Abstract. The aim of the current study was to investigate the role of enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) in the progression of bladder cancer. Human bladder cancer tissue samples were analyzed by immunohistochemistry, and the association between the clinicopathological parameters and EZH2 expression was analyzed. The proliferation, apoptosis and migration ability of the human bladder cancer cell lines E-J and 5637 with or without the EZH2 inhibitor UNC1999 was investigated. The effect of UNC1999 was further explored in a xenograft model of nude mice. The in vivo and in vitro expression levels of EZH2, janus kinase 2, signal transducer and activator of transcription 3 and their phosphorylated forms were examined by western blotting. The expression levels of EZH2, JAK2 and STAT3 were increased in bladder cancer tissue compared with normal adjacent tissue. Furthermore, the expression of EZH2 was increased in tumors with a higher TNM Classification of Malignant Tumors stage and histological grade compared with tumors with a lower stage and grade. The human bladder cancer cell lines E-J and 5637 treated with UNC1999 demonstrated reduced cell proliferation, apoptosis and migration compared with cells treated without UNC1999. Additionally, EZH2 may promote the proliferation and migration of bladder cancer via the JAK2/STAT3 pathway. EZH2 may serve an important role in the proliferation and migration of human bladder cancer cells, and may aid in the development of novel treatment strategies for bladder cancer.

Introduction

Bladder cancer is the most common tumor among genitourinary cancers and the fourth most common cancer among males (1). Bladder cancer has a high incidence and mortality rate among the malignant tumors of the genitourinary tract (2). Furthermore, bladder cancer is more prevalent than other urinary tract carcinomas (3). Cancer statistical data have indicated an estimated 549,393 new cases and 199,922 mortalities from bladder cancer worldwide in 2018 (4). The majority of the newly diagnosed tumors are superficial and may be treated by transurethral resection (5). However, a large number of patients have a high rate of tumor recurrence following the first surgery (6). The mechanisms underlying the development of bladder cancer have not been thoroughly elucidated. Therefore, the identification of the molecular mechanisms involved in the progression of bladder tumors may aid the development of novel diagnostic and therapeutic interventions. The signal transducer and activator of transcription 3 (STAT3) signaling pathway is implicated in the progression of lung cancer (7). STAT3 is a cytoplasmic transcription factor which is expressed in response to a large number of cytokines and growth factors (8). A previous study suggested that STAT3 may promote the growth of tumor cells and inhibit tumor cell apoptosis (9). STAT3 is activated by the phosphorylation of conserved tyrosine and serine residues in its C-terminal domains by janus kinase (JAK) proteins (9). The phosphorylation of proteins in the JAK2/STAT3 pathway may result in the growth and proliferation of bladder cancer cells (10). The enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) is a member of the polycomb group proteins (11). EZH2 serves important roles in embryonic stem cell pluripotency and self-renewal (12,13). Furthermore, EZH2 expression was upregulated in different types of malignant tumors with poor prognosis, including prostate, bladder, renal and breast carcinomas (14-17). Previous studies revealed that EZH2 may be a marker of aggressive urological neoplasms (18,19). However, the biological effect of EZH2 in bladder cancer and the associations between EZH2 and the JAK2/STAT3 signaling pathway have not been elucidated. The aim of the current study was to investigate the effects of EZH2 on bladder carcinogenesis and to explore its mechanism in human bladder cancer cells.

Materials and methods

Drugs and reagents. The EZH2 inhibitor UNC1999 was purchased from Selleck Chemicals (Shanghai, China) and
was dissolved in dimethyl sulfoxide (DMSO; concentrations used in experiments, 0.1, 1, 10 and 100 µM). Antibodies against phospho-JAK2 (1:1,000 dilution; cat. no. 3771S), JAK2 (1:1,000 dilution; cat. no. 3230S), phospho-STAT3 (1:1,000 dilution; cat. no. 9145S), STAT3 (1:1,000 dilution; cat. no. 9139S), EZH2 (1:1,000 dilution; cat. no. 5246S) and GAPDH (1:3,000 dilution; cat. no. 5174S), as well as horse- 
radish peroxidase-conjugated goat anti-rabbit and anti-mouse IgG (1:2,000 dilution; cat. nos. 7074S and 7076S), were purchased from Cell Signaling Technology, Inc. (Boston, MA, USA).

**Clinical specimens.** Human bladder tumors and corresponding adjacent normal tissues were collected from patients who under- 
went partial or radical cystectomy for urothelial carcinomas of 
the bladder at the Department of Urology of Renmin Hospital of 
Wuhan University (Wuhan, China) between June 2015 and 
March 2017. The study was approved by the Institutional Ethics 
Committee of Renmin Hospital of Wuhan University. Each 
participant provided signed informed consent prior to participa-
tion in the present study. Patients or their legal surrogate decision 
makers provided signed informed consent for the surgical 
procedures. All specimens were anonymized. Inclusion criteria 
were the following: i) ≤75 years; ii) histologically confirmed 
bladder cancer; iii) no severe major organ dysfunction; and iv) 
no prior cancer chemotherapy. Exclusion criteria were the fol-
lowing: i) ≥76 years; ii) severe major organ dysfunction; and 
iii) prior cancer chemotherapy. The clinical information of the 
patients, including sex, age, smoking history, the diameter and 
differentiation of the tumor, lymph node metastasis, stage of 
TNM, histological grade, chemotherapy received and EZH2 
expression status, were recorded. Half of the specimens were 
removed and fixed in 4% paraformaldehyde at room tempera-
ture for 1 week, followed by routine paraffin embedding, and the 
remaining half were preserved in liquid nitrogen at -196°C. All 
of the specimens were classified as bladder carcinoma or normal 
tissue by histological identification. Tumor stage and grade were 
determined according to the Union for International Cancer 
Control (UICC) guidelines (20).

**Cell lines and cell culture.** The human bladder cancer cell 
lines E-J and 5637 were purchased from the The Cell Bank of 
Type Culture Collection of Chinese Academy of Sciences 
(Shanghai, China). E-J and 5637 cells were authenticated by STR profi-
ing. E-J and 5637 cells were cultured at 37°C with 5% CO₂, in RPMI 1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal 
bovine serum (FBS; SciCell Research Laboratories, Inc., 
San Diego, CA, USA), 0.1 mg/ml streptomycin and 100 U/ml 
penicillin. The medium was replaced every 48 h.

**Cell viability assay.** E-J and 5637 cells were plated into 
96-well plates at a density of 5x10³ cells/well. Following 24 h 
of culture at 37°C, the cells were treated with different concen-
trations of UNC1999 (0.1, 1, 10 and 100 µM) for different 
times (24, 72 and 120 h) and subsequently incubated with 
10 µl of MTT dye/well for 4 h. Following the MTT incubation, 
the purple formazan crystals were dissolved by the addition 
of 150 µl DMSO per well. Cell viability was subsequently 
analyzed at a wavelength of 490 nm.

**Apoptosis assay.** E-J and 5637 cells were incubated with or 
without UNC1999 (100 µM). The control group was treated 
with DMSO, for 72 h. The cells were collected and washed 
twice with PBS and resuspended with 150 ml binding buffer 
(included in the Annexin V-FITC Apoptosis Detection kit; 
cat. no. CI062M; Beyotime Institute of Biotechnology). The 
cells were subsequently incubated with 10 µl Annexin 
V-fluorescein isothiocyanate (FITC) and 5 µl propidium 
iodide for 5 min at room temperature in the dark. The Annexin 
V-FITC Apoptosis Detection kit was used according to the 
manufacturer’s protocol. Apoptotic cells were subsequently 
analyzed using a flow cytometer (BD Biosciences, San Jose, 
CA, USA). Analysis was performed using the BD FACS 
itware (version 1.0; BD Biosciences).

**Wound healing assay.** E-J and 5637 cells (5x10³) were plated in six-well plates. Following a 24 h incubation period at 37°C 
with 5% CO₂, wounds were made in each well using a 200 µl 
pipette tip. The cells were washed three times with PBS and 
incubated with UNC1999 (100 µM) at 37°C for 24 h. The 
wound areas were subsequently quantified using a microscope.

**Cell migration assays.** A total of 5x10³ E-J and 5637 cells were 
plated in the upper chambers of Transwell plates (24 wells; 
8 µm pore size with polycarbonate membrane; Corning Inc., 
Corning, NY, USA) in 100 µl serum-free RPMI 1640 medium. 
RPMI 1640 medium containing 10% FBS and UNC1999 
(100 µM) was plated in the lower chambers. Following incu-
batation at 37°C for 24 h, the migratory cells were fixed with 
methanol at room temperature for 6 h and stained with 0.2% 
crystal violet (Beyotime Institute of Biotechnology). The 
numbers of stained cells in five randomly selected fields were 
counted using a light microscope (magnification, x400; Leica 
Microsystems, Ltd., Milton Keynes, UK).

**Immunohistochemistry.** The expression of EZH2 was 
assessed by immunohistochemical staining. The bladder 
cancer tissues and para-carcinoma tissues were cut into 
4-µm thick sections. Endogenous peroxidase activity was 
inhibited with 3% hydrogen peroxide at 37°C for 10 min. The 
sections were subsequently treated with 1:50 normal horse 
serum (cat. no. I6050130; Thermo Fisher Scientific, Inc.) in 
tris-buffered saline for 30 min at 37°C. Tissue sections were 
incubated with primary antibody directed against EZH2 
overnight at 4°C. The sections were subsequently washed 
three times with PBS. Sections were incubated with a 
horseradish peroxidase-labeled secondary antibody for 30 min 
at 20°C. The sections were incubated with 3,3'-diaminobenzi-
dine for 2 min at 20°C. Tissues were observed under a light 
microscope (magnification, x400) and images were captured. 
Immunostained sections were evaluated by two experienced 
pathologists under blinded conditions.

**Reverse transcription-semi-quantitative polymerase chain 
reaction (RT-qPCR).** RT-qPCR was used to assess the EZH2 
mRNA level in clinical specimens. Total RNA was extracted 
from the aforementioned clinical specimens and cells using 
TRizol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). EZH2 levels were assessed using a Roche LightCycler 
480 (Roche Diagnostics GmbH, Mannheim, Germany) and
BeyoFast™ SYBR Green qPCR Mix kit (cat. no. D7260; Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's protocols. GAPDH was used as an endogenous reference gene to analyse the relative gene expression levels. The thermocycling conditions were as follows: 1 cycle of 94˚C for 3 min, followed by 35 cycles of 94˚C for 5 sec, 56˚C for 30 sec and 72˚C for 30 sec, and a final extension at 60˚C for 30 sec. The quantification cycle fluorescence value (Cq) was calculated using SDS software (version 2.1; Applied Biosystems; Thermo Fisher Scientific, Inc.). The expression levels were analysed according to the 2^−ΔΔCq method (21). All experiments were performed in triplicate. The appearance of a single peak in the melting curve implicated the specificity of the PCR products. The primer sequences were as follows: EZH2 forward, 5'-GAC CCT GAC CTC TGT CTT ACT T-3', and reverse, 5'-GAT GGT GCC AGGCAA TAG ATG-3'; and GAPDH forward, 5'-AGG TCG GTG TGA ACG GAT TTG-3', and reverse, 5'-TGT AGA CCA TGT AGT TGA GGT CA-3'.

Figure 1. EZH2 levels in bladder cancer. Immunohistochemical analysis of EZH2 in (A) normal bladder tissues, (B) low expression and (C) high expression bladder tumor tissues (magnification, x400). (D) Reverse transcription-semi-quantitative polymerase chain reaction analysis of the expression level of EZH2 in human bladder cancer tissues from 34 patients. (E) Western blot analysis of the expression level of EZH2 in human bladder cancer tissues. (F) Quantification of protein bands in human bladder cancer tissues. *P<0.01 vs. normal group. EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit.

Flank xenograft model. The Institutional Animal Care and Use Committee of Wuhan University approved the experimental protocols and supervised the care of animals and experimental procedures. Nude mice (Balb/c nu/nu) were purchased from the Animal Center of Wuhan University. BALB/c nude mice (n=10; male) were used for in vivo experiments. The mice were bred and experiments were performed in laminar flow cabinets under specific-pathogen-free conditions in the Laboratory Animal Center of Renmin Hospital of Wuhan University. Nude mice were kept at the College Laboratory Animal Center of Conventional Breeding at a temperature of 25-27˚C with a relative humidity of 45-50%, in an aseptic environment, with adequate illumination (12-h light/dark cycle), moisture and feed. The mice had free access to food and water. Preliminary in vivo experiments revealed that the tumorigenic effect of E-J cells was better than that of 5637 cells; therefore, E-J cells were used in the in vivo experiments. A total of 5x10^6 E-J cells were inoculated in the flank region of six-week-old nude mice (weight, 16-20 g). Two perpendicular diameters (a, the largest; b, the smallest) of the tumor were measured once per week with calipers for 5 weeks of monitoring. Tumor volume (V) was calculated using the following formula: V=a x b^2 x 0.5 (22). UNC1999 (50 mg/kg) and saline in equal volumes were injected intraperitoneally twice per week when the mean tumor volume reached ~100 mm^3. Tumor growth rate was plotted and analyzed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA).
Table I. Clinicopathological features of the 34 patients with bladder cancer and their associations with EZH2 expression.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
<th>Low</th>
<th>High</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td>0.86</td>
</tr>
<tr>
<td>&gt;60</td>
<td>22</td>
<td>8</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.714</td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Histological stage</td>
<td></td>
<td></td>
<td></td>
<td>0.026&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ta-T1</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>T2-T4</td>
<td>20</td>
<td>4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>G1</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>G2-G3</td>
<td>19</td>
<td>2</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

P-values were calculated using a Fisher's exact test. <sup>2</sup>Statistically significant. The mean of the relative expression of EZH2 in tissues of all paired samples was used as the cut-off value to determine high and low expression groups. EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; Ta, non-invasive papillary carcinoma.

Results

Expression levels of EZH2 in bladder cancer. In order to investigate whether the expression level of EZH2 was associated with the progression of bladder cancer, the expression levels of EZH2 in bladder carcinoma tissue and adjacent non-neoplastic parenchyma were analyzed by immunohistochemical staining. The mean of the relative expression of EZH2 in tissues of all paired samples was used as the cut-off value to determine high and low expression groups. The expression levels of EZH2 were increased in tumor tissues compared with adjacent normal tissues (P<0.05; Fig. 1A-D). Similar results were obtained by western blot analysis (P<0.05; Fig. 1E, F). The tumor stage and grade were classified according to the TNM staging system of the UICC guidelines. The level of EZH2 expression in tumor tissues was significantly increased in the higher TNM stages (T2-T4) compared with the lower stages (Ta-T1; Table I). The EZH2 levels were associated with the tumor histological stage (P=0.026) and tumor histological grade (P<0.001). These results indicated that the expression level of EZH2 was closely associated with the progression of bladder cancer.

UNC1999 inhibits the proliferation and migration of the bladder cancer cell lines E-J and 5637. The MTT, apoptosis, wound-healing, and cell migration assays were used to investigate the effects of the EZH2 inhibitor UNC1999 on the proliferation and migration of bladder cancer cell lines E-J and 5637 (cells in the control groups were treated with an equal volume of DMSO; Fig. 2). The MTT assay revealed that treatment with UNC1999 inhibited the proliferation of the bladder cancer cell lines in a dose- and time-dependent manner (Fig. 2A and B). UNC1999 exhibited the greatest inhibitory effect at a final concentration of 100 µM and an incubation period of 72 and 120 h. The difference between the 72 and 120 h incubation periods was not statistically significant (P>0.05). Therefore, the bladder cancer cell lines were treated with 100 µM UNC1999 for 72 h in subsequent experiments. Apoptosis analysis revealed that UNC1999 induced significant apoptosis in E-J and 5637 cells (Fig. 2C-E).

Cell migration is a central process in the evolution and progression of tumors (23). The wound healing and Transwell migration assays were used to investigate the effects of UNC1999 on the cell migration of bladder cancer cell lines. E-J and 5637 cells treated with UNC1999 exhibited decreased migration compared with the control group which was treated with DMSO (P<0.05) in the wound healing assay (Fig. 2F and G). Similar results were obtained with the Transwell assay, where a fewer number of cells treated with UNC1999 migrated into the lower chamber compared with control cells (P<0.01; Fig. 2H and I). The data obtained from the apoptosis and migration assays suggested that UNC1999 reduced the proliferation and migration of the bladder cancer cell lines.

EZH2 inhibition suppresses bladder cancer via blocking of the JAK2/STAT3 signaling pathway. The JAK2/STAT3 signaling pathway controls the invasive and aggressive phenotype of cancer cells (24). The current study investigated the effect of EZH2 on the phosphorylation levels of JAK2 and STAT3, as the phosphorylation is required for the activity of...
Figure 2. Inhibition of EZH2 suppresses bladder cancer cell migration and viability. The viability of (A) E-J and (B) 5637 cells treated with UNC1999 was measured using the MTT assay. (C) Results of flow cytometry for E-J cells. (D) Results of flow cytometry for 5637 cells. (E) Quantitative analysis of the apoptotic cells. (F) The migration of bladder cancer cells treated with UNC1999. Magnification, x200. (G) The wound healing assay was used to measure the migration ability of bladder cancer cells treated with UNC1999. *P<0.01 vs. normal group (treated with DMSO); †P<0.05 vs. 24 H group.
the JAK2/STAT3 signaling pathway. Constitutive p-JAK2 and p-STAT3 levels were decreased in E-J and 5637 cells treated with UNC1999 compared with controls (P<0.05; Fig. 3). These data suggested that inhibition of EZH2 may regulate the activation of the JAK2/STAT3 signaling pathway in bladder cancer cell lines.

**UNC1999 inhibits bladder cancer growth in vivo.** To further explore the antitumor activity of UNC1999 in vivo, E-J tumor xenografts were analyzed. UNC1999 exhibited significant antitumor activity in nude mice bearing E-J tumor xenografts at a dose of 50 mg/kg (P<0.05; Fig. 4A). In order to examine the mechanism underlying the inhibition of tumor growth by UNC1999 in vivo, the expression levels of JAK2 and STAT3 were measured using western blotting. The expression levels of JAK2 and STAT3 were significantly decreased in the tumors treated with UNC1999 compared with the control tumors (P<0.05; Fig. 4B-E). These results suggested that UNC1999 inhibited tumor growth in vivo by inhibiting EZH2 and subsequent inhibition of the JAK2/STAT3 signaling pathway.

**Discussion**

Bladder cancer is a common cause of cancer-associated mortality worldwide (25). The majority of patients with bladder cancer are diagnosed at an advanced stage of the disease due to a lack of disease-specific makers in the early stages (26). Surgery and chemotherapy are currently the most effective treatments for bladder cancer; however, the prognosis remains poor in the advanced stages and the overall survival rate is
unsatisfactory (27). A number of oncogenes have been identified in recent years, but the molecular mechanisms underlying tumorigenesis of bladder cancer remain unknown (28). Therefore, the identification of novel molecular targets in bladder cancer may improve the diagnosis and prognosis of the disease.

EZH2 is a member of the polycomb repressive complex 2. EZH2, together with embryonic ectoderm development and SUZ12 polycomb repressive complex 2 subunit, catalyze the di- and tri-methylation of histone H3 lysine 27 (H3K27), which is essential for embryonic stem cell pluripotency and self-renewal (29). EZH2 is an adhesion protein expressed in a number of organs and tissues, and it can promote cancer metastasis (30). EZH2 has been reported to be a marker of the aggressive stages of prostate cancer (31). The ectopic overexpression of EZH2 can lead to the transformation of normal prostatic cells and the canceration of breast epithelium (32). EZH2 upregulation is a potential tumor biomarker and contributes to tumor progression; therefore, it is deemed to be an oncogene (33). A previous study suggested that EZH2 inhibition by small interfering RNA may decrease cancer cell proliferation, induce cancer cell apoptosis in vitro and decrease breast xenograft growth in vivo (34). Taken together, EZH2 may be a novel target for drug development for different types of cancer.

UNC1999 is a novel S-adenosyl-l-methionine-competitive EZH2 inhibitor that has demonstrated efficacy in different types of cancer, including leukemia, colon cancer and multiple myeloma (35). The results obtained in the present study indicated that the EZH2 inhibitor UNC1999 may reduce cancer cell proliferation and migration, induce bladder cancer cell apoptosis and contribute to the regression of bladder tumor xenografts in mice. Additionally, the present study revealed that EZH2 may be upregulated in bladder cancer and suggested that pharmacological inhibition of EZH2 may be a novel therapeutic strategy in bladder cancer. The increase of intratumoral EZH2 expression, which is associated with bladder cancer progression and poor prognosis, is indicated as an independent prognostic factor for the overall survival time in clinical patients (15,36,37). Lee et al (36) revealed a predictive value of high expression levels of EZH2 for prognosis in bladder tumors, and that the E2F transcription factor 1-EZH2-SUZ12 polycomb repressive complex 2 subunit-driven transcriptional events may regulate cancer aggressiveness and chemoresistance. This highlights the potential applications for intratumoral EZH2 immunostaining in future clinical prognostic stratification and therapeutic interventions.

JAK2 is a vital hormone signaling and intracellular mediator of cytokines (38). JAK2 can activate a series of downstream signaling pathways, including the STAT cascade (39,40). STAT3, a member of the STAT family, may be activated by phosphorylation at the tyrosine residue 705 to regulate the release of growth factors and cytokines in gastric cancer (41). The activation of the JAK2/STAT3 signaling pathway was associated with the growth, migration and metastasis of lung cancer cells (42). Lei et al (43) revealed that the activation of

Figure 4. UNC1999 inhibited growth of E-J xenografts and block the JAK2/STAT3 signaling pathway in vivo. (A) Graphs presenting the average tumor volumes of E-J xenografts treated with or without UNC1999. (B) The levels of EZH2, JAK2, STAT3 and their phosphorylated forms were analyzed by western blotting. The quantitative data of (C) EZH2, (D) JAK2 and (E) STAT3 and their phosphorylated forms in E-J xenografts treated with or without UNC1999. *P<0.01 vs. control group. EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; JAK2, janus kinase 2; p-, phosphorylated-; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3.
the JAK2/STAT3 signaling pathway may promote the proliferation and migration of cancer cells as well as inhibit apoptosis in renal cell carcinoma. The inhibition of JAK2/STAT3 phosphorylation in pancreatic cancer may reduce the proliferation of pancreatic cancer cells in vivo and in vitro (44). In the current study, the western blotting results revealed that the phosphorylation of the JAK2/STAT3 signaling pathway was associated with the proliferation of bladder cancer cells, and the downregulation of EZH2 expression levels was associated with low expression levels of p-JAK2 in vivo and in vitro. These results suggested that EZH2 activity may serve an important role in bladder cancer development.

In summary, the present study demonstrated that UNC1999 decreased the proliferation, and migration of bladder cancer cells and increased apoptosis by inhibiting the activity of EZH2. Furthermore, the inhibition of EZH2 activity was associated with inhibition of the JAK2/STAT3 signaling pathway. Thus, EZH2 may be a potential therapeutic target for the treatment of patients with bladder cancer.

The present study had a number of limitations. UNC1999 is the first orally bioavailable inhibitor that has high in vitro potency for wild-type and mutant EZH2 as well as EZH1. EZH1 is a H3K27 methyltransferase that shares 96% sequence identity with EZH2 in their respective catalytic domains (45). To the best of our knowledge, there are no previously published studies suggesting that EZH1 may be associated with tumor progression. Additionally, the current study did not investigate the mechanism of EZH1 in bladder cancer. Future experiments knocking out or silencing EZH2 may remove the potentially confounding results obtained by inhibiting EZH1. The results of these studies may be compared with the results obtained with or without UNC1999 inhibition. The current study suggested that the JAK2/STAT3 signaling pathway may serve a key role in the in the carcinogenic mechanism of EZH2 in bladder cancer. However, it is likely that several other pathways may be involved in the progression of bladder cancer. Long non-coding RNA, microRNA, circular RNA and exosomes may also be implicated. Future studies investigating other possible mechanisms underlying the progression of bladder cancer are required.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets produced during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

ZC, JG and LW conceived and designed the experiments. ZC and YD performed the experiments. XL, HC, MW, XW and XDW analyzed the data, contributed to the interpretation of results obtained and wrote the manuscript. JG edited the final draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of Renmin Hospital of Wuhan University (Wuhan, China), and written informed consent was obtained from all participants. The Institutional Animal Care and Use Committee of Wuhan University approved the experimental protocols and supervised the care of animals and experimental procedures.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.