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Jarid2 enhances the progression of bladder cancer through regulating PTEN/AKT signaling

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ABSTRACT

Aims: Jumonji AT-rich interactive domain 2 (Jarid2) is an interacting component of PRC2 which catalyzes methylation of H3K27 (H3K27me3) and causes the downregulation of PTEN. In the present study, we aimed to explore whether Jarid2 could interact with H3K27me3 to regulate PTEN expression in bladder cancer.

Main methods: Jarid2 expression in bladder cancer tissues and cells were determined by western blotting and RT-PCR. CCK-8, flow cytometry, transwell chamber and in vivo xenograft assays were performed to assess cell growth, apoptosis, migration and tumorigenesis, respectively. Chromatin immunoprecipitation (ChIP) assay was used to assess the methylation of PTEN.

Key findings: Jarid2 expression was increased in bladder cancer tissues and cells. Downregulation of Jarid2 with shRNA transfection obviously inhibited the proliferation, migration and tumorigenesis of bladder cancer T24 and HT-1376 cells and induced cell apoptosis. Jarid2 downregulation decreased the expression of p-AKT and increased PTEN expression. Besides, Jarid2 down-regulation repressed the epithelial-mesenchymal transition (EMT), whereas knockdown of PTEN impaired this effect. Moreover, upregulation of Jarid2 increased the combination of PTEN promoter and H3K27me3, and 5-aza-CdR rescued it. Meanwhile, 5-aza-CdR administration abolished Jarid2 roles in the promotion of EMT process and AKT activation, as well as the reduction of PTEN expression.

Significance: Overall, the present study elaborated that Jarid2 facilitated the progression of bladder cancer through H3K27me3-mediated PTEN downregulation and AKT activation, which might provide a new mechanism for Jarid2 in promoting bladder cancer progression.

1. Introduction

Bladder cancer is the sixth most common malignancy and one of the most widespread carcinomas worldwide [1,2]. In spite of only 25% of patients with bladder cancer are muscle-invasive bladder cancer (MIBC), they account for the majority of bladder cancer-related deaths [3]. Therefore, it is essential to characterize new regulatory mechanisms for bladder cancer.

Jumonji AT-rich interactive domain 2 (Jarid2)-encoded protein belongs to the Jumonji family, which contains a DNA-binding domain named as the AT-rich interaction domain (ARID), a jumonji N (JmjN) domain, a zinc finger domain and a JmjC domain [4]. Except for the maintenance of embryonic stem cell pluripotency [5,6], Jarid2 was considered to be deregulated in many kinds of cancers and promoted their progression. For instance, Jarid2 was overexpressed in hepatocellular carcinoma (HCC) tissues and its upregulation facilitated the epithelial-mesenchymal transition (EMT) and promoted invasion of HCC cells through repressing phosphatase and tension homolog (PTEN)-induced AKT overactivation [7]. Knockdown of Jarid2 inhibited EMT induced by transforming growth factor-β (TGF-β) in colon cancer HT29 cells and lung cancer A549 cells [8]. In bladder cancer, Zhu et al. [9] reported that knockdown of Jarid2 impaired the invasive and sphere-forming abilities of bladder cancer cells, as well as reduced the population of tumor initiating cells, suggesting that Jarid2 plays an important role in bladder cancer progression.

It's well documented that PI3K/AKT signaling pathway plays a pivotal role in regulation of individual development, cell proliferation, apoptosis and differentiation. Hyper-activation of it is strongly implicated in carcinogenesis through enhancing cancer cell viability and migration and inhibiting cell apoptosis [10], including bladder cancer. For instance, Tsuruta et al. [11] demonstrated that PTEN, a tumor suppressor gene and a negative regulatory factor of PI3K/AKT signaling

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was significant downregulated in nearly 94% patients with advanced bladder cancer. Besides, Wu et al. [12] found that upregulation of PTEN with adenovirus vector transfection obviously repressed bladder cancer cell viability and promoted cell apoptosis. And AKT activation significantly reduced cell apoptosis in bladder cancer T24 cells [13]. In addition, it was identified recently that PTEN could be epigenetically regulated by the transcriptionally repressive mark H3 lysine 27 trimethylation (H3K27me3) which is catalyzed by the Enhancer of Zeste Homolog 2 (EZH2), the functional enzymatic component of the polycomb repressive complex 2 (PRC2) [14–16]. In addition, Jarid2 was reported to play an essential role in PRC2 combination with its targeted genes, leading to the downregulation of gene expression via H3K27me3 [6,17,18]. Up to data, whether Jarid2 could interact with H3K27me3 to modulate PTEN expression in bladder cancer remains unclear.

In this study, with the purpose of exploration of the molecular mechanism of Jarid2 in bladder cancer progression, we first investigated Jarid2 expression patterns in bladder cancer and determined its role in cell function; secondly, we studied whether Jarid2 regulated bladder cancer progression through PTEN/AKT signaling; finally, we explored the relationship between Jarid2 and H3K27me3 to further clarify the molecular mechanism of Jarid2 in regulation of PTEN.

2. Materials and methods

2.1. Tissue samples

Twenty matched human bladder cancer tissue samples and the adjacent non-tumor bladder cancer tissue samples were obtained from patients with bladder cancer. The para-carcinoma tissues were isolated from > 2.5 cm of the cancer tissues to avoid the contaminations in tissue specimens. All patients didn't accept any form of chemo-radiotherapy before surgical operation and each patient had signed the informed consent. The clinical information for these patients was listed in Table 1. This study was approved by the Human Research Committee of Zhejiang University and had been carried out in accordance with the Helsinki Declaration.

2.2. Cell culture

Human normal ureteral epithelial immortalized cell line SV-HUC-1 and human bladder cancer cell lines HT-1376, T24, and 5637 were all purchased from American Type Culture Collection (ATCC, USA). For cell culture, SV-HUC-1 cells were maintained in F-12 K medium (Gibco, USA), supplemented with 10% fetal bovine serum (FBS, Gibco, USA). HT-1376 cells were maintained in Eagle's Minimum Essential Medium (Gibco, USA) with 10% FBS supplement. 5637 cells were cultured in RPMI-1640 medium while T24 cells were cultured in McCoy's 5a medium (Gibco, USA), with the supplementation of 10% FBS. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

2.3. Cell transfection

Short hairpin RNAs (shRNAs) used to downregulate Jarid2 expression (sh-Jarid2; No. TL312055), and the overexpressing plasmid of Jarid2 (OE-Jarid2; No. SC309940), and the small interfering RNAs (siRNAs) used to downregulate PTEN (si-PTEN, No. SR321496), as well as their negative controls (NC) were all purchased from OriGene (Beijing, China). For cell transfection, OE-Jarid2, OE-NC, si-PTEN or si-NC were transfected to T24 and HT-1376 cells with lipofectamine 2000 regent (Invitrogen, CA, USA) according to the manufactory's description.

2.4. Western blotting analysis

Protein extraction from tissues and cells was performed using radioimmunoprecipitation buffer containing phosphatase and protease inhibitors (Solarbio, Beijing, China). After quantification, 30 μg proteins from each sample were loaded into the 10% SDS-PAGE and then transferred to PVDF membranes (Thermo Fisher Scientific, MA, USA). Next, 5% non-fat milk was used to block the membranes at room temperature for 1 h, followed by incubation with the indicated primary antibodies, including JARID2 (No. #13594, Cell Signaling Technology, CA, USA), E-cadherin (No. #14472, Cell Signaling Technology), N-cadherin (No. #4061, Cell Signaling Technology), Slug (No.#9585, Cell Signaling Technology), Snail (No.ab53519, Abcam, MA, USA), phosphorylated-protein kinase B (p-AKT, No. sc-52940, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), AKT (No. sc-5298, Santa Cruz Biotechnology, Inc.), PTEN (No. 51–2400, Thermo Fisher Scientific, MA, USA) and GAPDH (No. MA5–15738-BTIN, Thermo Fisher Scientific) overnight at 4 °C. Subsequently, the membranes were incubated with the corresponding secondary antibodies (AmyJet Scientific Inc., Wuhan, Hubei province, China) for 1 h at room temperature. Bound signaling was detectable with ECL regent (Millipore, MA, USA), and image-Pro Plus software (Media Cybernetics, Inc.,

Table 1
The clinical information for patients with bladder cancer.

	Gender	Age (years)	Lymph node metastasis	Depth of invasion (T)	Histological grade	TNM stage	Tumor size (cm)	histological type (s)
1	Male	62	No	Ta	N/A	0	1.2	SCC
2	Female	68	No	T1	High	I	0.6	UC
3	Male	79	No	T3	High	II	3.5	UC
4	Female	81	Yes	T4	High	III	4.9	UC
5	Male	69	No	T2	High	I	2.6	UC
6	Male	73	No	T3	High	IV	3.7	UC
7	Male	41	No	Ta	Low	I	2.9	UC
8	Female	77	Yes	T4	High	IV	4.1	UC
9	Male	52	No	T1	Low	I	1.2	UC
10	Female	69	No	T3	High	III	2.1	UC
11	Male	46	No	T1	High	II	0.9	UC
12	Female	83	No	T2	High	III	3.6	UC
13	Male	41	Yes	T3	High	IV	2.8	UC
14	Male	38	No	T1	High	I	1.6	UC
15	Male	74	Yes	T4	High	IV	4.7	UC
16	Female	48	No	T2	High	II	3.3	UC
17	Male	79	Yes	T4	High	IV	2.9	UC
18	Male	42	No	T2	High	I	1.6	UC
19	Male	53	No	T2	High	I	3.7	UC
20	Male	61	No	T1	High	0	0.7	UC

N/A: not applicable; SCC: squamous cell carcinoma; UC: urothelial carcinoma.

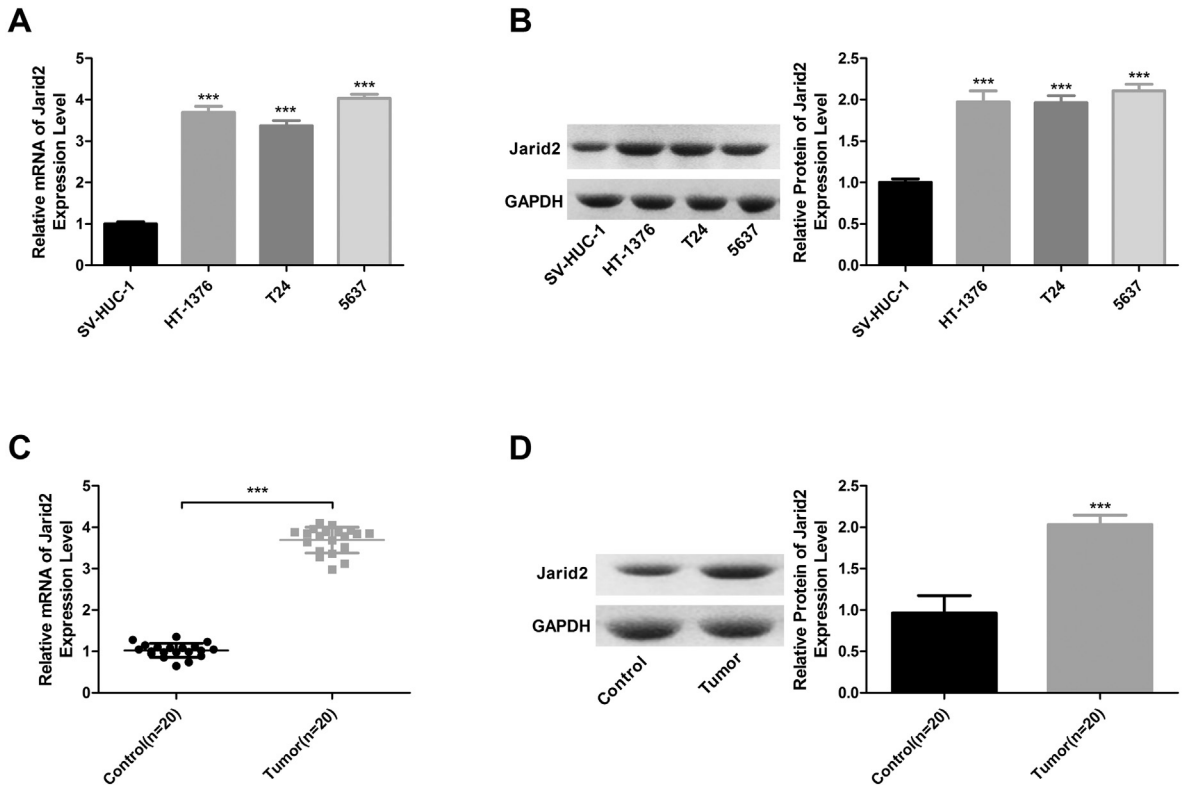

Fig. 1. Jarid2 was over-expressed in bladder cancer cells and tissues. (A-B) RT-PCR and western blotting analysis were carried out to test the mRNA and protein expression levels of Jarid2 in bladder cancer cell lines HT-1376, T24 and 5637 and normal bladder epithelial cell line SV-HUC-1. C-D. RT-PCR and western blotting analysis were used to test Jarid2 expression in 20 matched bladder cancer tissues and the adjacent normal tissues. (***P < 0.001).

Rockville, MD, USA) was used to quantify protein expression.

2.5. Quantitative real time PCR (RT-PCR)

TRIzol reagent (Thermo Fisher Scientific) was used to extract total RNA from tissue samples and cells. The total RNA was reversely transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, MA, USA). RT-PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SuperScript™ III Platinum™ SYBR™ Green One-Step qRT-PCR Kit (Thermo Fisher Scientific). The mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method. GAPDH was used as an internal reference to normalize Jarid2 expression. Primers were listed as follows: GAPDH: forward-5'-CTCCTGTTCGACAGTCAGCCG-3′, reverase-5′- CCTAGCCTCCCGGGTTTCTC-3′; Jarid2: forward-5′- GCTTCCCACCAGGATGACAG-3′, reverse-5′- TAGCTGGAGGGGGTAGCAAT-3′; PTEN-forward-5′- CTGCAGAAAGACTTGAAGGCG-3′, reverse-5′- GGGAATAGTTACTCCCTTTTTGTC-3′.

2.6. Chromatin immunoprecipitation (ChIP) assay

ChIP assay used to evaluate the interaction between H3K27me3 and PTEN promoter was performed as previously described [19]. The crosslinked chromatins were immunopreciptated with anti-H3K27me3 (No. ab6002, Abcam, MA, USA), anti-IgG (No. ab2410, Abcam, MA, USA) or anti-Jarid2 (No. ab178561, Abcam, MA, USA). The enrichment of the specific amplified region was analyzed by RT-PCR. Primers used to amplify PTEN promoter fragments are as follows: forward-5'-CCGTGCATTTCCCTCTACAC-3′ and reverse- 5′- GAGGCGAGGATAACGAGCTA-3′.

2.7. CCK-8 assay

T24 and HT1376 cells (2000 cells for each well) were seeded in 96-well plates and incubated at 37 °C overnight, then sh-NC or sh-Jarid2 were added to the culture medium. After 0, 1, 2, 3, 4 and 5 days of the treatment, 10 μL of cell counting kit-8 (CCK-8) regent (Sangon Biotech, Shanghai, China) were added into each well and incubated for another 4 h at 37 °C. Then, the optical density (OD) at 450 nm were examined using microplate reader.

2.8. Flow cytometry

After being treated with sh-NC or sh-Jarid2 for 48 h, T24 or HT-1376 cells were harvested and washed with PBS for one time. Then, Annexin V-FITC and PI solution were added to each sample for 15 min in the dark. The fluorescent signals reflecting cell apoptosis were evaluated by flow cytometry within 1 h. Cells in $FITC^-/PI^-$ quadrant were identified as living cells, $FITC^+/PI^-$ were early apoptotic cells and $FITC^+/PI^+$ were late apoptotic cells.

2.9. Transwell assay for cell migration

1×10^5 T24 or HT-1376 cells resuspended in 200 μL culture medium containing 1% FBS were seeded in the upper transwell chamber (BD Biosciences, USA). Meanwhile, 500 μL cell culture medium containing 10% FBS was added into the lower chamber in 24-well plates. After 24 h of incubated at 37 °C, T24 or HT-1376 cells were fixed with methanol for 10 min and incubated with 0.1% crystal violet for 8 min at room temperature. After wipe off cells attached in the upper chamber, the migrated cells were photographed with a microscope (Olympus IX73) and quantified by manual counting of five randomly selected fields.

2.10. In vivo xenograft assay

Ten male BALB/c nude mice aged 4–6 weeks old (18–23 g) obtained

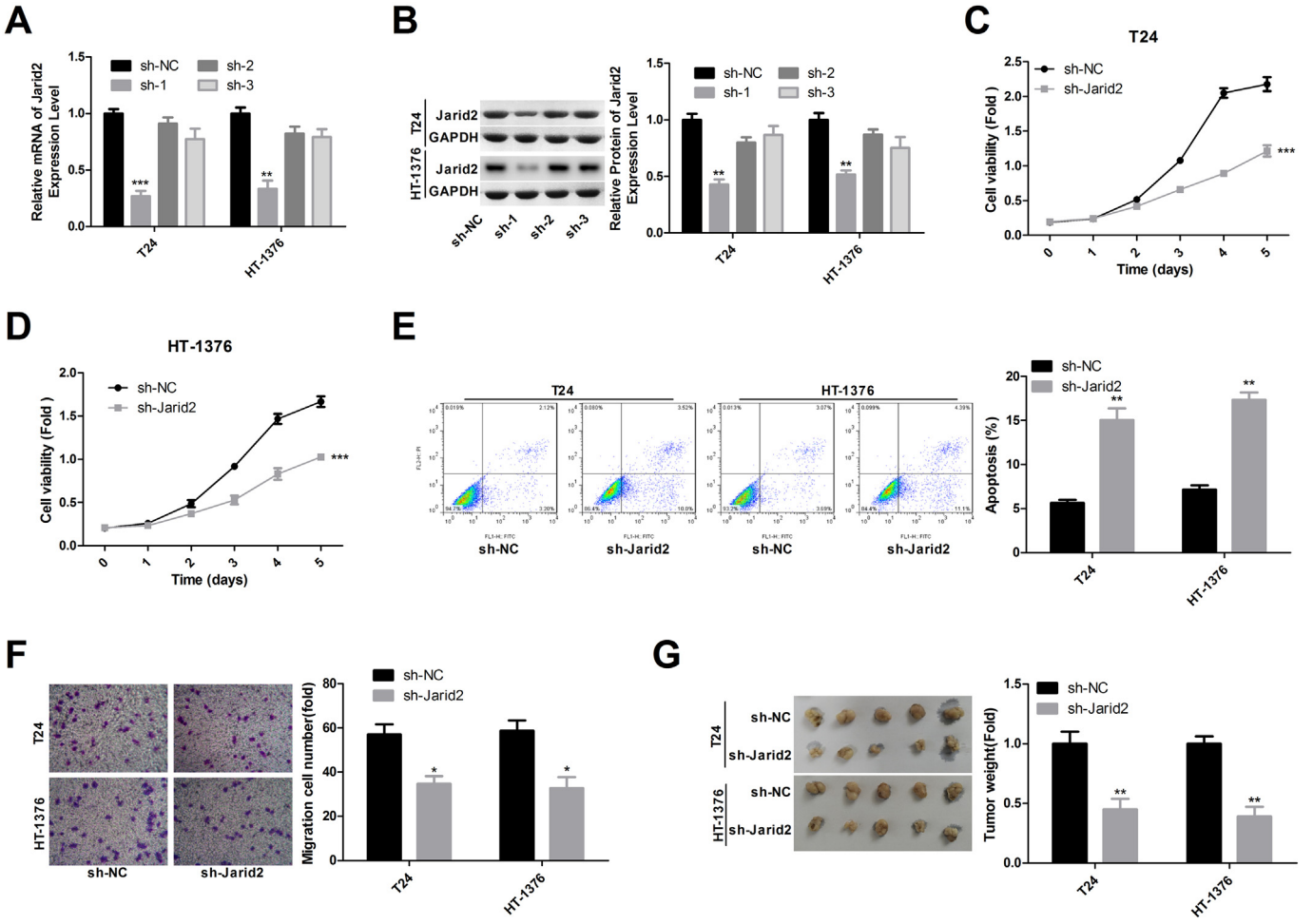

Fig. 2. Knockdown of Jarid2 repressed tumor growth in bladder cancer T24 and HT-1376 cells. A-B. The knockdown efficiency of sh-Jarid2 in mRNA and protein levels were determined by RT-PCR and western blotting, respectively. C-D. Cell proliferation ability was measured by CCK-8 regent after T24 or HT-1376 cells were transfected with sh-NC or sh-Jarid2 for the indicated times. E. After 48 h of cell transfection with sh-NC or sh-Jarid2, T24 and HT-1376 cells were collected for flow cytometry to detect cell apoptosis. F. Transwell migration assay was performed to assess cell migration ability after T24 or HT-1376 cells were transfected with sh-NC or sh-Jarid2. G. In vivo tumor-burdened experiments were used to evaluate the effects of Jarid2 downregulation in the tumorigenesis of T24 and HT-1376 cells. (***$P < 0.001$).

from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) were used for this study. All mice were housed in a temperature- and humidity-controlled room with food and water available and in a 12 h/12 h cycle. For the transplantation tumor experiment, T24 or HT-1376 cells infected with sh-NC or sh-Jarid2 were selected with puromycin (5 μg/mL) to form the stable transduced T24 or HT-1376 cells. Then, 5×10^6 stable transduced cells were resuspended in 200 μL PBS and were then injected subcutaneously into the left flank of mice (5 mice in each group). After 4 weeks, the mice were euthanized, and tumors were took out and weighed. This animal study was approved by the Institutional Animal Care and Use Committee of Zhejiang University.

2.11. Statistical analysis

Each experiment was performed at least three times and data were expressed as mean ± SD. Data comparison was carried out with SPSS20.0 software. Student's *t*-test or one-way analysis of variance (ANOVA) was used for statistical analysis, and $P < 0.05$ was thought to be statistically significant.

3. Results

3.1. Jarid2 is highly expressed in bladder cancer tissues and cells

To explore the function of Jarid2 in bladder cancer progression, we firstly detected its expression profiles in bladder cancer cells and tissues. Results showed that Jarid2 expression in bladder cancer cell lines HT-1376, T24 and 5637 were obviously higher than that in human ureteral epithelial immortalized cell line SV-HUC-1 at both mRNA and protein levels (Fig. 1A–B). Besides, we also detected Jarid2 expression in 20 paired bladder cancer tissues and the paracancerous normal bladder tissues. Consistently, Jarid2 expression was increased in bladder cancer tissues as compared to the adjacent normal tissues in both mRNA and protein levels (Fig. 1C–D). These findings suggested that the high expression of Jarid2 might play an important role in the carcinogenesis of bladder cancer

3.2. Knockdown of Jarid2 represses tumor growth in bladder cancer

Next, to explore the role of Jarid2 in bladder cancer development, we performed CCK-8, flow cytometry, transwell and tumor-burdened experiments to assess the effects of Jarid2 in bladder cancer cell proliferation, apoptosis, migration and tumorigenesis. Compared with sh-NC group, the expression of Jarid2 was significantly reduced by nearly

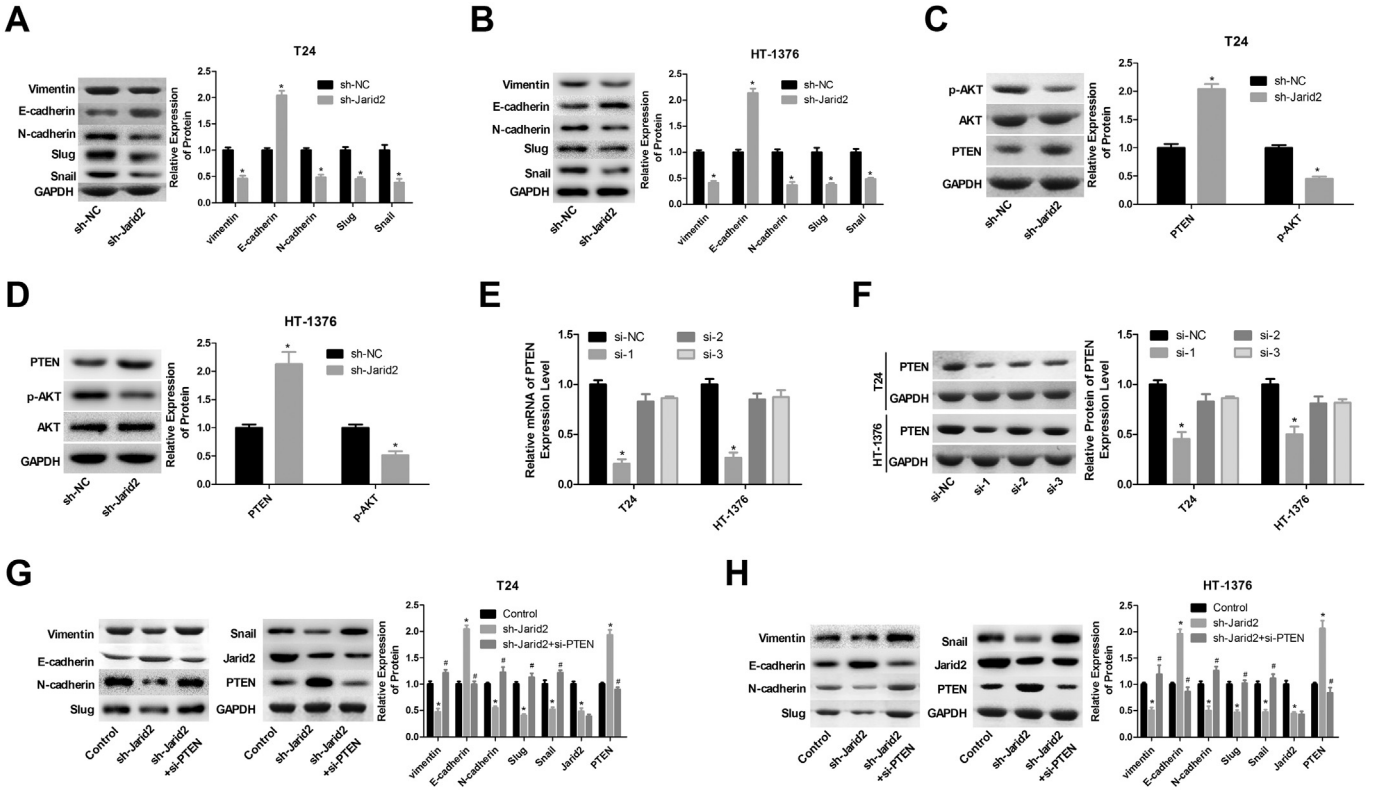

Fig. 3. Knockdown of Jarid2 inhibited EMT through regulation of PTEN/AKT signaling in bladder cancer. A-B. Western blotting was used to detect the protein levels of EMT-related proteins, such as Vimentin, N-cadherin, Slug, Snail and *E*-cadherin after T24 and HT-1376 cells were transfected with sh-Jarid2 or sh-NC (*$P < 0.05$). C-D. Western blotting was used to detect the protein levels of p-AKT, AKT and PTEN after T24 and HT-1376 cells were transfected with sh-Jarid2 or sh-NC (*$P < 0.05$). E-F. The knockdown efficiency of si-PTEN was detected by western blot and RT-PCR (*$P < 0.05$). G-H. The expressions of Vimentin, N-cadherin, Slug, Snail and E-cadherin proteins were examined by western blotting after 48 h of cell transfection. (*$P < 0.05$, compared to control group, $^{\#}P < 0.05$, compared to sh-Jarid2 group).

70% in mRNA level and 60% in protein level after T24 or HT-1376 cells were transfected with sh-Jarid2 (Fig. 2A–B). Cell proliferation (Fig. 2C–D), migration (Fig. 2F) and tumor formation (Fig. 2G) abilities were all obviously reduced, while cell apoptosis was enhanced (Fig. 2E) after Jarid2 was downregulated in T24 or HT-1376 cells, suggesting that Jarid2 served as an oncogene in the progression of bladder cancer.

3.3. Knockdown of Jarid2 inhibits EMT through regulation of PTEN/AKT signaling in bladder cancer

Next, we explored the effects of Jarid2 in EMT process and determined whether PTEN/AKT signaling was involved in Jarid2-mediated bladder cancer progression. Compared with the control group, knockdown of Jarid2 significantly decreased the expression levels of Vimentin, N-cadherin, Snail and Slug, while increased E-cadherin expression in both T24 (Fig. 3A) and HT-2376 cells (Fig. 3B), suggesting that downregulation of Jarid2 could hinder EMT process. Besides, knockdown of Jarid2 decreased the expression of p-AKT while increased PTEN expression (Fig. 3C–D). Then, we investigated the effects of PTEN in the progression of Jarid2-related EMT process in bladder cancer, the si-1 targeting PTEN significantly decreased PTEN expression in T24 and HT-1376 cells at both mRNA and protein levels (Fig. 3E–F). And downregulation of PTEN in T24 and HT-1376 cells significantly weakened sh-Jarid2 effects on E-cadherin expression increase and the decreased expression levels of Vimentin, N-cadherin, Slug and Snail (Fig. 3G–H). These results indicated that downregulation of Jarid2 intervened EMT process in bladder cancer through regulating PTEN/AKT pathway.

3.4. Downregulation of Jarid2 promotes PTEN expression through enhancing the combination of PTEN promoter with H3K27me3

Finally, we explored the relationship between Jarid2 and H3K27me3 to further clarify the molecular mechanism of Jarid2 in regulation of PTEN through gain−/loss-of-function assays. Transfection with OE-Jarid2 significantly increased Jarid2 expression in both mRNA and protein levels (Fig. 4A–B). CHIP assay showed that downregulation of Jarid2 induced a strong reduction in the combination between Jarid2 with PTEN promoter region (Fig. 4C–D) and a reduction in H3K27me3 levels at PTEN promoter region and vice versa (Fig. 4E–F). To further explore the effects of methylation in PTEN expression, we recruited 5-aza-CdR, a demethylate reagent. 5-aza-CdR treatment decreased the binding of PTEN promoter and H3K27me3 (Fig. 4G–H), and increased PTEN expression in both mRNA and protein levels (Fig. 4I–J). In addition, 5-aza-CdR significantly blunted OE-Jarid2 roles in the promotion of the binding of PTEN promoter with H3K27me3 and the reduction of PTEN expression (Fig. 4G–J). Moreover, 5-aza-CdR administration abolished Jarid2-mediated the increased expression levels of p-AKT, Vimentin, N-cadherin, Slug and Snail and the reduced expression of E-cadherin in T24 (Fig. 4K) and HT-1376 cells (Fig. 4L). These results illustrated that Jarid2 positively regulate EMT process in bladder cancer through H3K27me3-induced PTEN downregulation.

4. Discussion

It's reported that approximately 20% of patients with MIBC are associated with a strong propensity toward deadly metastases [20]. Due to the complex mechanisms of carcinogenesis, the specific molecular

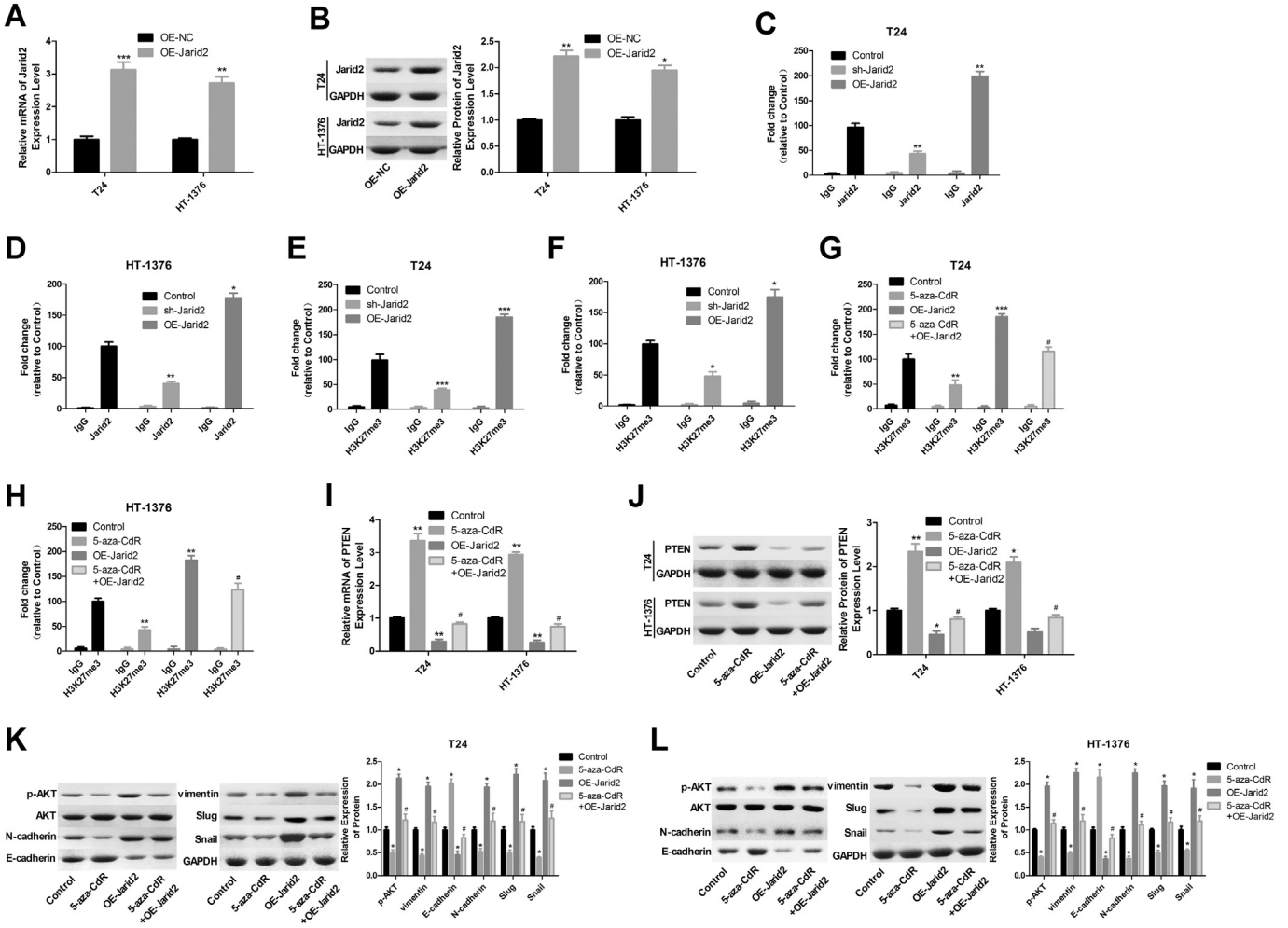

Fig. 4. Jarid2 reduced PTEN expression through enhancing its methylation with H3K27me3. A-B. Jarid2 expression was determined by RT-PCR and western blotting analysis after T24 and HT-1376 cells were transfected with OE-Jarid2 or OE-NC. C—F. CHIP assay was performed to analyze the interaction of H3K27me3/Jarid2 with PTEN promoter after T24 or HT-1376 cells were treated with control vector, OE-Jarid2 or sh-Jarid2. G-H. CHIP assay was performed to analyze the interaction of H3K27me3 protein with PTEN promoter after T24 or HT-1376 cells were treated with OE-Jarid2, 5-aza-CdR or 5-aza-CdR + OE-Jarid2. I-J. RT-PCR and western blotting analysis of the mRNA and protein expression of PTEN after T24 or HT-1376 cells were treated with OE-Jarid2, 5-aza-CdR or 5-aza-CdR + OE-Jarid2. K-L. Western blotting analysis of the protein levels of p-AKT, AKT, Vimentin, N-cadherin, Slug, Snail and E-cadherin after T24 or HT-1376 cells were treated with OE-Jarid2, 5-aza-CdR or 5-aza-CdR + OE-Jarid2. ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to control group; $^{\#}P < 0.05$, compared to OE-Jarid2 group).

mechanisms underlying the migration of bladder cancer are not completely understood till now. Recently, Zhu et al. [9] found that ectopic upregulation of Jarid2 enhanced the invasive and sphere-forming abilities of bladder cancer cells through modulation of p16, suggesting that Jarid2 functioned as an oncogene in bladder cancer. In the present study, we focused on the effects and mechanisms underlying Jarid2 on bladder cancer metastases. We found that Jarid2 was overexpressed in bladder cancer tissue samples and cell lines, and knockdown of Jarid2 with shRNA significantly inhibited cell proliferation, migration and tumorigenesis and promoted cell apoptosis in bladder cancer T24 and HT-1376 cells, suggesting that Jarid2 might be a putative novel therapeutic target for inhibiting the metastases of malignant bladder cancer. Up to now, JARID2 has been reported to play different roles in various cancers. Jarid2 serves as an oncogene in majority tumors including bladder cancer [9], lung and colon cancer [8], ovarian cancer [21], HCC [7] and glioma [22]. However, Jarid2 functions as a tumor suppressive gene in leukemia, in which Jarid2 was identified to be downregulated in B-chronic lymphocytic leukemia and acute monocytic leukemia, and knockdown of it significantly promoted cell proliferation and ectopic overexpression of it inhibited cell malignant phenotypes [23].

Increasing evidence has confirmed that EMT process shows high correlation with cancer cell migration and invasion through transforming polarized and adherent epithelial cells into motile and invasive mesenchymal cells [24,25]. In this regard, we explored the functions of Jarid2 in EMT to further explore the mechanism of Jarid2 in regulation of bladder cancer metastases. The expressions of Vimentin, Slug, Snail and N-cadherin, markers of mesenchymal cells, were significantly decreased when Jarid2 was downregulated in bladder cancer T24 and HT-1376 cells while the expression of E-cadherin, marker of epithelial cells was elevated as compared with the control group, suggesting that knockdown of Jarid2 possessed the ability to repress EMT in bladder cancer. Our results were consistent with previous studies [7,8].

To study the mechanisms underlying Jarid2 in bladder cancer, we carried out western blot to assess the effects of Jarid2 in the activation of PTEN/AKT signaling. Downregulation of Jarid2 significantly decreased the phosphorylation of AKT protein and increased PTEN expression, and the inhibition of EMT induced by Jarid2 downregulation was abolished when PTEN was downregulated, suggesting that knockdown of Jarid2 repressed EMT process through PTEN-mediated AKT signaling inactivation. Similarly, PTEN/AKT pathway was reported to be strongly implicated in the EMT process of multiple kinds of cancers [26,27].

Histone methylation is an important process of chromatin-based

modifications that modulates many cellular processes such as DNA repair, DNA replication, and gene transcription, while histone demethylase gene modulates gene expression via removing the methyl marks on histone tails to either facilitate or repress gene transcription [28]. And PRC2 is identified as an important regulator of histone methylation [29,30]. As a component element of PRC2, Jarid2 also plays a vital role in DNA methylated modification to regulate gene expression [31,32]. In this study, we demonstrated that knockdown of Jarid2 caused an obvious reduction in the combination between Jarid2 and PTEN promoter region, as well as a reduction in the combination of H3K27me3 in PTEN promoter region, suggesting that Jarid2 was indispensable for H3K27me3 modification of PTEN and subsequently downregulated PTEN expression and promoted EMT process in bladder cancer. This result is in accordance with a previous studyin HCC [7]. Moreover, to verify our speculation, we used 5-aza-CdR to demethylate of PTEN and the results showed that PTEN expression was significantly increased while AKT activation and EMT process were repressed, which further confirmed that Jarid2 promoted AKT activation and EMT through H3K27me3 modification to PTEN promoter to inhibit its expression.

In conclusion, this study demonstrated another molecular mechanism of Jarid2 in bladder cancer process, in which Jarid2 was high expression and downregulation of it could obviously inhibit cancer cell EMT process through the inhibition of AKT activation via increasing the H3K27me3 modification of PTEN promoter.

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Declaration of Competing Interest

The authors declare that there were no conflict of interest.

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