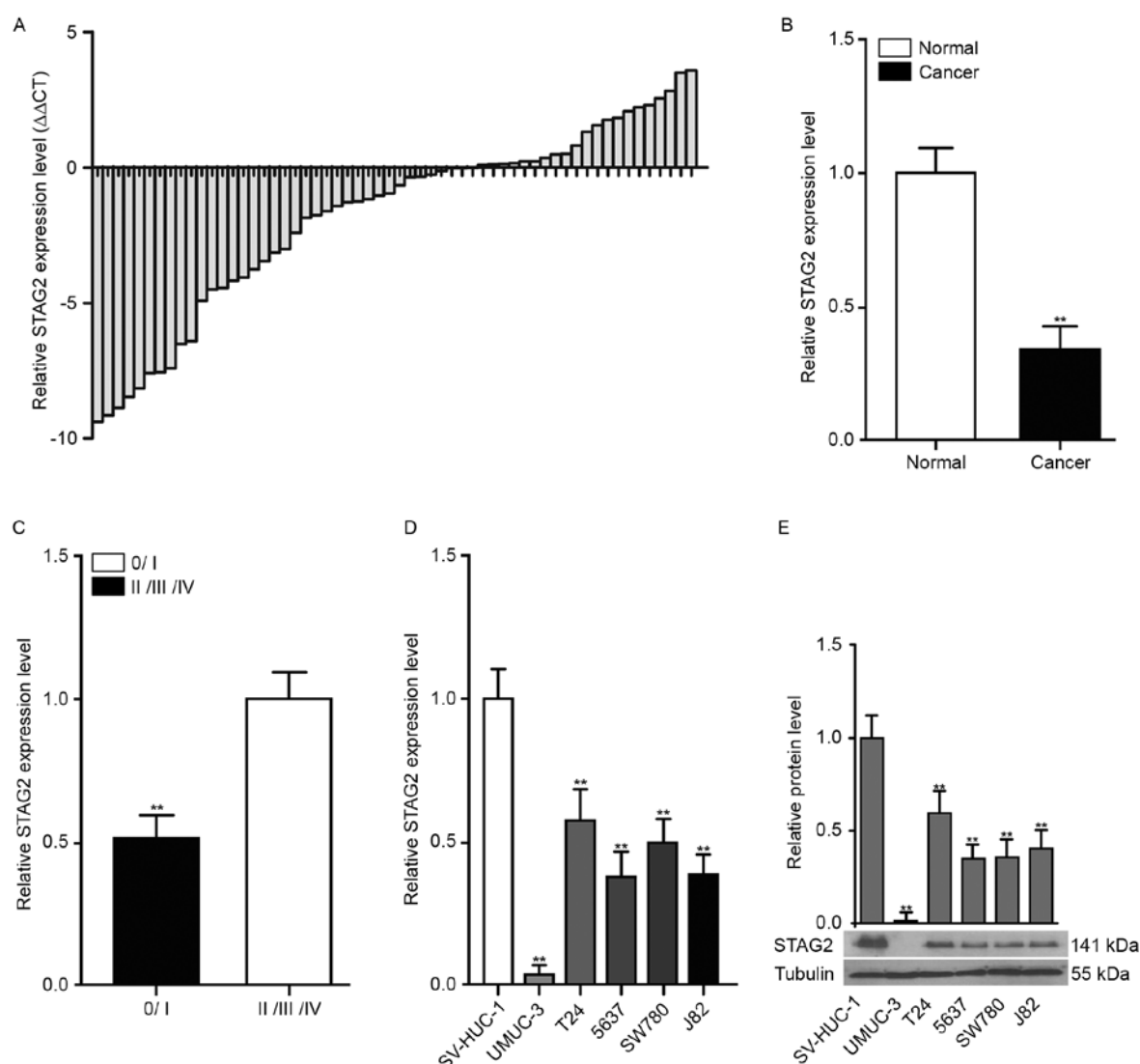
Figure 1. STAG2 expression in bladder cancer cell lines, bladder cancer tissues, and paracancer tissues. (A) STAG2 expression levels in bladder cancer tissues. (B) Relative expression level of STAG2 in bladder cancer tissues versus paracancer tissues. (C) STAG2 expression levels in patients with different TNM stages of bladder cancer. (D) STAG2 expression levels in bladder cancer cell lines versus SV-40-immortalized human uroepithelial cells. (E) STAG2 protein expression was detected by western blotting. Data (B-D) are shown as the means ± SDs. **P<0.01.

Briefly, transfected cells were detached using trypsin, resuspended in complete medium, harvested by centrifugation at 1,300 rpm for 5 min, and then incubated in 500 μl cold 1X binding buffer (Invitrogen) with 5 μl Annexin V-FITC in the dark for 15 min at room temperature. Then, the stained cells were collected by centrifugation and incubated in 500 μl cold binding buffer with 3 μl PI for 15 min. Apoptosis was assessed using a flow cytometer (Navi 105; Beckman Coulter).

Statistical analysis. Statistical analyses were performed using the SPSS 19.0 software package (SPSS, Chicago, IL, USA). Differential STAG2 expression in bladder cancer and paracarcinoma tissue was analyzed using paired samples t-test, and the relationships between STAG2 expression and clinicopathological characteristics were analyzed using Chi-square test. Other data were analyzed using unpaired t-test. All values are expressed as means ± standard deviation (SD) of at least three independent experiments. P<0.05 was considered statistically significant.

Results

Analysis of STAG2 expression in bladder cancer tissues and cell lines. STAG2 expression was examined in 58 pairs of bladder cancer tissues and adjacent non-cancerous tissues, as well as bladder cancer and normal cell lines. The patients' clinical parameters are shown in Table I. Notably, STAG2 expression was significantly decreased in 63.8% (37 of 58) of cancer tissues compared with that in adjacent normal bladder tissue samples (Fig. 1A and B) and significantly correlated with TNM stage (Fig. 1C). Similarly, STAG2 expression was also markedly reduced in bladder cancer cell lines (Fig. 1D and E).

Generation of stable cell lines. To explore its functional significance in bladder cancer, STAG2 was overexpressed or knocked down in UMUC-3 and T24 cells with lentiviral vectors expressing STAG2 cDNA or shRNA, respectively (Fig. 2A), resulting in four stable cell lines (UMUC-3-NC, UMUC-3-STAG2, T24-shNC, and T24-shSTAG2). As shown
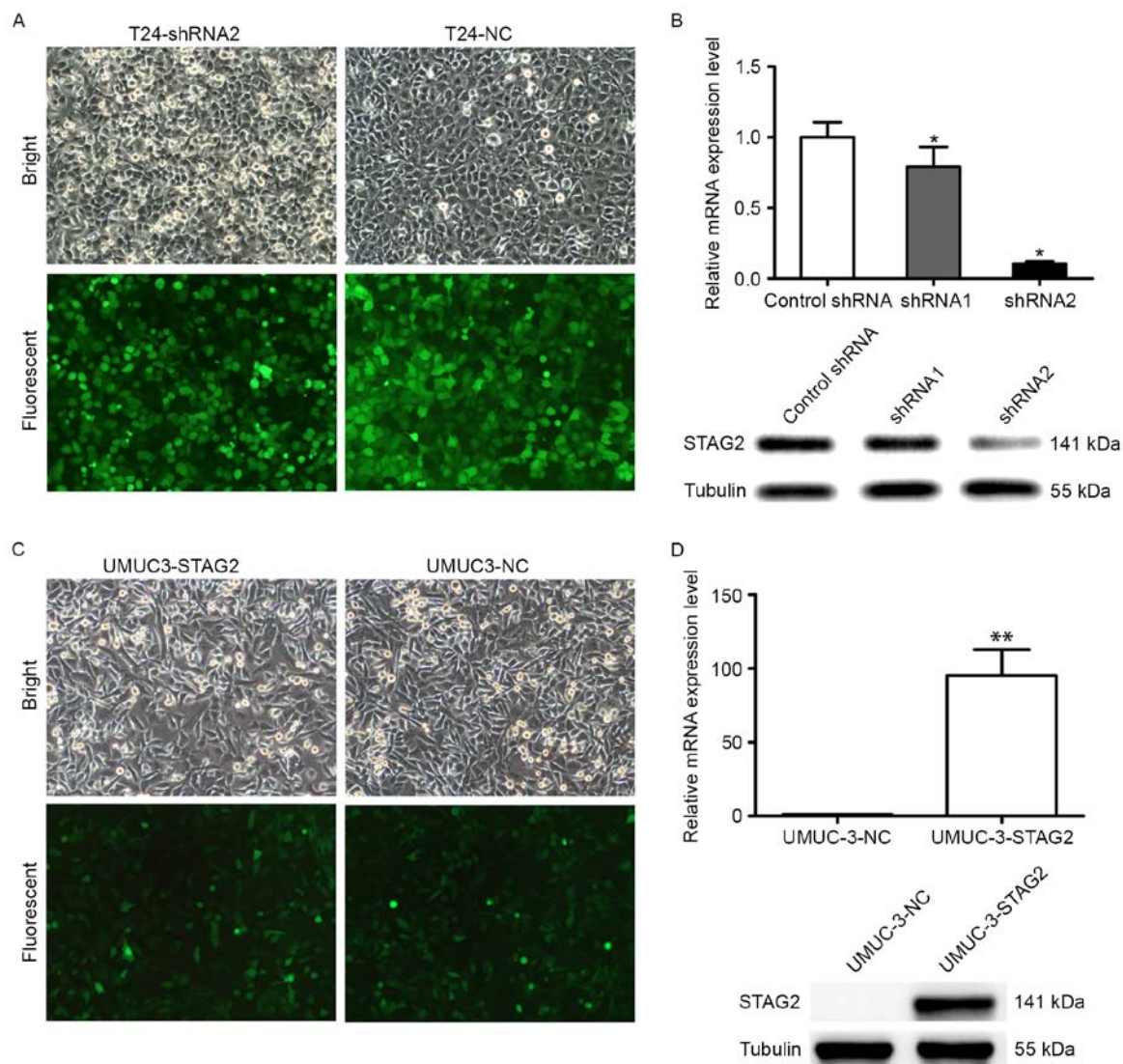
Figure 2. STAG2 knockdown in T24 cells and overexpression in UMUC-3 cells. (A and B) STAG2 knockdown in T24 cells. (C and D) Overexpression of STAG2 in UMUC-3 cells. Data are shown as the means ± SDs. *P<0.05, **P<0.01.

in Fig. 2, STAG2 was successfully knocked down in T24 cells at both the RNA and protein level, as shRNA2 knockdown effect is better, following experiment to select shRNA2 cells (Fig. 2A and B). Furthermore, STAG2 was successfully overexpressed in UMUC-3 cells at the RNA and protein levels (Fig. 2C and D). The infection efficiencies in all four cell lines were >80%, which was considered to be sufficient for subsequent experiments.

Effect of STAG2 expression on cell proliferation. To investigate the impact of STAG2 on bladder cancer cell proliferation, the effect of STAG2 knockdown or overexpression on cell proliferation was examined in UMUC-3 and T24 cells with CCK-8 and Edu assays. Notably, UMUC-3-STAG2 cells with higher STAG2 levels showed a marked delay in proliferation when compared to the negative control group (Fig. 3A, C and E), whereas T24-shRNA cells exhibited accelerated proliferation (Fig. 3 B, D and F). Collectively, these data suggest that STAG2 expression is negatively correlated with bladder cancer cell proliferation.

Role of STAG2 in cell cycle progression. We next investigated whether STAG2 was necessary for cell cycle progression in bladder cancer by PI staining. Consistent with our observations on proliferation, flow cytometry analysis demonstrated that STAG2 overexpression induced G_0/G_1 arrest in UMUC-3 cells (Fig. 4B), whereas knockdown promoted S-phase entry in T24 cells (Fig. 4A).

Participation of STAG2 in cell migration and invasion. To further determine the relationship between STAG2 and bladder cancer cell invasion and migration, the role of STAG2 in cell migration and invasion was assessed with transwell migration and invasion assays. In migration assays, significantly more T24-shRNA cells migrated to the lower chamber than that observed with T24-shNC cells (Fig. 5A and B), whereas fewer UMUC-3-STAG2 cells migrated to the lower chamber than UMUC-3-NC counterparts (Fig. 5D and E). Similar results were observed with invasion assays. Specifically, STAG2 knockdown increased invasion in T24 cells (Fig. 5A and C), while overexpression blocked invasion
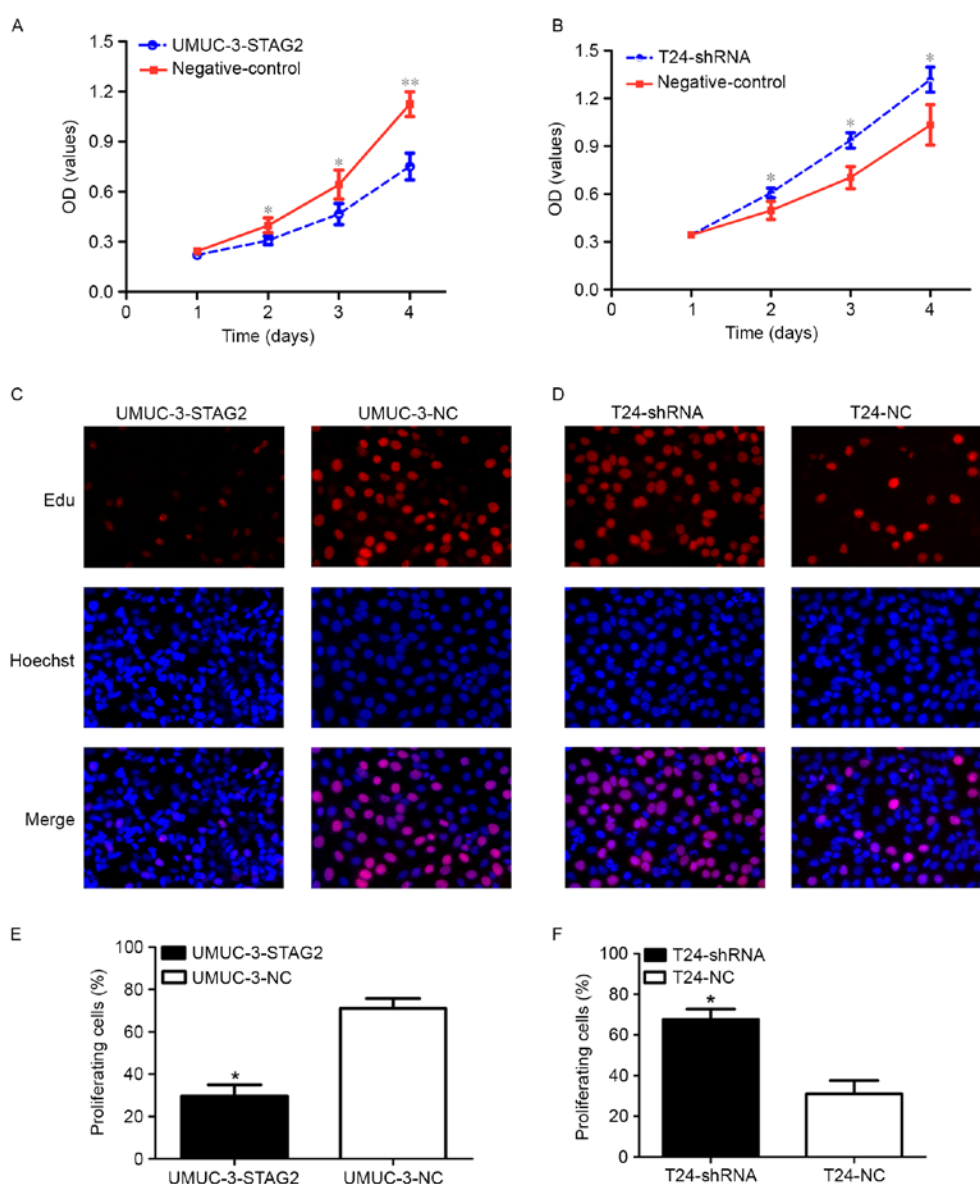
Figure 3. STAG2 inhibits cell proliferation in bladder cancer cells. (A, C and E) The effects of STAG2 knockdown on T24 cell proliferation. (B, D and F) The effects of STAG2 overexpression on UMUC-3 cell proliferation. Data are shown as the means ± SDs. *P<0.05, **P<0.01. All assays were performed three times independently.
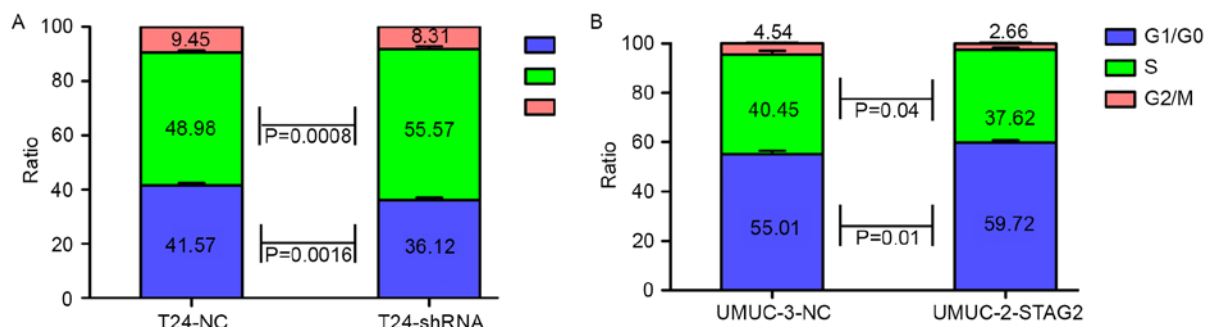

Figure 4. Cell cycle analysis in lentivirus-infected T24 and UMUC-3 cells. The relative percentages of T24 and UMUC-3 cells at G_1, S, and G_2/M phases were compared. Data are shown as the means ± SDs. *P<0.05. Each assay was carried out in triplicate.

in UMUC-3 cells (Fig. 5D and F). Therefore, these results suggested that STAG2 inhibited cell migration and invasion in bladder cancer.

Role of STAG2 gene in cell apoptosis. We examined whether STAG2 expression affected apoptosis in bladder cancer cells. As shown in Fig. 5, apoptosis was significantly decreased
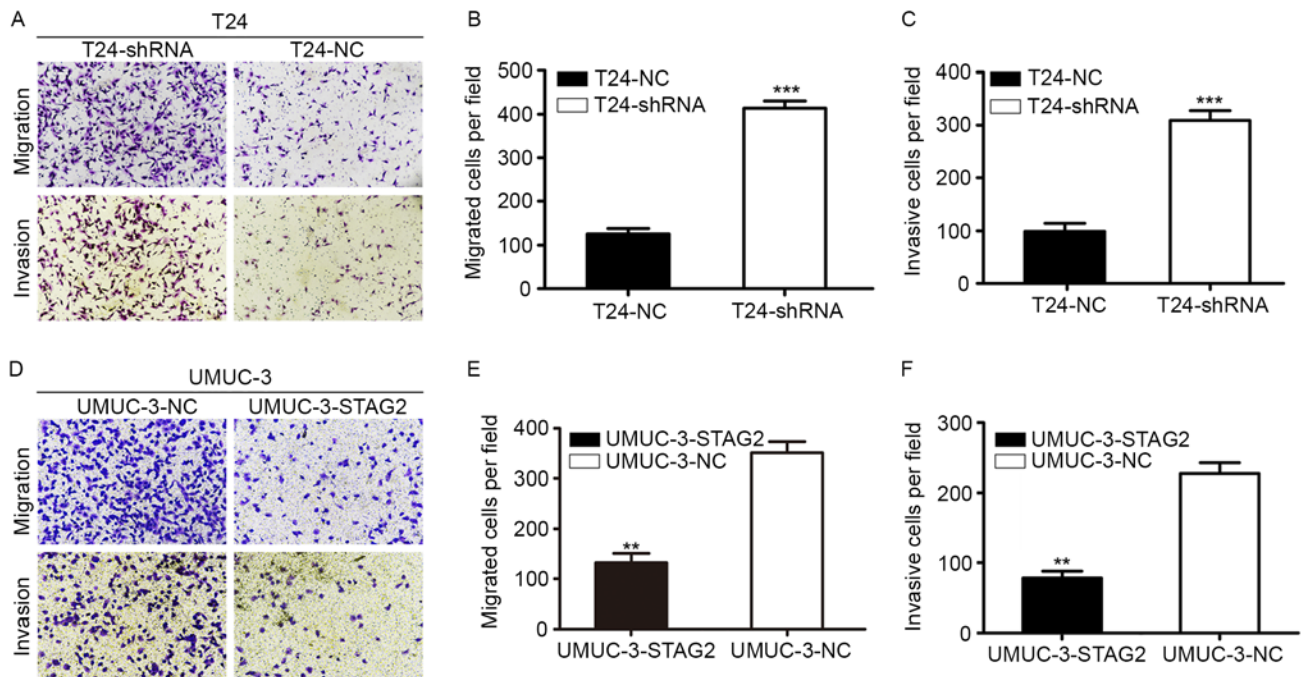
Figure 5. STAG2 inhibits T24 and UMUC-3 bladder cancer cell migration and invasion. (A-C) The effects of STAG2 knockdown on T24 cell migration and invasion. (D-F) The effects of STAG2 overexpression on UMUC-3 cell migration and invasion. Data are shown as the means ± SDs. **P<0.01, ***P<0.001. Each assay was performed in triplicate.
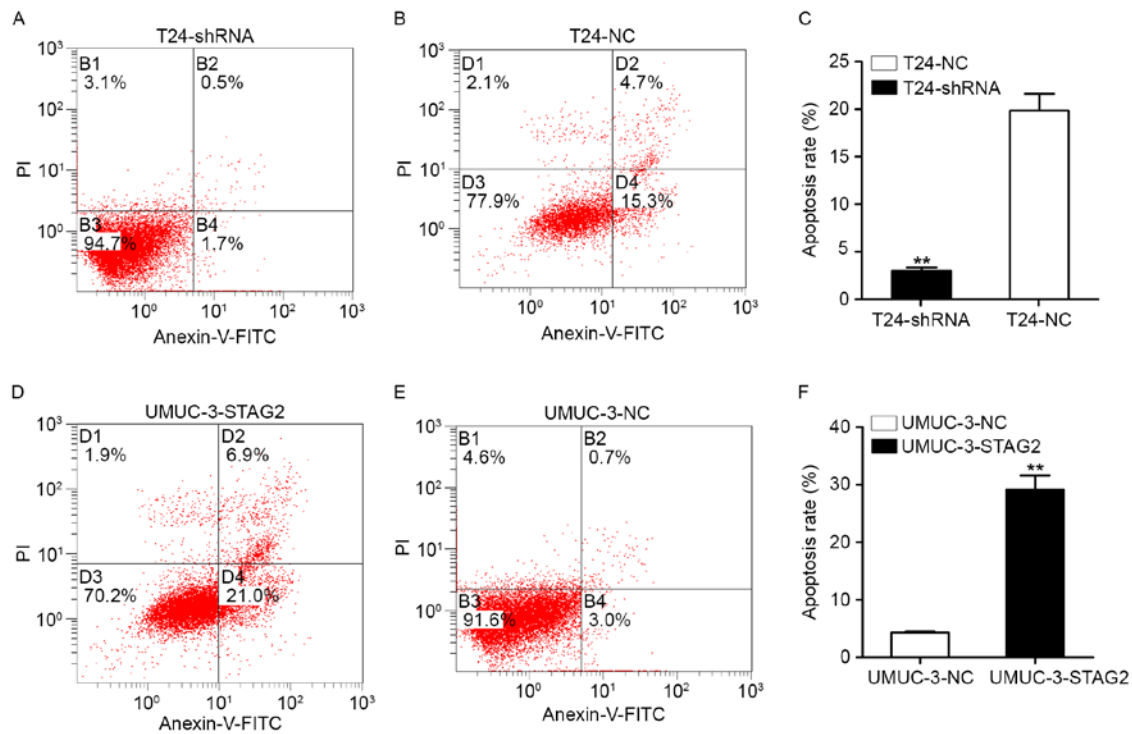

Figure 6. STAG2 induced apoptosis in bladder cancer cells. Lentivirus-infected T24 and UMUC-3 cells were subjected to apoptosis assays. (A-C) The effects of STAG2 knockdown on T24 cell apoptosis. (D-F) The effects of STAG2 overexpression on UMUC-3 cell apoptosis. Data are shown as the means ± SDs. **P<0.01. Each experiment was repeated three times independently.

in T24-shRNA cells compared with that of T24-shNC cells (Fig. 6A-C). Conversely, apoptosis was significantly increased in UMUC-3-STAG2 cells compared with that in UMUC-3-shNC cells (Fig. 6D-F). These results suggest that STAG2 induces apoptosis in bladder cancer cells.

Effects of STAG2 on signaling pathways. Finally, we analyzed the signaling pathways likely to be affected by STAG2 expression by western blotting. As shown in Fig. 7, E-cadherin, caspase-3, and caspase-7 expression was significantly enhanced in UMUC-3-STAG2 cells compared with that in
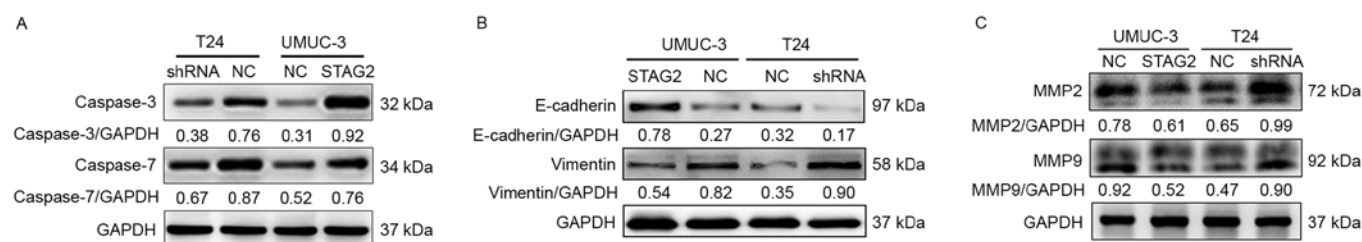
Figure 7. The effect of STAG2 overexpression or knockdown on the expression of downstream signaling factors. (A) Caspase-3 and caspase-7, (B) E-cadherin and vimentin, and (C) expression of MMP2 and MMP9 was examined by western blotting. GAPDH was included as a loading control.

UMUC-3-shNC cells (Fig. 7A and B). In contrast, vimentin, matrix metalloproteinase (MMP) 2, and MMP9 were markedly increased in T24-shRNA cells compared with T24-shNC cells (Fig. 7B and C).

Discussion

Bladder cancer is one of the most common malignant tumors worldwide (19). Although early detection and treatment can improve survival rates, only ~50% of patients are diagnosed and treated prior to the development of invasive lesions (20). Therefore, new tumor markers and therapeutic targets are urgently needed to improve treatment outcomes. In this study, we evaluated the expression and function of STAG2, an important subunit of cohesin (16), in bladder cancer. Our findings demonstrate that low STAG2 expression is associated with TNM stage in bladder cancer, where it plays important roles in mediating cell motility and cell cycle progression. *STAG2* is stably expressed by non-tumor tissues; however, truncating mutations have been identified in 36% of non-invasive bladder cancer samples and 16% of invasive bladder cancer samples. In bladder cancer, STAG2 mutations have been observed as truncated mutations, including nonsense, frameshift, and splice site mutations, resulting in absence of the protein's C-terminal domain. In some cases, STAG2 expression is completely abolished independent of gene mutations, suggesting that other mechanisms may also cause loss of STAG2 expression, such as CpG promoter methylation (10,21,22). Balbás-Martinez *et al* previously reported that STAG2 inactivating mutations are significantly associated with low tumor stage and grade, multicentricity, and tumor size and inversely related to chromosomal copy number changes (18). In addition, the absence of STAG2 expression was associated with lower tumor stage and grade and with female sex (10). In the present study, we concluded that loss of *STAG2* mRNA expression was closely related to low tumor grade in bladder cancer; however, no significant correlations were found between STAG2 loss and other clinical features, indicating that STAG2 inactivation was an early event that did not affect cancer progression. Recent studies have provided contradictory findings regarding the relationship between the STAG2 expression and the prognosis in patients with bladder cancer. For instance, the study by Balbás-Martinez *et al* determined that STAG2 deletion associated with a better prognosis in both NMIBC and MIBC (18); however, Guo *et al* found that loss of STAG2 expression correlated with poor prognosis (8). Moreover, STAG2 deletion is associated with different prognoses in patients with MIBC

and NMIBC (22). The variability amongst these studies may be attributed to the surgical approach or postoperative treatment of patients, or regional and ethnic differences. Notably, Balbás-Martinez *et al* reported that STAG2 knockdown had no effect on aneuploidy, whereas Solomon *et al* found that STAG2 inactivation resulted in cohesin downregulation and aneuploidy (18,22), for the chromosomal aneuploidy studies, the inconsistent results were likely attributed to differences in the selected cell lines. Specifically, 639V, RT112, and UMUC-11 were used in Balbás-Martínez *et al* study, whereas Solomon *et al* used RT4. Furthermore, *STAG2* is reportedly downregulated in colorectal cancer, gastric cancer, breast cancer, non-small cell lung cancer, and prostate cancer, suggesting that *STAG2* gene inactivation is a common feature of solid cancers (13).

In this study, we found that STAG2 was downregulated in bladder cancer tissues when compared to matched, normal bladder tissues, suggesting that STAG2 may exhibit inhibitory effects on bladder cancer development. Significantly, we found that STAG2 downregulation correlated with clinical stage and pathological grade, and inhibited the migration and invasion of bladder cancer cells. Previous studies have shown that tumor cell migration and invasion are related to the EMT process, which is characterized by loss of epithelial cadherin (E-cadherin). Notably, EMT is also required for cancer progression and metastasis, wherein the zinc-dependent endopeptidases MMP2 and MMP9 degrade extracellular matrix components to mediate migration in tumor cells. Consistent with this notion, both MMP2 and MMP9 have been shown to play important roles in bladder cancer progression (23,24). Similarly, our data showed that STAG2 knockdown caused the downregulation of E-cadherin protein expression as well as the upregulation of vimentin, MMP2, and MMP9, suggesting that STAG2 regulates invasion and migration via EMT signaling in bladder cancer cells. However, additional studies are needed to determine the specific mechanisms regulating these processes.

Interestingly, our findings demonstrated that STAG2 also regulated cell cycle progression in bladder cancer cells independent of proliferation rate. Thus, further studies are necessary to elucidate the mechanisms by which this occurs. Interestingly, our findings indicated that caspase-7 - a critical effector caspase in apoptosis - may be a target of STAG2. Caspase-7 is frequently downregulated in gastric cancer and colorectal cancer, and inactivating mutations have been found in head/neck cancer, esophageal cancer, and colon cancer. Such mutations can result in loss of capacity for apoptosis, thereby promoting cancer. In addition, caspase-7 is highly associated with caspase-3, a major executioner in apoptosis

frequently downregulated in solid tumors and associated with a poor prognosis (25). Our findings also identified caspase-3 as a target of STAG2, further supporting the pro-apoptotic role of STAG2 in bladder cancer. In view of the impact of STAG2 on cell apoptosis, cell invasion, and migration, we examined cells by western blotting and found that STAG2 likely regulates various signaling pathways associated with these processes. However, this was a preliminary analysis, and further research is needed to clarify the mechanisms by which this occurs.

In conclusion, our findings demonstrated that STAG2 downregulation, deletion, or loss of function is a common event in bladder cancer and may be associated with cancer metastasis through the regulation of MMP2, MMP9, and EMT. Additionally, STAG2 may be involved in apoptosis by regulating caspase-3 and caspase-7 expression. Thus, these data suggest that STAG2 may be a potential therapeutic target in patients with bladder cancer.

Acknowledgements

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