Downregulation of BLM RecQ helicase inhibits proliferation, promotes the apoptosis and enhances the sensitivity of bladder cancer cells to cisplatin

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Abstract. Bloom syndrome protein (BLM) is known to maintain genomic integrity including DNA repair, recombination, replication and transcription. Its dysregulation affects the genomic instability of cells, which results in a high risk of developing various types of cancer and even Bloom syndrome. However, to date, to the best of our knowledge, no association has been made between human BLM and bladder cancer. Thus, the aim of the present study was to investigate the role of BLM in human bladder cancer. The expression pattern of BLM in bladder cancer tissue was detected by immunohistochemistry. The viability, proliferation, cell cycle and apoptosis of bladder cancer cell lines were determined by Cell Counting Kit-8, EdU and flow cytometry following transfection of BLM small interfering RNA. Finally, the effect of BLM on sensitivity of bladder cancer cell lines to cisplatin was investigated by reverse transcription-quantitative PCR and western blot. It was demonstrated that the expression of BLM in human bladder cancer was increased compared with adjacent healthy bladder tissues. In addition, silencing of BLM inhibited the proliferation and promoted the apoptosis of bladder cancer cells and it also enhanced the sensitivity of bladder cancer cells to cisplatin. Together, the findings of the present study demonstrated that the regulation of BLM activity may have potential for use as a novel therapeutic target and a predictor for the prognosis of bladder cancer.

Introduction

Bladder cancer is the fourth leading cause of cancer-related mortality worldwide. In 2012, the economic burden associated with bladder cancer was 4.9 billion in the European Union, accounting for 5% of the total health care costs for cancer (1). In China in 2015, it was estimated that there were 66,8000 new cases of bladder cancer, which led to a considerable loss in productivity and a heavy economic burden, also due to the fact that the nature of cancer requires lifetime surveillance (2). The diagnosis, treatment and 5-year survival rates of patients with bladder cancer have greatly improved over the past 30 years (3). However, patient outcomes, particularly those of patients who are not responsive or intolerant to chemo-immunotherapy remain poor. Therefore, there is an urgent need for the development of novel markers which can be used to identify the most effective treatment regimens for patients.

Genomic instability is a trademark of cancer (4,5). Pathological studies show that 70-75% of newly diagnosed bladder cancer cases are non-muscle-invasive bladder cancers (NMIBCs) and the remainder are classified as muscle-invasive bladder cancers (MIBCs). NMIBCs are easily manageable; however, ~15% of high-grade cases progress to MIBC (6). Studies demonstrate that non-homologous end joining (NHEJ) is involved in double-strand break repair in MIBCs; in MIBCs, the failure to repair stalled replication forks is observed (7,8). As proteins directly participate in these molecular alterations and mediate cancer cell biology mechanisms, proteins may be more accurate and specific markers for cancer (9).

The human RecQ helicases are a highly conserved protein family which serve vital roles in DNA metabolism and genetic stability. There are five human RecQ helicases and they contain a deeply conserved helicase domain that can uncoil double strand structure in an ATP-dependent and 3'-to-5' manner (10). Bloom syndrome protein (BLM), also known as RECQL2, belongs to this family. The mutation of BLM leads to Bloom syndrome, which is mostly characterized by a predisposition to developing various types of cancers, including bladder cancer (11,12). BLM interacts with proteins involved in replication fork migration and the NHEJ pathway. Due to the lack of BLM, human epithelial cells exhibit hyper-recombination and mice or yeast exhibit a cancer-prone phenotype (13,14).

The loss or inactivation of BLM has been shown to lead to cancer development via structural changes in oncogenes or tumor suppressor genes (15,16). It has been demonstrated that BLM may serve important roles in the progress of oncogenesis (17). Furthermore, previous studies have found that the

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nonsense mutation of BLM increases the risk of developing breast or ovarian cancer (18,19). RecQ helicase expression has also been used to predict whether cancers are sensitive to DNA-damaging chemotherapeutic agents (20,21). However, to date, to the best of our knowledge, there are no reports on the mechanisms of action of BLM in bladder cancer. Thus, in order to elucidate the role of BLM in bladder cancer, the present study analyzed BLM expression patterns in bladder cancer and matched adjacent healthy tissues. In addition, in order to further determine the functions of BLM in bladder cancer, the cell cycle, proliferation and apoptosis, as well as sensitivity to chemotherapeutic drugs was analyzed in an *in vitro* model.

Materials and methods

Tissue microarray (TMA) and immunohistochemical (IHC) analysis. TMA chips that covered 68 bladder cancer tissue specimens and 54 adjacent normal bladder cancer specimens were purchased from Shanghai Outdo Biotech Co., Ltd. The present study was approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University (approval number 2017-ke-47) and was conducted in accordance with the Declaration of Helsinki. Patients gave written consent for their information to be stored on the hospital database and to be used in research. Immunohistochemical staining was performed according to the instructions of the S-P kit (OriGene Technologies, Inc.). The TMAs were firstly fixed with 4% paraformaldehyde and blocked with 10% goat serum for 30 min at room temperature. Then, the TMAs were stained with BLM primary antibody (cat. no. NBP1-89929; Novus Biologicals, Ltd.) at a dilution of 1:100 overnight at room temperature. Negative and positive controls (by omission of the primary antibody and IgG-matched serum) were included in each test.

Evaluation of immunohistochemistry score. The BLM protein staining scores were evaluated by two experienced pathologists and the concordance between the two pathologists was excellent. The whole field of the section was scored and intensities of staining were grouped as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining. The cut-off value used to separate patients into high and low BLM expression groups was ≥2.

Cell lines and culture. Human bladder cancer cell lines (J82 and 5637) were obtained from China Infrastructure of Cell Line Resources. Cells were cultured routinely in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.), supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) in a standard culture conditions (5% CO_2 at 37˚C).

Transient siRNA transfection. Cells were transfected with 20 nM negative control [short interfering (si)RNA-CON] or BLM siRNA (siRNA-BLM) (sense: 5'-CCCACUACUUUG CAAGUAATT-3', antisense: 5'-UUACUUGCAAAGUAG UGGGAA-3') according to the manufacturer's instructions (Ambion; Thermo Fisher Scientific, Inc.). Briefly, $15x10^4$ cells were seeded and cultured in 6-well plates and medium was changed to Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.) at ~70% confluence. Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) and siRNA-CON or siRNA-BLM was diluted into Opti-MEM separately and incubated for 5 min at room temperature then mixed and incubated for 20 min to allow the formation of lipid-siRNA complex. After 6 h incubation, the medium was changed to RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.). After 48 to 72 h, the cells were harvested and used for experiments.

Cell viability assay. Cell viability was assessed by the Cell Counting Kit-8 (CCK-8) (Beyotime Institute of Biotechnology) for three times independently. Briefly, the cells was plated into a 96 well plate and treated with corresponding agents for the indicating time. CCK-8 reagent (10 μl/well) was added to each well and incubated for 2 h. Then the cell viability was determined by using a micro-plate reader at a 450 nm wavelength (Multiskan; Thermo Fisher Scientific, Inc.).

Cell proliferation assay. A total of $4x10^4$ cells were cultured in 96-well plates and exposed to 50 μM EdU solution (Guangzhou RiboBio Co., Ltd.) for 4 h at 37˚C and then fixed in 4% formaldehyde overnight at 4˚C and permeabilized in 0.5% Triton X-100 for 10 min. Cells were incubated with 100 μl Apollo reaction cocktail for 30 min at room temperature in the dark and DNA was stained with Hoechst 33342 (100 μl/well) for 30 min at room temperature in the dark. The stained cells were analyzed using a fluorescent microscope (6 fields were selected randomly; magnification, x40).

Cell cycle assay. A total of $1x10^6$ cells were fixed with pre-cooled 70% ethanol and treated with 10 μg/ml RNase (Sigma-Aldrich; Merck KGaA), then the cells incubated at 37˚C for 30 min before staining with 50 μg/ml propidium iodide (PI) for 30 min at 4˚C (Invitrogen; Thermo Fisher Scientific, Inc.). Cell cycle distribution was analyzed using a Gallios flow cytometer (Beckman Coulter, Inc.) and ModFit software version 3.2 (Verity Software House) was utilized for cell cycle analysis.

Cell apoptosis assay. The experiment was performed following the protocols described by the kit manufacturer (BD Biosciences). A total of $5x10^5$ cells were harvested and re-suspended in binding buffer at a concentration of $1x10^6$ cells/ml. 100 μl of the single cells suspension was mixed with 5 μl of Annexin V-FITC and 5 μl of PI and further incubated for 15 min at room temperature in the dark. Finally, 400 μl of binding buffer was added to the mixture and the cells were analyzed by a Gallios flow cytometer (Beckman Coulter, Inc.). The FlowJo software (BD Biosciences; Version 7.6) was used to analyze the early apoptosis rate, late apoptosis rate and total apoptosis rate respectively. The cells undergoing early apoptosis were defined as Annexin V-FITC-positive/PI-negative, and the late apoptotic cells were defined as Annexin V-FITC-positive/PI-positive. The total apoptosis rate was the percentage of early + late apoptotic cells.

IC_{50} determination. The transfected cells were seeded into a 96-well plate at a density of $5x10^4$ cells per well. Then the cells were treated with different concentrations of cisplatin

(0, 2, 4 and 8 μmol/l) for different periods of time (12, 24 and 48 h). The IC_{50} value of cisplatin in the J82 and 5637 was calculated at 24 h by probit regression. Cisplatin was obtained from Qilu Pharmaceutical Co., Ltd.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from $5x10^5$ cells using TRIzol reagent (Thermo Fisher Scientific, Inc.), in accordance with the manufacturer's instructions. RT-qPCR was performed according to the manufacturer's instructions (Taq Plantinum PCR MasterMix, Tiangen Biotech Co., Ltd.). The primers were designed using the software Primer Premier (BLM: forward 5'-GGATCCTGGTTCCGTCCGC-3', reverse 5'-CCT CAGTCAAATCTATTTGCTCG-3', β-actin: forward: 5'-TGA CGTGGACATCCGCAAAG-3', reverse: 5'-TCTTCATTG TGCTGGGTGCC-3'). The PCR program included a cycle of 95˚C for 15 min, followed by 40 cycles of denaturation at 95˚C for 10 sec, annealing/extension at 60˚C for 32 sec. All samples were normalized against the internal control (β-actin) and analyzed using the $2^{-\Delta\Delta Cq}$ method (22).

Western blot analysis. Cells were lysed with RIPA buffer (10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1% NP-40; 0.25% sodium deoxycholate) and quantified using the BCA protein assay kit (Beyotime Institute of Biotechnology). Equal amounts (40 μg) protein were separated on a 10% SDS-polyacrylamide gel and electrotransferred to PVDF membranes (MilliporeSigma). Membranes were immersed in blocking buffer with 10% fat free milk for 1 h at room temperature. The blocked PVDF membrane was incubated with antibodies against BLM (1:1,000; cat. no. 2742; Cell Signaling Technology, Inc.) for 3 h at room temperature. Following incubation with the primary antibodies, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibodies for 1 h (1:5,000; cat. nos. 31466 and PA174421; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature. Protein bands were detected by using enhanced chemiluminescence. β-actin (1:1,000; cat. no. 3700; Cell Signaling Technology, Inc.) was used as the loading control. Proteins bands were visualized using an ECL reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and the immunoblots were quantified using ImageJ software (version 3.0; National Institutes of Health).

Statistical analysis. Two groups were compared using Mann Whitney test if data were non-parametric or using unpaired Student's t-test if data were parametric. Multiple groups were compared using one-way analysis of variance, followed by Sidak's or Tukey's multiple comparisons test. Survival curves were estimated using the Kaplan-Meier analysis and were compared using the log-rank test. A Cox regression model was used to identify prognostic factors for survival of patients with bladder cancer. The contingency table presented in Table I was analyzed using a chi-squared test or Fisher's exact test when the expected count in <20% of the cells of the analyzed contingency table is 5 or fewer (i.e. age, gender, differentiation, muscle invasion and lymph node metastasis). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Differential expression of BLM in bladder cancerous and non-cancerous tissues. A total of 68 bladder cancer and 54 adjacent healthy bladder tissues were analyzed in the present study. The patient demographics are summarized in Table I. The expression of BLM in cancer tissues was assessed using immunohistochemistry. Bladder cancer tissues expressed a high level of BLM, while the adjacent healthy tissue expressed a low level of BLM (Fig. 1). When the immunostaining scores of BLM were further compared between cancerous tissue and adjacent healthy tissue, a significantly higher expression level of BLM was found in the cancerous tissues in all patients ($P<0.0001$) or in patients with bladder cancer of grade II or higher ($P<0.001$; Fig. 1B and C). These results suggested that the expression of BLM was significantly higher in bladder cancer tissues than in adjacent normal tissues.

Survival analysis was then performed for the patients with bladder cancer. In the low BLM expression group (non-staining group and weak staining group), 33 of the 49 (67.35%) patients survived, while in the high BLM expression group (moderate staining group and strong staining group), 8 of the 19 (42.10%) patients survived. There was a significant difference in the survival rate between the high and low BLM expression groups ($P=0.013$; Fig. 1D).

In Cox regression analysis, two prognostic factors for the overall survival of patients with bladder cancer were identified using univariate analysis: The American Joint Committee on Cancer (AJCC) stage (I-II vs. >II, $P=0.008$) and TNM stage (I/II vs. III/V, $P=0.001$). However, the BLM expression level (low vs. high) was not a statistically significant prognostic factor ($P=0.981$). Furthermore, multivariate analysis revealed that the AJCC ($P=0.030$) and TNM stage ($P=0.008$) were significant independent predictors of the poor survival of patients with bladder cancer (Table II). Additionally, a nomogram of the median survival time was drawn, containing BLM expression, age and sex (data not shown).

Expression and silencing of BLM in bladder cancer lines. To investigate the role of BLM in bladder cancer, the expression of BLM was first assessed in two bladder cancer lines (J82 and 5637). As shown in Fig. 2A and B, both cell lines expressed BLM. The mRNA and protein expression level of BLM in the J82 cells was lower compared with that in the 5637 cells.

Subsequently, both bladder cancer cell lines were transfected with a control siRNA (siRNA-CON) or a BLM-specific siRNA (siRNA-BLM). As shown in Fig. 2C and D, both the mRNA and protein expression levels of BLM were markedly decreased in these two cell lines ($P<0.0001$) following transfection with siRNA-BLM.

Silencing of BLM inhibits the viability and proliferation and promotes the apoptosis of bladder cancer cells in vitro. The cell cycle was analyzed to identify whether cell cycle perturbation occurs after the silencing of BLM expression. The results of cell cycle assay (Fig. 3) revealed that both cell lines transfected with siRNA-BLM were arrested in the G_0G_1 phase ($P<0.001$). In the siRNA-BLM group, the J82 cells in the S phase were significantly decreased compared with

Table I. Association between BLM expression and clinicopathological characteristics in bladder cancer patients.

Characteristics	Number	BLM expression		P-value
		High (n=19)	Low (n=49)	
Age (years)				
<60	17	4	13	0.761
≥60	51	15	36	
Sex				
Male	57	17	40	0.715
Female	11	2	9	
Pathology grade				
I-II	22	6	16	1.000
>II	46	13	33	
Differentiation				
Well, moderate	65	18	47	1.000
Poor	3	1	2	
Muscle invasion				
No	11	3	8	1.000
Yes	57	16	41	
Tumor invasion depth				
Tis, T1, T2	39	13	26	0.287
T3, T4	29	6	23	
Lymph node metastasis				
No	59	17	42	1.000
Yes	9	2	7	
Distant metastasis				
M0	68	19	49	-
M1	0	0	0	
AJCC stage				
I-II	41	15	26	0.059
>II	27	4	23	

AJCC, American Joint Committee on Cancer.

Table II. Risk factors associated with overall survival of bladder cancer patients.

Variables	HR (95% CI)	P-value
Age	1.004 (0.972-1.036)	0.802
Gender	0.854 (0.389-1.876)	0.694
Differentiation	0.496 (0.237-1.040)	0.063
Neural invasion	1.787 (0.858-3.723)	0.121
TNM stage	2.345 (1.087-5.057)	0.030[a]
AJCC	3.040 (1.432-6.457)	0.008[a]
BLM expression	2.439 (0.732-5.255)	0.981

[a]P<0.05. HR, hazard ratio; CI, confidence interval.

the siRNA-CON group (P<0.0001) and the 5637 cells in the G_2M phase were significantly decreased compared with the siRNA-CON group (P<0.05). These results indicated that the silencing of BLM expression led to G_1 arrest in the bladder cancer cells.

It was hypothesized that cell proliferation was inhibited as there were fewer in the S phase. To verify this hypothesis, a cell proliferation assay was performed. The numbers of EdU-labeled J82 and 5637 bladder cancer cells transfected with siRNA-CON were 335.25±97.79 and 118.71±19.20 in each field, respectively. In addition, the numbers of EdU-labeled J82 and 5637 bladder cancer cells transfected with siRNA-BLM were 185±24.90 and 76.34±16.45, respectively (P<0.05). Therefore, the silencing of BLM significantly decreased bladder cancer cell proliferation (Fig. 4).

To examine cell functions in response to BLM knockdown in the J82 and 5637 cells, cell apoptosis assay was performed. The results revealed that cell apoptosis was significantly increased following transfection with siRNA-BLM compared with the siRNA-CON group (P<0.05, Fig. 5).

Silencing of BLM sensitizes bladder cancer cell lines to cisplatin. Previous studies have demonstrated that the RecQ

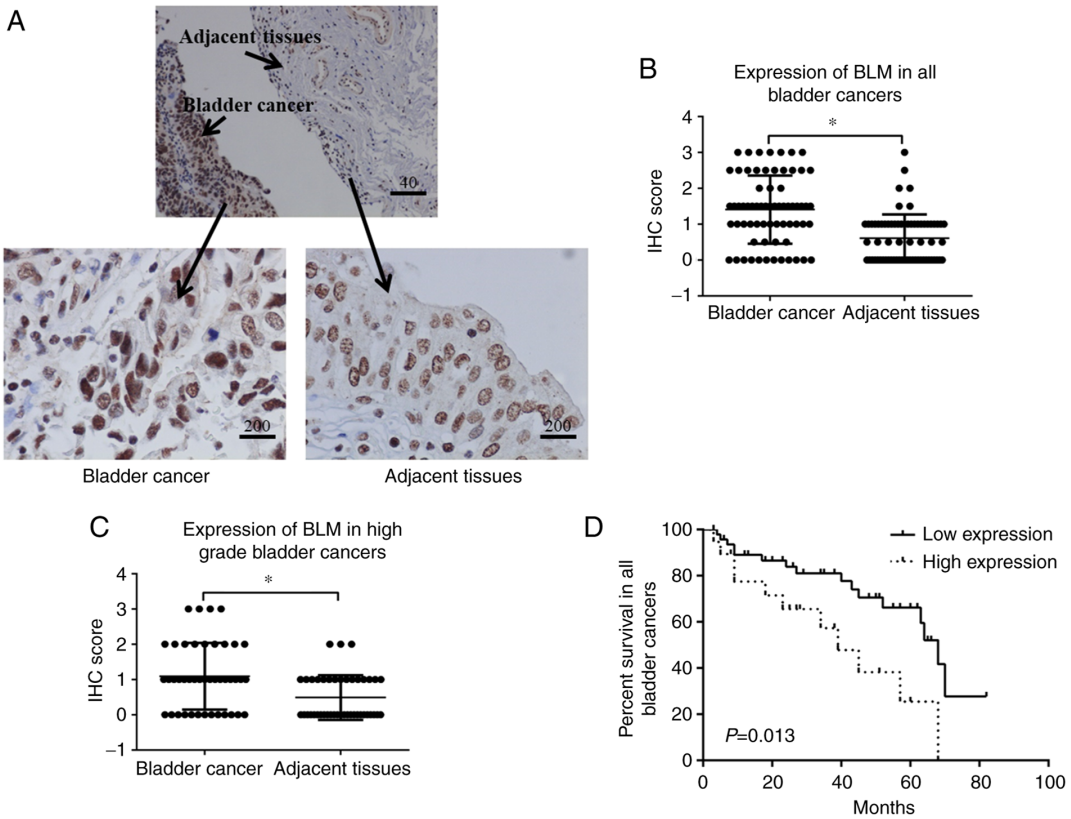

Figure 1. Higher expression of BLM in patients with bladder cancer. (A) Immunohistochemical staining illustrating the expression and localization of BLM in bladder cancer tissues and adjacent normal tissues (magnification: Top panel, x40; bottom panel, x200). BLM was detected in the nucleus in both cancer and normal tissues and the expression of BLM in bladder cancer tissues was significantly higher than that in the adjacent normal tissues. (B) In all bladder cancer tissues of all grades, BLM exhibited a higher expression in cancerous tissues than in normal ones (*P<0.0001). (C) In high-grade bladder cancer tissues, BLM exhibited a higher expression in cancerous tissues than in normal ones (*P<0.001). (D) There was a significant difference in the survival rate between the high BLM expression group (8 of 19 patients survived) and the low BLM expression group (33 of 49 patients survived) (P>0.05). BLM, Bloom syndrome protein.

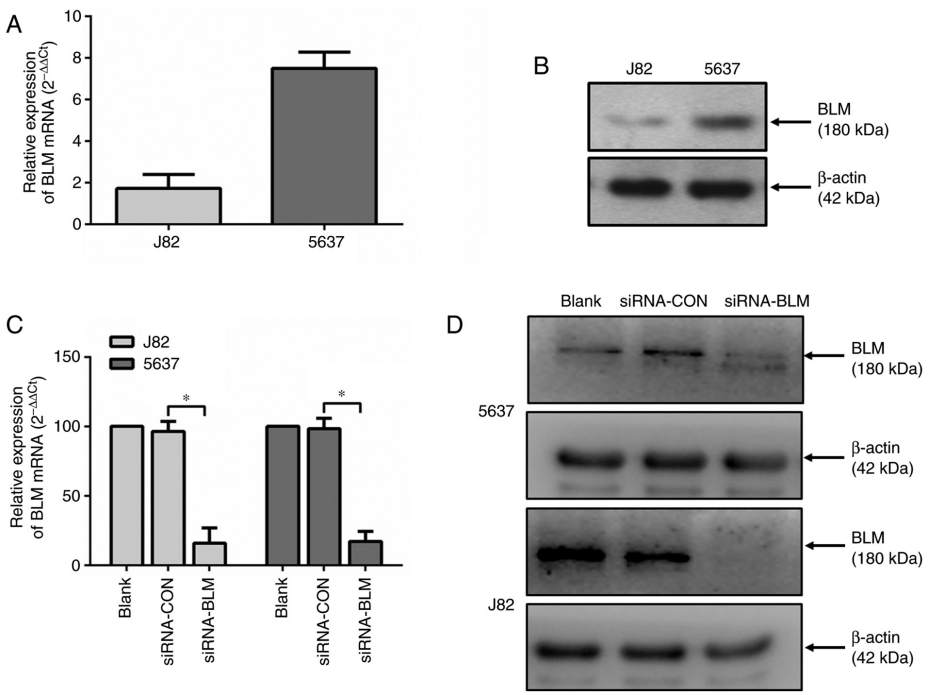

Figure 2. Expression and silencing of BLM in two bladder cancer cell lines. (A) Results of reverse transcription-quantitative PCR showing the relative mRNA expression of BLM in the invasive and well-differentiated bladder cancer cell line, 5637 and in the invasive and poorly differentiated cell line, J82. (B) Western blot analysis of the protein level of BLM in the 5637 and J82 cells. (C) BLM mRNA level in bladder cancer cells was silenced by transfection with siRNA-BLM compared with siRNA-CON-transfected cells (*P<0.0001). (D) Western blot analysis demonstrated that the BLM protein level significantly decreased in the siRNA-BLM-transfected 5637 and J82 cells compared with the siRNA-CON-transfected cells. BLM, Bloom syndrome protein; siRNA, small interfering RNA; CON, control.

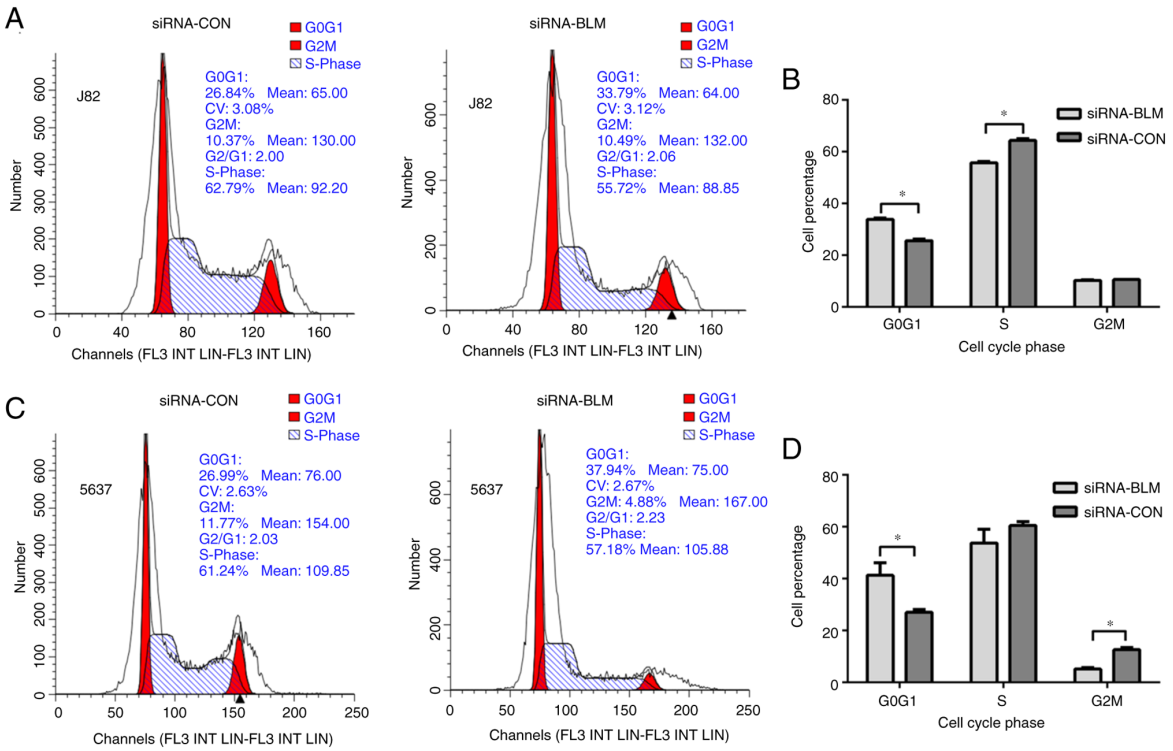

Figure 3. Cell viability is inhibited after the silencing of BLM. (A) J82 cells were transfected with siRNA-CON or siRNA-BLM and cell cycle distribution was analyzed using flow cytometry. (B) The percentages of J82 cells in the G_0G_1, S and G_2/M phase are presented in the histograms. The cells transfected with siRNA-BLM were arrested in the G_0G_1 phase (*P<0.001) and the numbers of cells in the S phase were significantly decreased compared with the siRNA-CON group (*P<0.0001). (C) The 5637 cells were transfected with siRNA-CON or siRNA-BLM and the cell cycle distribution was analyzed using flow cytometry. (D) The percentages of 5637 cells in the G_0/G_1, S and G_2/M phases are presented in the histograms. The cells transfected with siRNA-BLM were arrested in the G_0G_1 phase (*P<0.001) and the numbers of cells in the G_2M phase were significantly decreased compared with the siRNA-CON group (*P<0.05). BLM, Bloom syndrome protein; siRNA, small interfering RNA; CON, control.

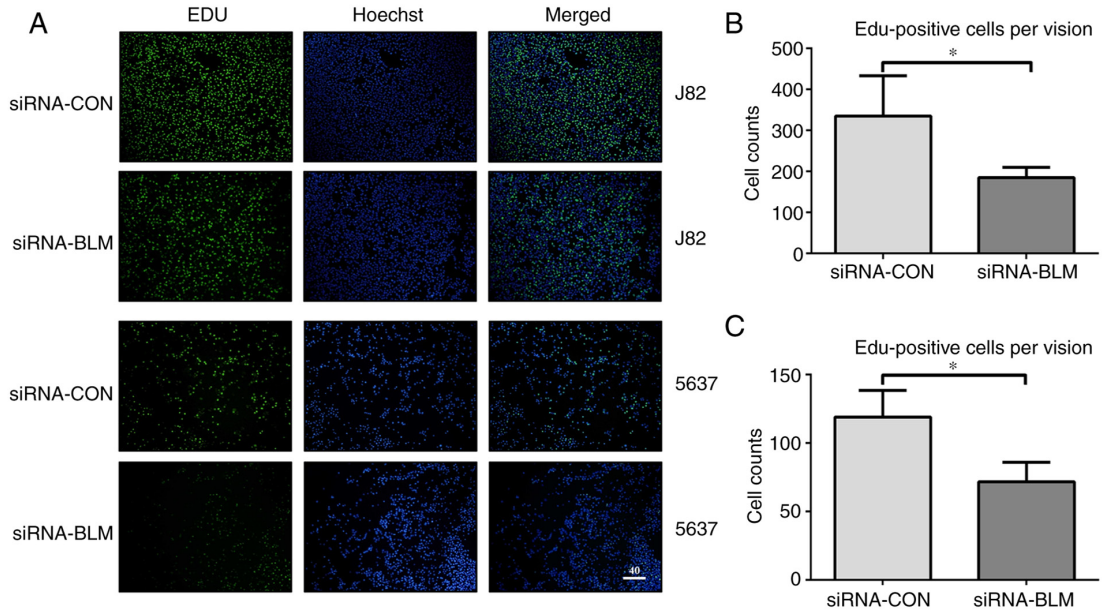

Figure 4. Silencing of BLM decreases bladder cancer cell proliferation. (A) Representative results of the effect of siRNA-BLM on bladder cancer cell proliferation (magnification, x40). (B) Quantitative analysis of EdU-positive J82 cell numbers. The numbers of EdU-positive J82 bladder cancer cells transfected with siRNA-BLM were decreased compared with the siRNA-CON group. *P<0.05. (C) Quantitative analysis of EdU-positive 5637 cell numbers. The numbers of EdU-positive 5637 cells transfected with siRNA-BLM were decreased compared with the siRNA-CON group *P<0.05. BLM, Bloom syndrome protein; siRNA, small interfering RNA; CON, control.

helicase level is negatively associated with genomic instability induced by DNA damaging agents in various type of cells (21,23). As platinum-containing anticancer drugs are widely used in intravesical instillation, cisplatin was selected

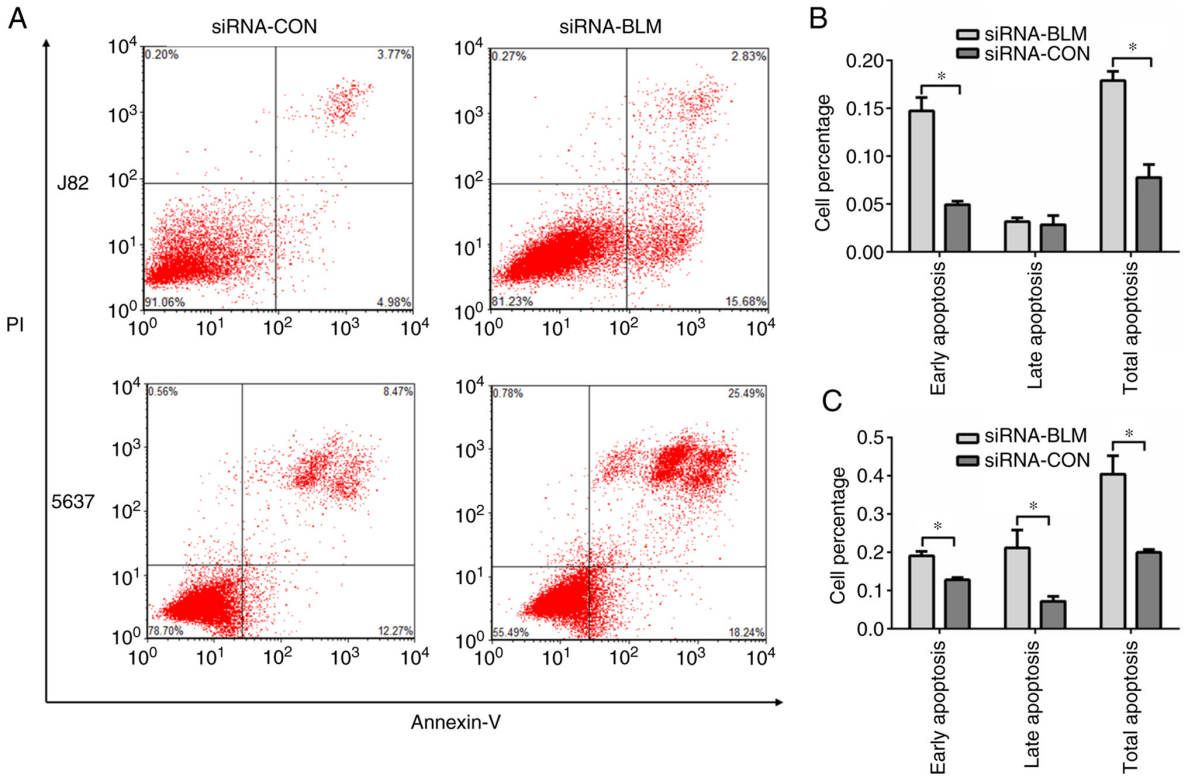

Figure 5. Silencing of BLM promotes bladder cancer cell apoptosis. (A) Representative results of cell apoptosis measured using flow cytometry. (B) The quantification of apoptotic cell numbers indicated that the J82 cells transfected with siRNA-BLM exhibited enhanced apoptosis compared with the siRNA-CON group. *P<0.05. (C) The quantification of apoptotic cell numbers indicated that the 5637 cells transfected with siRNA-BLM exhibited an increase in apoptosis compared with the siRNA-CON group. *P<0.05. BLM, Bloom syndrome protein; siRNA, small interfering RNA; CON, control.

to induce DNA damage in the present study. To determine the cytotoxicity of cisplatin in bladder cancer cell lines, the J82 and 5637 cells were first treated with various concentrations of cisplatin (0, 2, 4 and 8 μmol/l) for different periods of time (12, 24 and 48 h). The results revealed that cisplatin significantly increased the death of the J82 and 5637 cells in a concentration- and time-dependent manner (Fig. 6A and B). The IC_{50} of cisplatin to J82 and 5637 at 24 h were (10.44±1.00) μmol/l and (6.91±0.32) μmol/l respectively. Subsequently, the siRNA-BLM-siRNA-CON-transfected J82 and 5637 cells were treated with cisplatin at the IC_{50} concentration for 24 h. As shown in Fig. 6C and D, the silencing of BLM significantly sensitized the J82 and 5637 cells to cisplatin; the death rate of the siRNA-BLM-transfected cells was significantly higher than that of the control group (P<0.05).

Discussion

BLM monitors genomic integrity and functions as a genome 'caretaker'; however, to the best of our knowledge, the mechanisms of action of BLM in bladder cancer have not yet been reported. The present study first confirmed that BLM was highly expressed in human bladder cancer tissues when compared with adjacent healthy tissues. These results were consistent with those of other studies which found that BLM mRNA expression is significantly deregulated in breast (24) and colorectal cancer (25). It was hypothesized this may due to the varied expression of BLM throughout the cell cycle, with its highest expression being found during the S phase (26). Even minimal changes in BLM expression can disrupt genomic integrity and function. However, the molecular mechanisms which lead to the upregulation of BLM are not yet fully understood. In addition, no association between BLM expression and the survival rate of patients with bladder cancer of all grades or of a high grade was found. Therefore, the present findings suggested that further studies on BLM are required to determine whether it can be used as a predictive biomarker for bladder cancer.

The present study examined the function of BLM in bladder cancer progression *in vitro* using the J82 and 5637 bladder cancer cells. Silencing of BLM significantly led to cell cycle arrest in the G_0G_1 phase and inhibited cell proliferation while it promoted cell apoptosis. As is known, homologous recombination occurs during the S and G2/M phase of the cell cycle and NHEJ is pivotal for the repair of DNA double-strand breaks, particularly during the G_0 and G_1 phase of the cell cycle (27). Therefore, without BLM, NHEJ cannot be processed smoothly and the cell cycle is arrested in the G_0 and G_1 phase. With the accumulation of cells in the G_1 phase, cell death eventually occurs. Similar with the findings of the present study, Mao *et al* (20) found that the silencing of BLM in the GM639 and U-2 OS cells significantly suppresses cell proliferation. Chen *et al* (28) also demonstrated that BLM knockdown leads to a reduction in prostate cancer cell proliferation. On the other hand, BLM can resolve DNA double-strand breaks, a type of cell DNA damage followed by apoptosis. Therefore, cells lacking BLM eventually undergo apoptosis (29). These

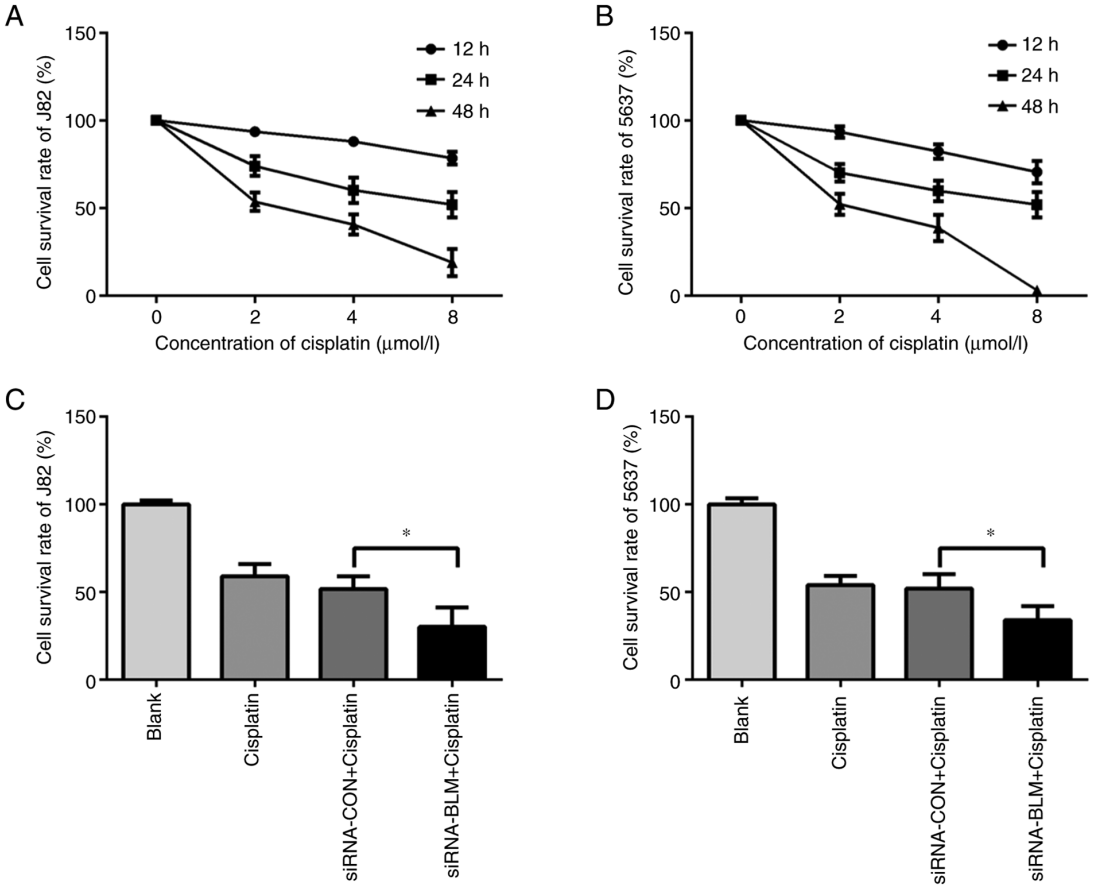

Figure 6. Silencing of BLM enhances cisplatin-induced bladder cancer cell death. (A) J82 cells were treated with cisplatin for 12, 24 and 48 h. The cell survival rate was analyzed using CCK-8 assay and the IC_{50} value at each time point and for each cell line was calculated using probit regression. (B) 5637 cells were treated with cisplatin for 12, 24 and 48 h and the cell survival rate was analyzed using CCK-8 assay. The IC_{50} value at each time point and for each cell line was calculated using probit regression. (C) J82 cells transfected with siRNA-BLM or siRNA-CON were treated with cisplatin for 24 h. The siRNA-BLM-transfected cells exhibited a significantly lower survival rate than the siRNA-CON group following treatment with cisplatin. *P<0.05. (D) 5637 cells transfected with siRNA-BLM or siRNA-CON were treated with cisplatin for 24 h. The siRNA-BLM-transfected cells also exhibited a significantly lower survival rate than the siRNA-CON group following treatment with cisplatin. *P<0.05. BLM, Bloom syndrome protein; siRNA, small interfering RNA; CON, control; CCK-8, Cell Counting Kit-8.

results suggested that the discovery of specific BLM inhibitors may greatly enhance the therapeutic effect in a variety of cancers.

In the present study, in light of the biological behavioral changes, a drug sensitivity test *in vitro* was conducted and the results revealed that the silencing of BLM expression sensitized bladder cancer cells to cisplatin. Although immense efforts have been made to explore genetic therapeutics over the past few years, the cisplatin-based chemotherapeutic regimen remains the first-line therapy for MIBCs. However, resistance to cisplatin is a major obstacle to successful treatment. Therefore, a better understanding of the mechanisms of cisplatin resistance may provide crucial information which would be of clinical significance. DNA repair pathway alterations are known to drive cancer behavior and therapeutic efficacy (30). In the present study, the inhibition of BLM expression could enhance cell sensitivity to cisplatin. Over the past few years, the role of BLM as a DNA sensor protein that recognizes DNA damage has been noted (31). As an important component of the DNA damage response, the response of BLM to DNA damage signals may occur through secondary nucleic acid structures. The dysregulated expression of BLM in cancer cells is observed in the cytoplasm in response to these lesions (32). Accordingly, the findings of the present study suggest that the inhibition of BLM in bladder cancer using small molecules or inhibitors may prove to be an effective therapy, similar to the study by Zhang *et al* (33), which found that a small molecule termed HJNO inhibited breast cancer cell expansion by targeting BLM helicase.

However, there are some limitations to the present study. Although it confirmed that the silencing of BLM significantly sensitized J82 and 5637 cells to cisplatin, whether the alterations of survival rate could be recognized by the difference of the BLM expression in MIBC patients receiving cisplatin treatment remains to be elucidated. Therefore, the influence of BLM differential expression on the survival rate of MIBC patients receiving cisplatin treatment and its related molecular mechanisms will be explored in future work.

In conclusion, the findings of the present study suggested that BLM served an oncogenic role in bladder cancer. The results provided preliminary evidence that BLM may be a predictive biomarker and a promising therapeutic target in

bladder cancer. However, further studies are required to determine the precise regulatory mechanisms of BLM in bladder cancer.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SF was responsible for study design, data collection, statistical analysis, data interpretation and literature search. XQ was responsible for study design, statistical analysis, data interpretation, literature search and drafting the manuscript. DF was responsible for data collection, statistical analysis and data interpretation. XZ was responsible for data interpretation, literature search, critical revision of the manuscript for scientific and factual content and sourcing funds. SF and XQ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University (approval number 2017-ke-47) and was conducted in accordance with the Declaration of Helsinki. Patients gave written informed consent for their information to be used in the research.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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