Regulation of Drp1 and enhancement of mitochondrial fission by the deubiquitinating enzyme PSMD14 facilitates the proliferation of bladder cancer cells

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Abstract. The protein Dynein-related protein 1 (Drp1) plays a crucial role in regulating the process of mitochondrial fission, which is known to be associated with the onset and progression of various human diseases. However, the specific impact of Drp1 on bladder cancer has yet to be fully understood. In previous studies, evidence to support the theory that the deubiquitinating enzyme proteasome non-ATPase regulatory subunit 14 (PSMD14) is responsible for stabilizing and promoting the activity of Drp1, ultimately resulting in increased mitochondrial fission, has been presented. The levels of PSMD14 in both bladder cancer tissues and cells were elevated, as confirmed through immunohistochemical and immunofluorescent staining. Co-immunoprecipitation and reciprocal co-IP tests demonstrated that PSMD14 and Drp1 interacted with each other. Upon knockdown of PSMD14, there was a corresponding decrease in Drp1 expression and subsequent inhibition of mitochondrial fission. However, when the Drp1 agonist Mdivi-1 was applied to cells where PSMD14 expression had been knocked down, a significant increase in cell growth was observed, partially restoring the cancer-promoting effects of PSMD14 on cell proliferation. In conclusion, these findings suggest that PSMD14 may stimulate bladder cancer cell proliferation by promoting mitochondrial fission through the stabilization of Drp1.

Introduction

Mitochondria provides energy for almost all cellular activities, thereby maintaining the cell's physiological functions (1). In addition, mitochondria play a vital role in cell physiological processes such as cell growth, cell division, innate immunity, calcium homeostasis, stem cell reprogramming, energy metabolism, and apoptosis (1,2). Mitochondria can adjust their tubular network through morphological changes, and fission and fusion affect the size and number of mitochondria, which is termed mitochondrial dynamics (3).

Mitochondrial fission is primarily driven by Dynein-related protein 1 (Drp1) (4), which is located in the cytoplasm under physiological conditions. The activated Drp1 translocates to the outer mitochondrial membrane, where it binds to its mitochondrial adaptors (including FIS1 and MFF) to promote fission events (5). In certain cases, cancer cells exhibit high levels of fission mitochondrial fragmentation and enhanced function, which leads to tumor growth and the development of chemoresistance (6). The activation and upregulation of Drp1 have been observed in various types of cancer, such as lung cancer, breast cancer, glioblastoma, colorectal cancer, pancreatic cancer, thyroid tumor, nasopharyngeal carcinoma, and melanoma (7-13). Previous studies have found that the loss of Drp1 leads to prolongation of the mitochondrial network, inhibition of cell proliferation, and induction of spontaneous apoptosis in a variety of cancer cells (6,14). However, the role of Drp1 in bladder cancer remains unclear.

The ubiquitin-proteasome pathway is the primary mechanism of protein catabolism in the cytoplasm and nucleus of mammals and is involved in the degradation of >80% of the proteins in the cell (15). The deubiquitinating enzyme (DUB) catalyzes the process of removing ubiquitin from ubiquitinated substrates, which is termed deubiquitylation (16). Due to the wide range of substrates, deubiquitylation plays an important role in a variety of cellular functions. Previous studies have reported that deubiquitylation dysregulation may the induction of various diseases, including cancer (17,18). As a subunit of the 19S regulatory particle in the 26S proteasome, the 26S proteasome non-ATPase regulatory subunit 14 (PSMD14) belongs to the JAB1/MPN+/MOV34 (JAMM) domain protease family of DUB proteins and has been reported to be involved in the occurrence and development of various types of cancer (19). Downregulation of PSMD14 can induce G0/G1 phase arrest, and apoptosis, and reduce the proliferation and epithelial-mesenchymal transition (EMT) of lung adenocarcinoma,

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prostate cancer, and breast cancer cells (20-24). A previous study found that PSMD14 exerts carcinogenic effects in head and neck squamous cell carcinoma and glioma by inhibiting the ubiquitination and degradation of cancer-related transcription factor E2F1 (25,26). PSMD14 also promotes EMT in esophageal squamous cell carcinoma by targeting SNAIL for deubiquitylation and stabilization (27). Therefore, PSMD14 acts as an oncogene, deubiquitinating a variety of protein substrates. However, the function and mechanism of PSMD14 in bladder cancer remain to be explored.

In the present study, it was found that PSMD14 functions upstream of Drp1 and is upregulated in patients with bladder cancer, which indicated a potential mechanism of PSMD14 and mitochondrial fission in cancer. The results of the present study revealed the importance of PSMD14 in tumorigenesis and a novel treatment strategy to attenuate mitochondrial fission by inhibiting the PSMD14-Drp1 pathway.

Materials and methods

Clinical tissues. This study was approved by the Institutional Ethics Committee of the Hunan Provincial People's Hospital and in accordance with the Ethical Management Guidelines of Hunan Provincial People's Hospital (approval no. 210139). A total of 32 bladder cancer tissue specimens and matched normal specimens were collected from Hunan Provincial People's Hospital. Informed written consent was obtained from all the participants.

Cell culture. T24 and 5637 cells (American Type Culture Collection) were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS, 100 mg/ml streptomycin, and 100 units of penicillin. All the cells were cultured in a humidified incubator at 37˚C, supplied with 5% CO_2, 21% O_2, and 74% N_2.

siRNA transfections. Cells were first seeded in six-well plates and cultured until they reached 30-50% confluence. siRNAs were introduced to the cells through transfection. Cells were transfected with Lipofectamine® 3000 Transfection Reagent (Beyotime Institute of Biotechnology). For the present study, six groups of cells were used: i) Lipofectamine® 3000 Transfection Reagent alone; ii) Lipofectamine® 3000 Transfection Reagent combined with 50 nM/μl NC siRNA; iii) Lipofectamine® 3000 Transfection Reagent combined with 50 nM/μl si-PSMD14 (5'-AAGGTTGTT ATTGATGCCTTCAGAT-3'); iv) Lipofectamine® 3000 Transfection Reagent combined with 50 nM/μl si-NC for PSMD14 (5'-AAGTTGTGTTACGTACTTCATGGAT-3'); v) Lipofectamine® 3000 Transfection Reagent combined with 50 nM/μl si-Drp1 (5'-AAGTGGTGACTTGTCTTCTTC GTAA-3'); vi) Lipofectamine® 3000 Transfection Reagent combined with or 50 nM/μl si-NC for DRP1 (5'-AAGAGT GGTTCTTCTCTTCGGTTAA-3'). Cells were transfected for 24 h before subsequent experiments. Drp1 agonist Mdivi-1 (50 mg/ml; Beyotime Institute of Biotechnology) was also used to manipulate Drp expression. The manufacturer's instructions were followed during the transfection process, and the efficiency of the transfection was confirmed using qPCR.

Immunohistochemical staining. The bladder tissue sections were treated first by removing the paraffin using xylene and ethanol, and then performing antigen retrieval. Following this, tissues were blocked using 3% BSA and then stained using two different types of antibodies anti-PSMD14 (cat. no. 4197S; Cell Signaling Technology, Inc.; 1:100) or anti-Drp1 (cat. no. 8570S; Cell Signaling Technology, Inc.; 1:100) at 4˚C overnight. To ensure the reliability of the results, a control was also included by using mouse serum at the same protein concentration as the antibody solution. Under a light microscope at an x100 magnification, the positively stained tissue sections could be seen as appearing brown or tawny-brown in color. Images of the tissue were taken using an optical microscope, and the integrated optical density (IOD) values of the tissue sections were then measured using Image-Pro Plus version 6.0 (Media Cybernetics, Inc.). To ensure accuracy, five different fields of view were selected from each tissue section, and three tissue sections were used for each group to determine the positive IOD values. The mean IOD values were then used to calculate the relative expressions of PSMD14 and Drp1.

Immunofluorescent staining. Cells that had been grown on slides were fixed with 4% paraformaldehyde solution for 15 min at room temperature, and then permeabilized with 0.1% Triton X-100 at room temperature for 15 min. The samples were washed three times with PBS and then sealed with a 5% BSA solution at room temperature for 45 min. Next, the primary antibody was incubated with the sample overnight at 4˚C. The primary antibodies used were: Anti-PSMD14 (cat. no. 4197S; 1:200; Cell Signaling Technology, Inc.), anti-Drp1 (cat. no. 8570S; 1:200; Cell Signaling Technology, Inc.), or anti-TOM20 (cat. no. 42406S; 1:200; Cell Signaling Technology, Inc.). DAPI (cat. no. 4083S; Cell Signaling Technology, Inc. 1:200) was used to counterstain the nuclei. The secondary antibodies included Alexa FluoR 555 goat anti-rabbit (cat. no. A27017; 1:400; Invitrogen; Thermo Fisher Scientific, Inc.). A total of five different fields of view were selected and randomly scored using a fluorescence microscope (magnification, x40, Olympus Corporation).

Western blotting. Cells were collected and lysed using RIPA buffer (Beyotime Institute of Biotechnology) for 30 min at 4˚C. The nuclear protein was collected using cytoplasmic and nuclear extraction kit (cat. no. A37487; Invitrogen; Thermo Fisher Scientific, Inc.). The protein lysates were quantified by BCA Protein Assay Kit (Beyotime Institute of Biotechnology). SDS gels (8-12%) were used for SDS-PAGE. The resolved proteins were then transferred to PVDF. To block the membranes, a 10% BSA solution was dissolved in TBS buffer containing 0.05% Tween 20 and added to the membranes at room temperature for 1 h. The primary antibody was then added to TBS containing 0.05% Tween 20 and incubated for at least 10 h at 4˚C. The primary antibodies used were anti-PSMD14 (cat. no. 4197S; Cell Signaling Technology, Inc.; 1:1,000) and anti-Drp1 (cat. no. 8570S; Cell Signaling Technology, Inc.; 1:1,000). To ensure accuracy, the protein content was normalized using GAPDH (cat. no. 5174S; Cell Signaling Technology, Inc.; 1:8,000) as the

internal control. After washing the membrane three times, the anti-rabbit second antibody (cat. no. 7074S; Cell Signaling Technology, Inc.; 1:8,000) and anti-mouse mice secondary antibody (cat. no. 7076S; Cell Signaling Technology, Inc.; 1:8,000) in TBS containing 0.05% Tween 20 were used at room temperature for 1 h. After three additional washes, the bands were detected by enhanced chemiluminescence and autoradiography. The experiments were repeated three times, and the results were evaluated using Image Lab 6.0 (Bio-Rad Laboratories, Inc.). Drp1 agonist Mdivi-1 (50 mg/ml; Beyotime Institute of Biotechnology) was also used to manipulate the expression of Drp1. The blots were grouped and cropped from the same gel.

RNA extraction and qPCR. Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and RNA purity was assessed using a spectrophotometer. First-strand cDNA was synthesized using a cDNA synthesis kit (Promega Corporation) at 65˚C for 5 min according to the manufacturer's protocol. Subsequently, cDNA was amplified by qPCR using an Applied Biosystems SYBR Green mix kit and the ABI 7900 Real-Time PCR system (both Applied Biosystems; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. qPCR was performed as follows: Denaturation at 94˚C for 4 min; followed by 40 cycles of amplification at 94˚C for 30 sec, hybridization at 56˚C for 30 sec, and extension at 72˚C for 30 sec. Quantification of mRNA was automatically conducted using SDS version 1.3 software (Applied Biosystems; Thermo Fisher Scientific, Inc.), and the $2^{-\Delta\Delta Cq}$ method was employed to calculate mRNA expression (28,29). The sequences of the primers used were: PSMD14 forward, 5'-GTCAGTGTGGAGGCAGTTGATC-3', and reverse, 5'-CCACACCAGAAAGCCAACAACC-3'; DRP1 forward, 5'-TCACCCGGAGACCTCTCATTC-3' and reverse, 5'-GGTTCAGGGCTTACTCCCTTAT-3', and β-actin forward, 5'-CGAGCGCGGCTACAGCTT-3' and reverse, 5'-TCCTTAATGTCACGCACGATTT-3'.

Cell viability assay. T24 and 5637 cells in the cell culture flask were digested with 0.25% trypsin. Then these cells were seeded in a 96-well culture plate at a density of $1x10^4$ cells/ml for 24 h. After 24 h of incubation, 10 μl CCK-8 reagent was added to each well for 2 h. The optical density was measured at a wavelength of 450 nm using a Bio-Rad 680 microplate reader. Each experiment was performed in triplicates.

In vitro ubiquitination assay. Cells were transfected with si-PSMD14. After transfection for 48 h, the cells were treated with MG132 (15 μm; Beyotime Institute of Biotechnology) overnight and then lysed with RIPA lysis buffer, follow by passing 5 times through a 21-gauge needle. Whole-cell extract (1 mg) was used for immunoprecipitation with 1 μg anti-PSMD14 (cat. no. 4197S; Cell Signaling Technology, Inc.) or anti-Drp1 (cat. no. 8570S; Cell Signaling Technology, Inc.). The proteins that had been pulled down were subjected to SDS/PAGE for immunoblot analysis.

Statistical analysis. Each experiment was repeated at least three times. The categorical data were assessed using a Fisher's exact test, and the quantitative data are presented as the mean ± SEM. All statistical analyses were performed using SPSS version 19.0 (IBM Corp.). The differences between groups were compared using a Student's t-test or ANOVA followed by a post hoc Dunnett's test or Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Drp1 and PSMD14 expression are upregulated in bladder cancer. To reveal the potential function of PSMD14 in cancer, immunohistochemical staining and western blotting were used to determine the expression of Drp1 and PSMD14 in bladder cancer tissues, and it was found that compared with normal tissues, Drp1 and PSMD14 expression were abnormally upregulated in bladder cancer tissues (Fig. 1). These results suggest that Drp1 and PSMD14 may play important roles in the occurrence and development of bladder cancer.

Knockdown of PSMD14 inhibits the growth of bladder cancer cells. The expression of PSMD14 of T24 and 5637 cells transfected with PSMD14 siRNA was detected by western blotting (Fig. 2A). Then cell viability assay and colony formation assays were used to detect the proliferation and colony formation of the transfected bladder cancer cells compared with the respective control cells. The results showed that the knockdown of PSMD14 significantly reduced the growth and proliferation capacity of T24 and 5637 cells (Fig. 2B and C). Mitochondrial fusion in the bladder cancer cells was assessed to explore the relationship between PSMD14 and mitochondrial dynamics. PSMD14 knockdown did not affect the expression of mitochondrial fusion factors (OPA1, Mfn1, and Mfn2) by immunofluorescent staining, and the levels of mitochondrial factor-Drp1 were downregulated by PSMD14 knockdown (Fig. 2D). Drp1 induced mitochondria to produce several fragments, and PSMD14 knockdown blocked this process (Fig. 2E).

The interaction mode between PSMD14 and Drp1. Next, whether endogenous Drp1 interacted with endogenous PSMD14 was assessed. Endogenous Drp1 and PSMD14 proteins were co-immunoprecipitated (Fig. 3A), and this finding was confirmed through reciprocal co-IP test (Fig. 3B). Then, si-PSMD14 transfected T24 and 5637 cells were treated with exogenous PSMD14. The results showed that the PSMD14 resulted in an increase in Drp1 in T24 and 5637 cells (Fig. 4A and B).

Knockdown of Drp1 inhibits the growth of bladder cancer cells. The results of western blotting results showed that Drp1 was significantly downregulated in T24 and 5637 cells transfected with si-Drp1 (Fig. 5A). Then cell viability and colony formation assays were used to detect the proliferation and colony formation ability of Drp1 knockdown bladder cancer cells. The results showed that the knockdown of Drp1 significantly reduced the growth and proliferation capacity of T24 and 5637 cells (Fig. 5B and C). Additionally, Drp1 knockdown significantly increased mitochondrial length and inhibited mitochondrial fission (Fig. 5D).

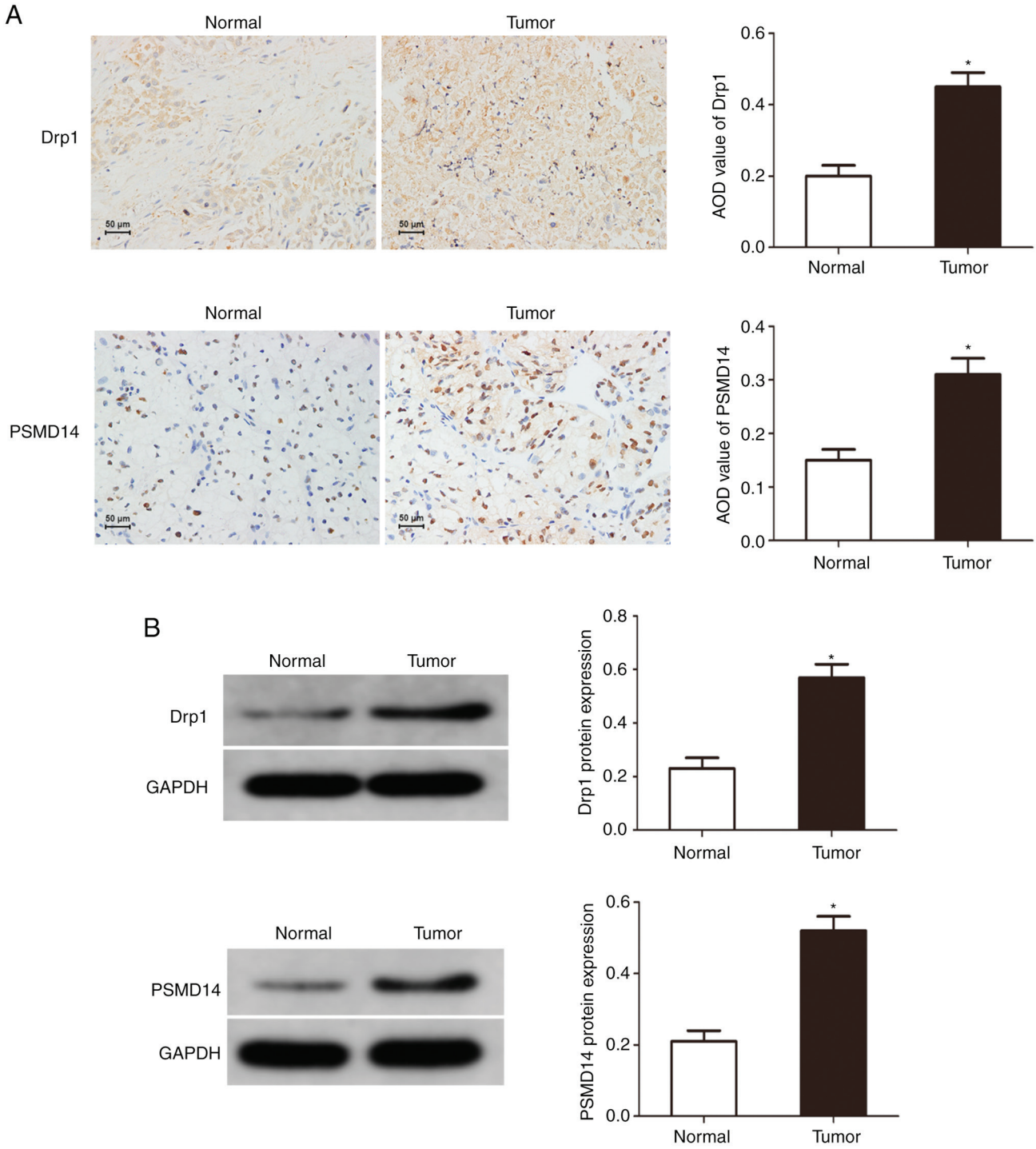

Figure 1. Drp1 and PSMD14 are upregulated in bladder cancer tissues. (A) Immunohistochemical staining of Drp1 and PSMD14 in human bladder cancer tissues. Scale bar, 50 μm. (B) Western blot analysis of Drp1 and PSMD14 expression in human bladder cancer tissues. *P<0.05 vs. normal group. Drp1, Dynein-related protein 1; PSMD14, proteasome non-ATPase regulatory subunit 14.

Upregulation of Drp1 partially restores the carcinogenic effects of PSMD14. The above results confirmed that Drp1 plays an important role in PSMD14-mediated bladder cancer. Next, whether the upregulation of Drp1 reversed the anti-cancer effects mediated by PSMD14 knockdown was assessed. Drp1 agonist Mdivi-1 (50 mg/ml) was used to upregulate Drp1 in bladder cancer cells (Fig. 6A). Then the proliferation of si-PSMD14 transfected bladder cancer cells treated with Mdivi-1 was determined. The results of the cell viability assays showed that Mdivi-1 significantly increased the growth of T24 and 5637 cells transfected with si-PSMD14 (Fig. 6B). Mdivi-1 treatment significantly reduced mitochondrial length and promoted mitochondrial fission (Fig. 6C).

Discussion

The ubiquitin-proteasome system is the primary mechanism of protein catabolism in the mammalian cytoplasm

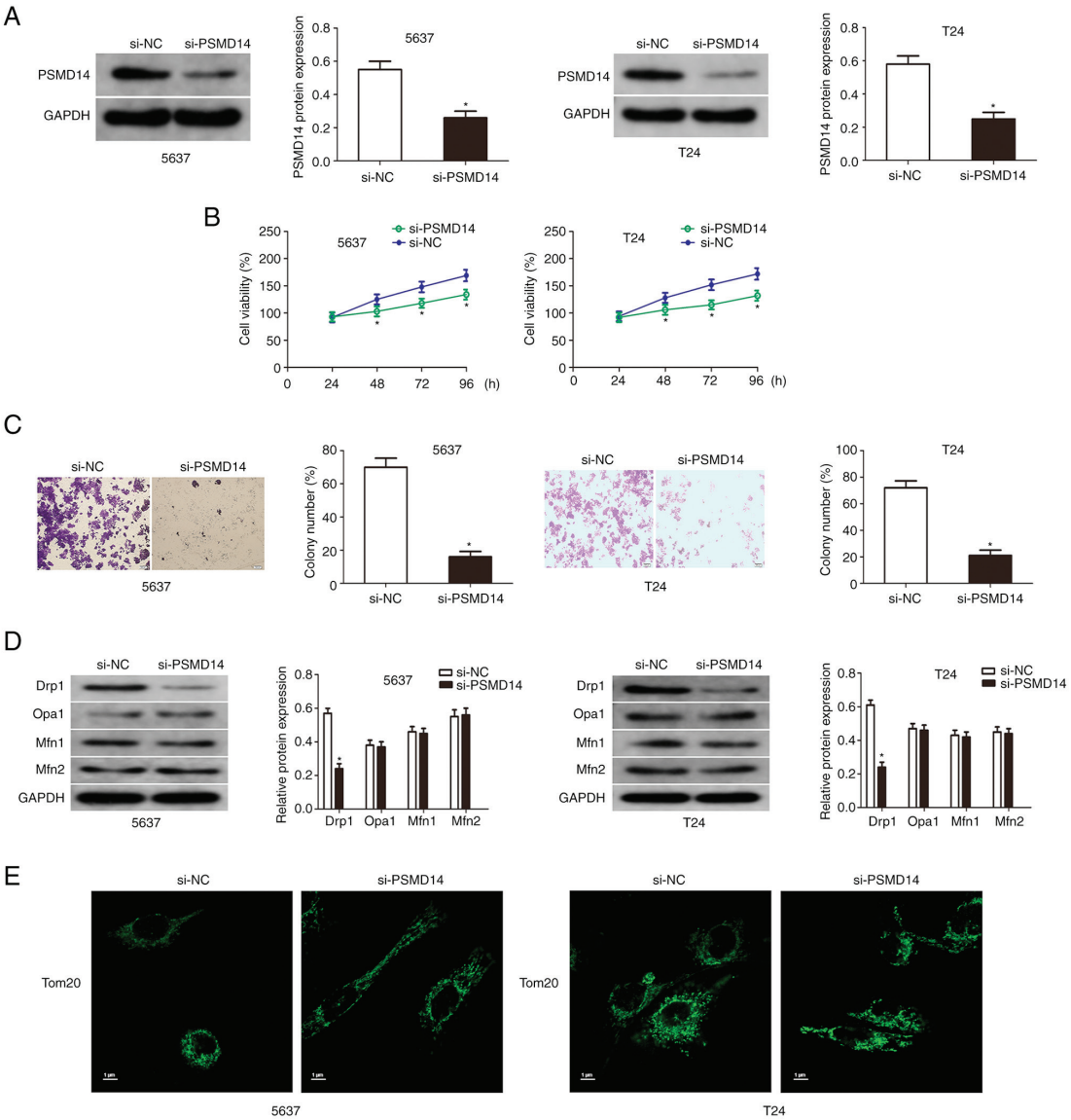

Figure 2. PSMD14 knockdown inhibits the growth of bladder cancer cells. (A) The expression of PSMD14 in T24 and 5637 cells with/without knockdown of PSMD14. (B) Cell viability assay of T24 and 5637 cells with/without knockdown of PSMD14. (C) Colony formation assay of T24 and 5637 cells with/without knockdown of PSMD14. (D) The expression of Drp1, OPA1, Mfn1, and Mfn2 were detected by western blotting. (E) Immunofluorescent staining of Tom20 in bladder cancer cells. Scale bar, 1 μm. *P<0.05 vs. si-NC group. Drp1, Dynein-related protein 1; PSMD14, proteasome non-ATPase regulatory subunit 14; si, small interfering; NC, negative control.

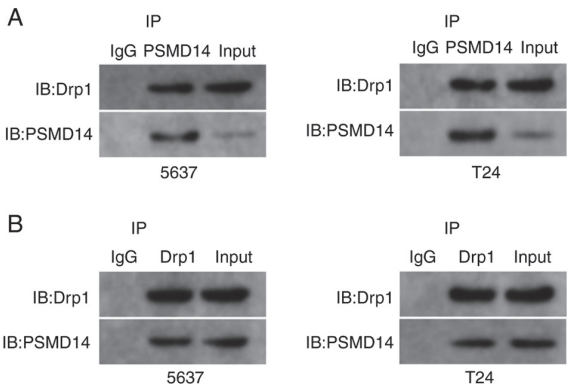

Figure 3. The interaction mode between PSMD14 and Drp1. (A) Co-IP analysis and reciprocal co-IP analysis of PSMD14 and Drp1 in T24 and 5637 cells. (B) Reciprocal co-IP analysis of PSMD14 and Drp1 in T24 and 5637 cells. Drp1, Dynein-related protein 1; PSMD14, proteasome non-ATPase regulatory subunit 14; co-IP, co-immunoprecipitation; IB, immunoblotting.

and nucleus, and it participates in the degradation of most proteins in the cell (30). The ubiquitin-proteasome system is modified by ubiquitination and deubiquitylation to maintain the dynamic balance of intracellular proteins and is one of the important balance systems for maintaining the stability of the intracellular environment. Additional evidence has shown that the changes in expression levels of deubiquitinating enzymes are closely related to human diseases, such as autoimmune diseases and tumors (31-34). PSMD14 plays an important role under normal physiological conditions, including the repair process of DNA damage, embryonic cell development, cell differentiation, and cell apoptosis (33-35). The abnormal expression of PSMD14 disrupts the dynamic balance of proteins in cells, leading to a variety of diseases including tumors (36,37). The results confirmed that the progression of bladder cancer is significantly associated with upregulated expression of PSMD14. PSMD14 can affect the malignant

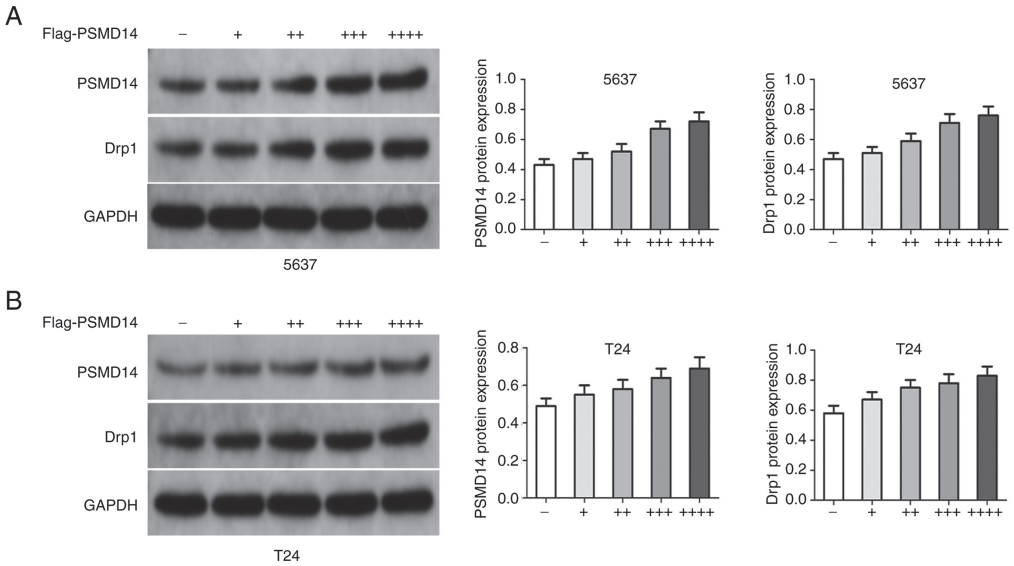

Figure 4. PSMD14 deubiquitinates and stabilizes Drp1. (A) Exogenous expression of PSMD14 induces the accumulation of Drp1 in T24 and 5637 cells. (B) Quantitative analysis of Drp1 expression. Drp1, Dynein-related protein 1; PSMD14, proteasome non-ATPase regulatory subunit 14.

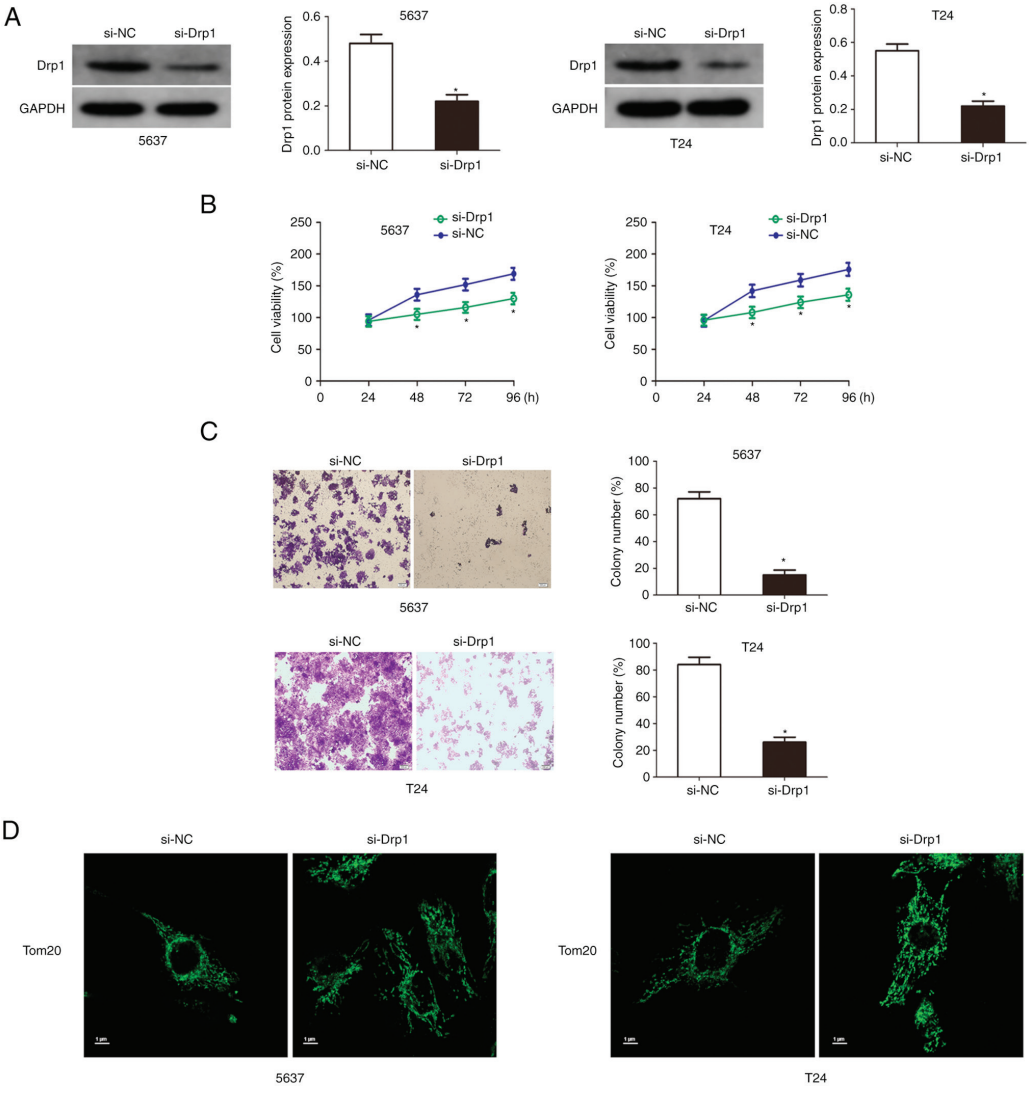

Figure 5. Drp1 knockdown inhibits the growth of bladder cancer cells. (A) The expression of Drp1 in T24 and 5637 cells with/without knockdown of Drp1. (B) Cell viability assay of T24 and 5637 cells with/without knockdown of Drp1. (C) Colony formation assay of T24 and 5637 cells with/without knockdown of Drp1. (D) Immunofluorescent staining of Tom20 in bladder cancer cells. Scale bar, 1 μm. *P<0.05 vs. si-NC group. Drp1, Dynein-related protein 1; si, small interfering; NC, negative control.

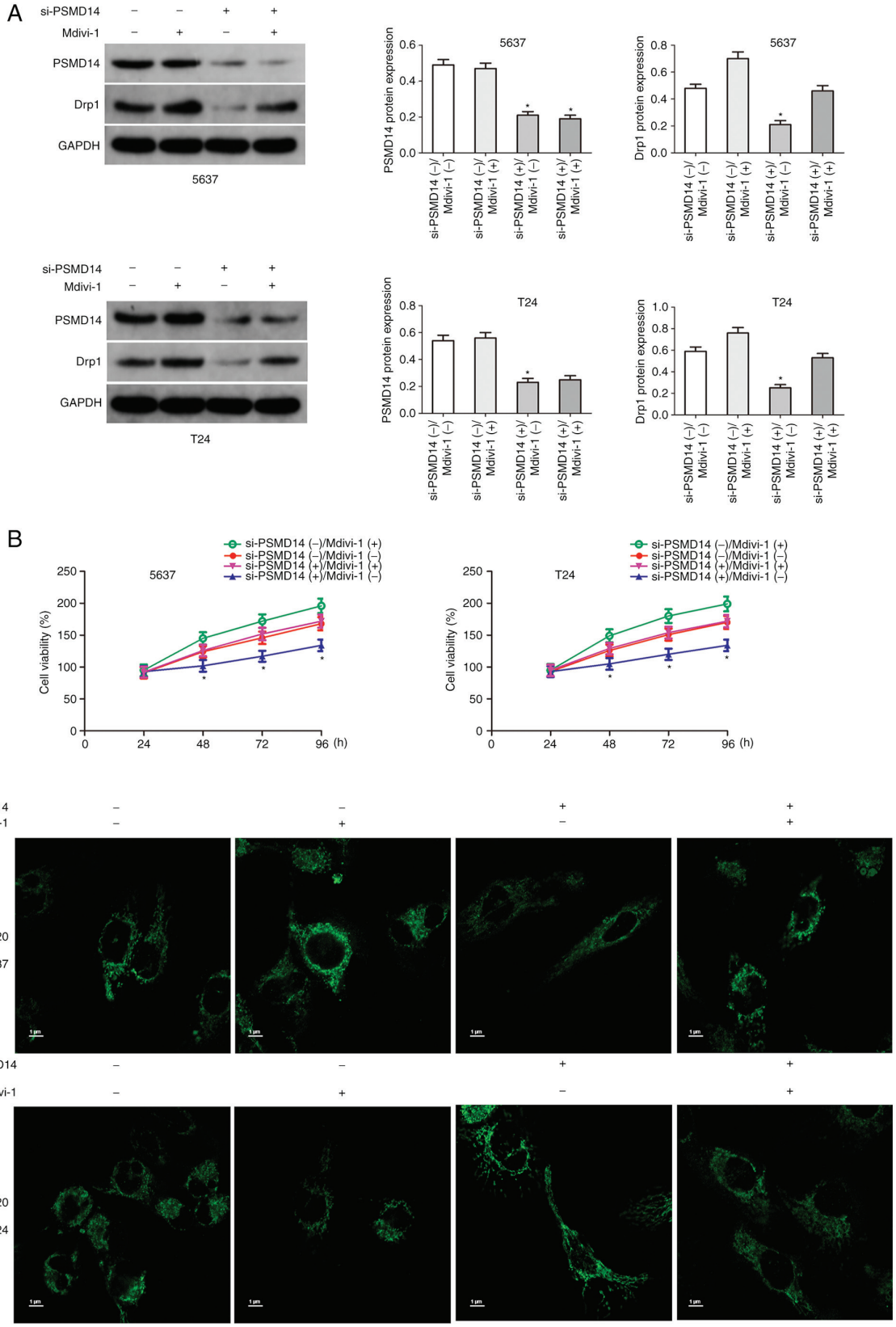

Figure 6. Upregulation of Drp1 partially restores the carcinogenic effect of PSMD14. (A) The expression of Drp1 in T24 and 5637 cells treated with Mdivi-1. (B) Cell viability assay of T24 and 5637 cells treated with Mdivi-1. (C) Immunofluorescent staining of Tom20 in bladder cancer cells treated with Mdivi-1. Scale bar, 1 μm. *P<0.05 vs. Mdivi-1(-) group. Drp1, Dynein-related protein 1; PSMD14, proteasome non-ATPase regulatory subunit 14; si, small interfering.

biological behavior of tumor cells, such as apoptosis resistance, proliferation, chemotherapy resistance, invasion, and metastasis by inhibiting the ubiquitination and degradation of specific proteins. A previous study showed that high expression of PSMD14 increased the resistance of colon cancer cells to chemotherapeutic drugs (38). Zhu *et al* (36) found that

PSMD14 induced the invasion and metastasis of esophageal cancer by regulating the ubiquitination and degradation of the EMT transcription factor Snail. PSMD14 reversed the ubiquitination of GRB2 and promoted cell growth and metastasis of hepatocellular carcinoma (20). PSMD14 can also regulate the stability of ALK2 to make colorectal cancer cells more resistant to chemotherapy (37). PSMD14 can also determine the fate of its substrate protein. PSMD14 promotes substrate transfer and proteolysis by removing the ubiquitin label and induces the release of substrate proteins from the proteasome to avoid degradation (36,39). Therefore, an increasing number of studies are suggesting that PSMD14 may be used as a marker in the ubiquitin-proteasome system to determine the fate of substrates. Here, it was found that PSMD14 knockdown can inhibit cell growth of bladder cancer, suggesting that PSMD14 plays an important role in the progression of bladder cancer.

Mitochondria are the central hub of cellular energy metabolism, biosynthesis, and signal transduction, and can help cells sense stress and adapt to the environment; thus, they play a special role in the occurrence and development of tumors (40). Disturbances in mitochondrial dynamics have been observed in different tumors. Mitochondrial fusion and fission proteins of tumor cells are often abnormally expressed, and mitochondrial morphology has also been shown to be altered (41-43). The expression levels of the mitochondrial division protein Drp1 were upregulated in liver cancer, breast cancer, and lung cancer (44-46). The increased mitochondrial fission in tumor cells suggests the changes in mitochondrial dynamics in tumors, and the intervention of disordered mitochondrial fusion or fission may be a potential tumor therapeutic target for tumors (47). The present study found that Drp1 expression is upregulated, and mitochondrial fission increased significantly in bladder cancer cells. Furthermore, PSMD14 could deubiquitinate and stabilize Drp1.

Mitochondrial dynamics also play a vital role in the regulation of the cell cycle. Mitra *et al* (48) revealed that the cycle of mitochondrial division and fusion is highly coupled with cell cycle progression. In the process of cell division, the mitochondria assigned to each daughter cell are divided from those in the parent cells. In the G1 and G2 phases, a network is formed between the mitochondria. However, in the S phase and mitotic phase, mitochondria are present in a highly fragmented state, which may be due to the increased expression of Drp1 during the cell cycle, which promotes the division of mitochondria (6). Mitra *et al* (48) also found that inhibiting the expression of Drp1 in cells can induce cell cycle arrest in the G1 phase. Previous studies have confirmed that overexpression of Drp1 promotes the proliferation of cancer cells by accelerating the transition of cells in the G1/S phase (6,49). Kitamura *et al* (50) found that Drp1 knockdown can arrest cell cycle progression of skin squamous cell carcinoma at the G2/M phase. Studies have found that overexpression of Drp1 can promote the proliferation of breast cancer, lung cancer, and glioma cells; however, the underlying mechanism has not been fully elucidated (51-53). The present study confirmed that the upregulation of Drp1 could reverse the anticancer effects mediated by PSMD14 knockdown. These results have shown that the overexpression of Drp1 may be related to tumor cell proliferation. The primary limitation of the present study is the lack of *in vivo* experiments, and thus, this will be taken into consideration in our future studies.

In conclusion, at present, the role of PSMD14 in the occurrence and development of cancer has not been fully elucidated. The results indicated that PSMD14 is closely related to the occurrence and development of bladder cancer. In addition, the present study proposes for the first time that PSMD14 mediates the mitochondrial fission of bladder cancer cells by deubiquitinating and stabilizing Drp1. PSMD14 as an important oncogenic protein of bladder cancer, may thus serve as a novel anti-cancer target.

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Availability of data and material

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

Authors' contributions

WX contributed to the conception and design of the study. WS performed the experiments. ZL and MX analyzed data and wrote this manuscript. WX revised and reviewed the manuscript. WS, MX, ZL and WX confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of the Hunan Provincial People's Hospital and in accordance with the Ethical Management Guidelines of Hunan Provincial People's Hospital (approval no. 210139). Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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