# The Role of NPM1 in the Invasion and Migration of Drug Resistant Bladder Cancer

Chenshuo Luo[1], Ting Lei[2], Man Zhao[2], Qian Meng[2], Man Zhang[1,2,3]*

**Purpose:** To study the difference of tumor progression caused by differential expression of NPM1 in drug-resistant bladder cancer.

**Materials and Methods:** The expression of NPM1 was analyzed by PCR and Western blot. NPM1 silencing bladder cancer cells (T24/DDP Lv-NPM1, PUMC-91/DDP Lv-NPM1) and overexpressing bladder cancer cells (T24/DDP Lv5-NPM1, PUMC-91/DDP Lv5-NPM1) were established by lentivirus and limited dilution method. The efficiency of gene interference was detected by fluorescence microscopy and Western blot. The migration ability and invasion ability of tumor in vitro were analyzed by wound healing assay and transwell cell invasion test, and the tumorigenic ability in vivo was judged by nude mouse tumorigenicity assay.

**Results:** Compared with the corresponding negative control group, both NPM1 silencing cell lines T24/DDP Lv-NPM1 and PUMC-91/DDP Lv-NPM1 showed strong migration ability and high invasive ability. At the same time, there was no significant difference in migration ability and the invasive cells proportion between NPM1 overexpressing cell line and related negative control group. NPM1 silencing bladder cancer cells had obvious tumorigenicity in vivo.

**Conclusion:** NPM1 silencing cells had significant migration and invasion ability. The silencing of NPM1 will accelerate tumorigenicity of drug resistant bladder cancer. Differential expression of NPM1 is of great value in monitoring the progression of drug-resistant bladder cancer.

**Keywords:** bladder cancer; NPM1; tumorigenicity; cisplatin

## INTRODUCTION

Bladder cancer is the second most common genitourinary malignancy, with a growing population of patients globally.(1) Because of drug resistance, conventional operation combined with drug perfusion cannot treat bladder cancer well, which makes it easy to recur and difficult to cure.(2) Considering the non-invasive and reliability of tumor markers, it is necessary to find a tumor marker that can timely monitor the progress of drug-resistant bladder cancer. In the process of tumor progression, the ability of invasion and migration of drug-resistant tumor is an important factor that can affect the prognosis of patients.(3)

Nucleocapsmin (NPM1) is one of the most important nucleocapsid protein, which can shuttle between nucleolus and cytoplasm.(4,5) Abnormal expression of NPM1 has been found in colon cancer, lung adenocarcinoma and hematology tumor.(6-8) Many studies have shown that NPM1 plays a role in many important cell activities and is related to the invasion and migration of tumor cells.(9) NPM1 can reflect the therapeutic effect and prognosis of various tumors, such as leukemia.(10)

When our laboratory analyzed the urine samples of bladder cancer patients, we identified 14 differential expression protein spots, including NPM1.(11) Then we found that NPM1 is one of the differentially expressed proteins between bladder cancer cell lines based on 2D-PAGE proteomics approaches. And then using bladder cancer cell lines against different gradient doses of Adriamycin and bladder cancer tissue samples, we showed NPM1 was independently associated with drug resistance and recurrent frequency of bladder cancer, suggesting that NPM1 was a key regulator in drug-resistant bladder cancer model.(12)

To explore the role of NPM1 in cell invasion and migration, we analyzed the role of NPM1 in the biological characteristics of tumorigenicity in vitro and vivo. This experiment will reveal the influence of NPM1 in bladder cancer, and provide guidance for the clinical recognition and intervention of bladder cancer.

## MATERIALS AND METHODS

### *Subject and Groups*

### *Bladder cancer cell lines and groups*

Drug resistant cell groups: The cisplatin resistant bladder cancer cell lines (T24/DDP, PUMC-91/DDP) were purchased from Beijing Shijitan Hospital Affiliated to Capital Medical University.

Negative control groups: Bladder cancer cell lines T24 and PUMC-91. T24 is an American type culture collection (ATCC), PUMC-91 purchased from the cell biology laboratory of Peking Union Medical College

[1]Clinical Laboratory Medicine, Peking University Ninth School of Clinical Medicine, Beijing, 100038, China
[2]Clinical Laboratory Medicine, Beijing Shijitan Hospital, Capital Medical University, Beijing, 100038, China
[3]Beijing Key Laboratory of Urinary Cellular Molecular Diagnostics, Beijing, 100038, China
*Correspondence: Clinical Laboratory Medicine, Peking University Ninth School of Clinical Medicine, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China.
Tel: 0086 010 63926389 FAX: 0086 010 63926283 Email address: zhangman@bjsjth.cn.
Received April 2020 & Accepted July 2021

**Table 1.** The primer design of real-time quantitative PCR (Q-RT-PCR).

| Gene | Forward primer | Reverse primer |
|---|---|---|
| NPM1 | 5'-TGGTGCAAAGGATGAGTTGC-3' | 5'-GTCATCATCTTCATCAGCAGC-3' |
| β-actin | 5'-CTACAATGAGCTGCGTGTGGC-3' | 5'-CAGGTCCAGACGCAGGATGGC-3' |

Hospital.

***NPM1 differential expression cell groups***

NPM1 silencing bladder cancer cell lines (T24/DDP Lv-NPM1, PUMC-91/DDP Lv-NPM1): NPM1 silenced stable infection cell line was constructed by lentivirus infection.

NPM1 silencing negative control groups (T24/DDP Lv-NC, PUMC-91/DDP Lv-NC): The infected cell lines were constructed by lentivirus infection.

NPM1 over-expressing groups (T24/DDP Lv5- NPM1, PUMC-91/DDP Lv5- NPM1): The infected cell lines were constructed by lentivirus infection.

NPM1 over-expressing negative control groups (T24/DDP Lv5-NC, PUMC-91/DDP Lv5-NC): The infected cell lines were constructed by lentivirus infection.

***Animal groups***

According to the condition of tumor injection, xenograft model were randomlyized divided into two groups: the negative animal control (Lv-NC) and NPM1 silencing animal group (Lv-NPM1). 4 mice in each group.

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

All procedures performed in studies involving animals were in accordance with the ethical standards of the Pecking University Ethics Committee. (Permit number: LA2017287)

***Reagents***

Cisplatin (P4394, Sigma, USA), RPMI-1640 medium (AE244464298, Thermo scientific, USA), FBS

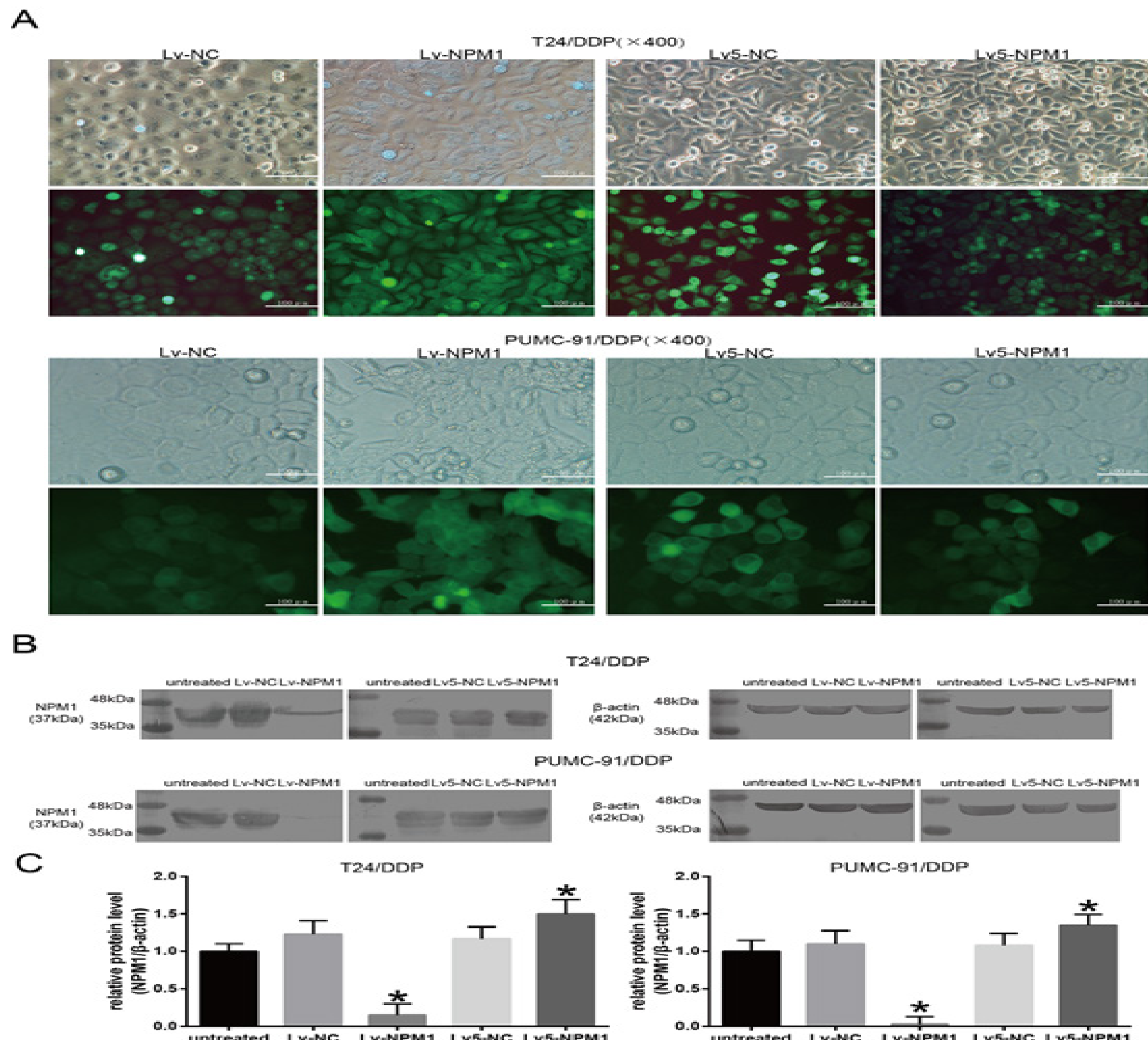


**Figure 1.** NPM1 silencing, NPM1 overexpressing stable cell lines and corresponding negative control stable cell lines.
**(A)** The infection efficiency of lentivirus (immunofluorescence microscopy, 10 × 40). **(B)** The expression of NPM1 protein (Western blot). **(C)** The expression level of NPM1 silencing stable cell line and NPM1 over-expressing stable cell line

**Table 2.** Effect of NPM1 differential expression on oncological characteristics of bladder cancer cells.

| Experimental groups | the ratio of NPM1 protein expression | Cell migration rate | Invasive cell count |
|---|---|---|---|
| T24/DDP Lv-NPM1 / T24/DDP Lv-NC | 0.15 ($P$ <.001) | 24h: 73.33% vs 13.83% ($P$ <.001)<br>48h: 86.53% vs 44.83% ($P$ <.001) | 1983 vs 788 ($P$ <.001) |
| PUMC-91/DDP Lv-NPM1 / PUMC-91/DDP Lv-NC | .03 ($P$ <.001) | 24h: 20.13% vs 8.47% ($P$ =.046)<br>48h: (90.43% vs 9.76%, $P$ <.001) | 755 vs 133 ($P$ <.001) |
| T24/DDP Lv5-NPM1 / T24/DDP Lv5-NC | 1.52 ($P$ =.019) | 24h: 8.03% vs 14.80% ($P$ =.261)<br>48h: 20.90% vs 41.00% ($P$ =.064) | 503 vs 605 ($P$ =.545) |
| PUMC-91/DDP Lv5-NPM1 / PUMC-91/DDP Lv5-NC | 1.35 ($P$ =.045) | 24h: 10.73% vs 6.70% ($P$ = .102)<br>48h: 15.23% vs 12.33% ($P$ = .630) | 139 vs 149 ($P$ =.635) |

(1861242, Gibco, ThermoFisher, USA), 0.25% trypsin (17518012, Gibco, ThermoFisher, USA), Trizol (84804, Gibco, ThermoFisher, USA), reverse transcription kit (64J00101, Dingguo, China). SYBR Green fluorescent quantitative PCR kits (K20524, TransGen Biotech, China), polyvinylidene fluoride (PVDF) membranes (ISEQ00010 Sigma, USA), Mouse anti-Nucleophosmin antibody (ab10530, Abcam, USA), Mouse IgG H&L (HRP) (ab205719, Abcam, USA), 96-well plates (E161134L, Thermo, USA), NPM1-silencing,NPM1 over-expressing and negative control lentivirus (GenePharma, China), growth factor reduced matrigel (3432-001-01, R&D Systems, USA), Transwell chambers (140644, Thermo, USA)

### *Cell culture and subculture*

T24, PUMC-91, T24/DDP and PUMC-91/DDP cells were cultured in RPMI-1640 medium containing 15% fetal bovine serum at 37 °C and 5% $CO_2$ incubator respectively. After digestion of 0.25% trypsin, they were used for subculture.

### *NPM1 expression detection*

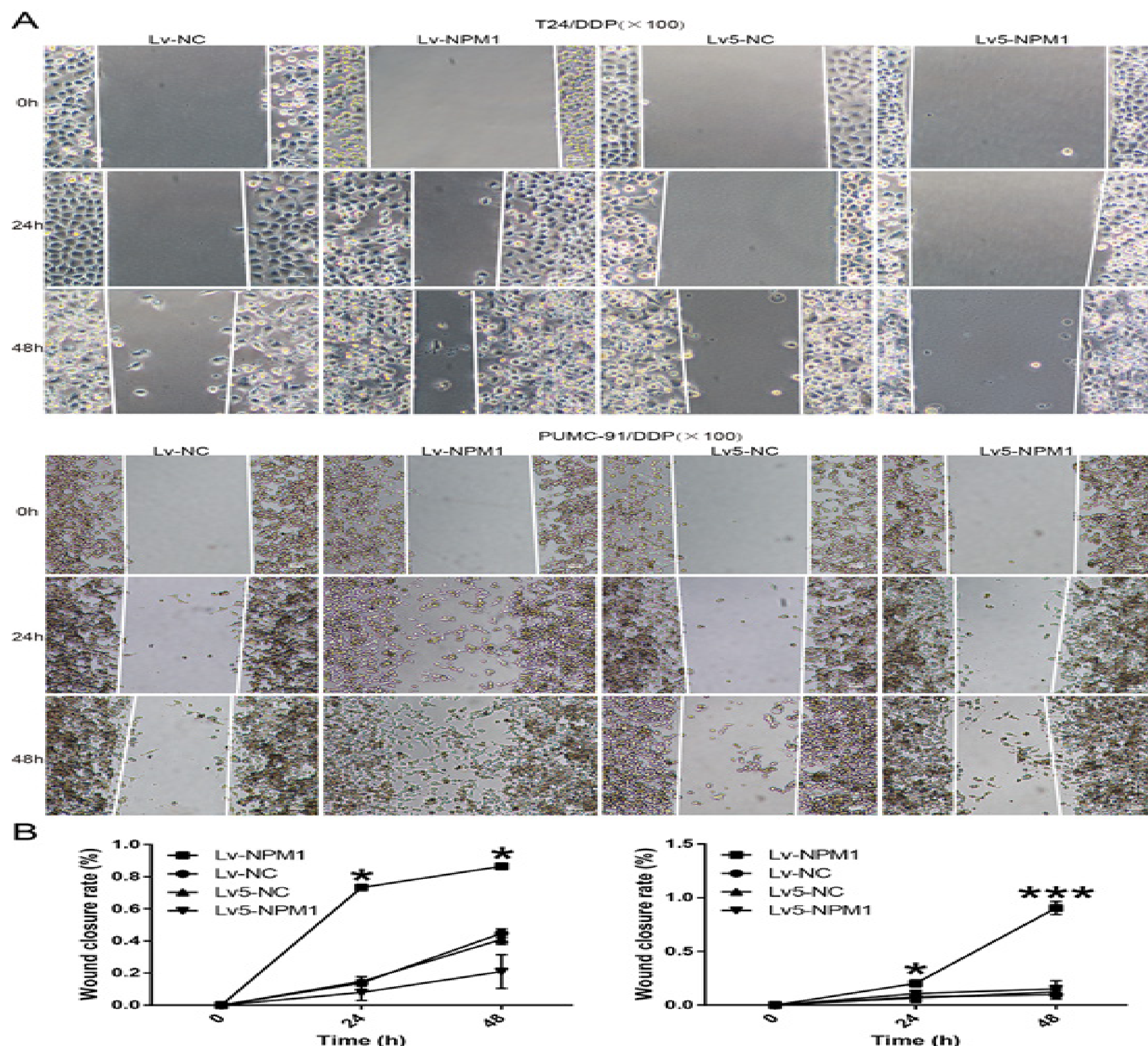


**Figure 2.** The effect of NPM1 silencing and NPM1 overexpressing on cisplatin resistant bladder cancer cell migration.
With experimental times lasting (0-48 hours), the cell migration rate of NPM1 silencing cisplatin resistant bladder cancer cells was higher than that of negative control and NPM1 overexpressing bladder cancer cells.

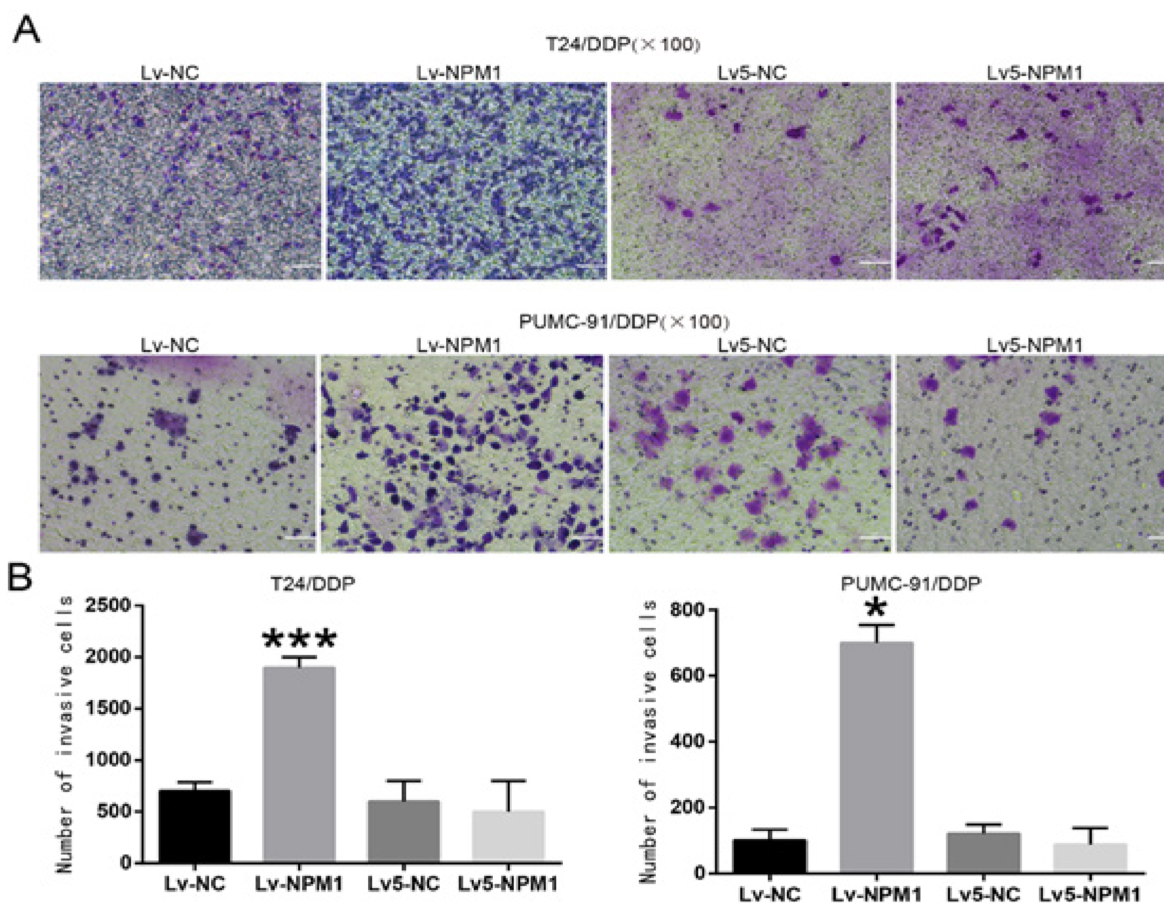


**Figure 3.** The effect of NPM1 silencing and NPM1 overexpressing on the cell invasion of cisplatin resistant bladder cancer cells. 48 hours after the experiment, NPM1 silencing cisplatin resistant bladder cancer cells were more invasive than negative control cells.

NPM1 gene mRNA and protein expression were detected by real-time fluorescent quantitative PCR (Q-PCR) and Western blot, respectively.

### *Q-PCR*

Primer design: Primer 5.0 Software is used for the primer design (**Table 1**). Total RNA extraction: When the cells grow to the logarithmic growth stage, discard the medium, wash the cells with PBS for three times, digest the cells with trypsin, and extract the total RNA by Trizol method. The operation was carried out according to the instructions of the total RNA extraction kit. Determination of RNA concentration and purity: RNA concentration and purity were determined by ultraviolet spectrophotometer. The OD value of sample RNA is between 1.8 and 2.0. cDNA synthesis: Reverse transcription of RNA samples was performed by reverse transcription kit. The total reaction volume of 20 µL. Fluorescent quantitative PCR: All operations were carried out according to the instructions of SYBR green fluorescent quantitative PCR kit. Fluorescent quantitative PCR analysis was carried out by PCR analysis instrument. Calculate the corresponding CT value. Determination of mRNA expression: The relative expression of NPM1 gene mRNA was analyzed by relative quantitative analysis, and the expression of β-actin was taken as a reference, and the relative expression multiple was N (N = 2-ΔΔCT).ΔΔ CT = (Mean CT value of target gene in the group - Mean CT value of β-actin gene in the group) - (Mean CT value of target gene in the control group - Mean CT value of β-actin gene in the control group).

### *Western Blot*

When cells grew to logarithmic growth phase, they were washed with PBS for 3 times. RIPA lysis buffer containing proteinase inhibitor was used to lyse the cells. The volume of the sample was calculated according to the protein mass (20 µg). After SDS-PAGE gel electrophoresis, the protein was transferred to the PVDF membrane and sealed with skim milk powder for 2 hours. PVDF membrane was immersed in the diluted antibody of skimmed milk powder for 4 °C overnight. The PVDF membrane was cleaned by TBST 3 times. Add the corresponding diluted antibody (1: 5000), shake it on the horizontal shaker for 2 hours at room temperature, and clean it with TBST for 3 times at room temperature. DAB developer was used for development, and pictures were exposed and saved. Image J system was used to analyze the band optical density, the optical density value of the target protein band and β-actin band were compared with that of the control group. The relative optical density value of the control group is set to 1, and calculate the relative optical density value of each target protein.

### *Establishment of stable bladder cancer cell line*

NPM1 silenced and NPM1 over expressed cell lines

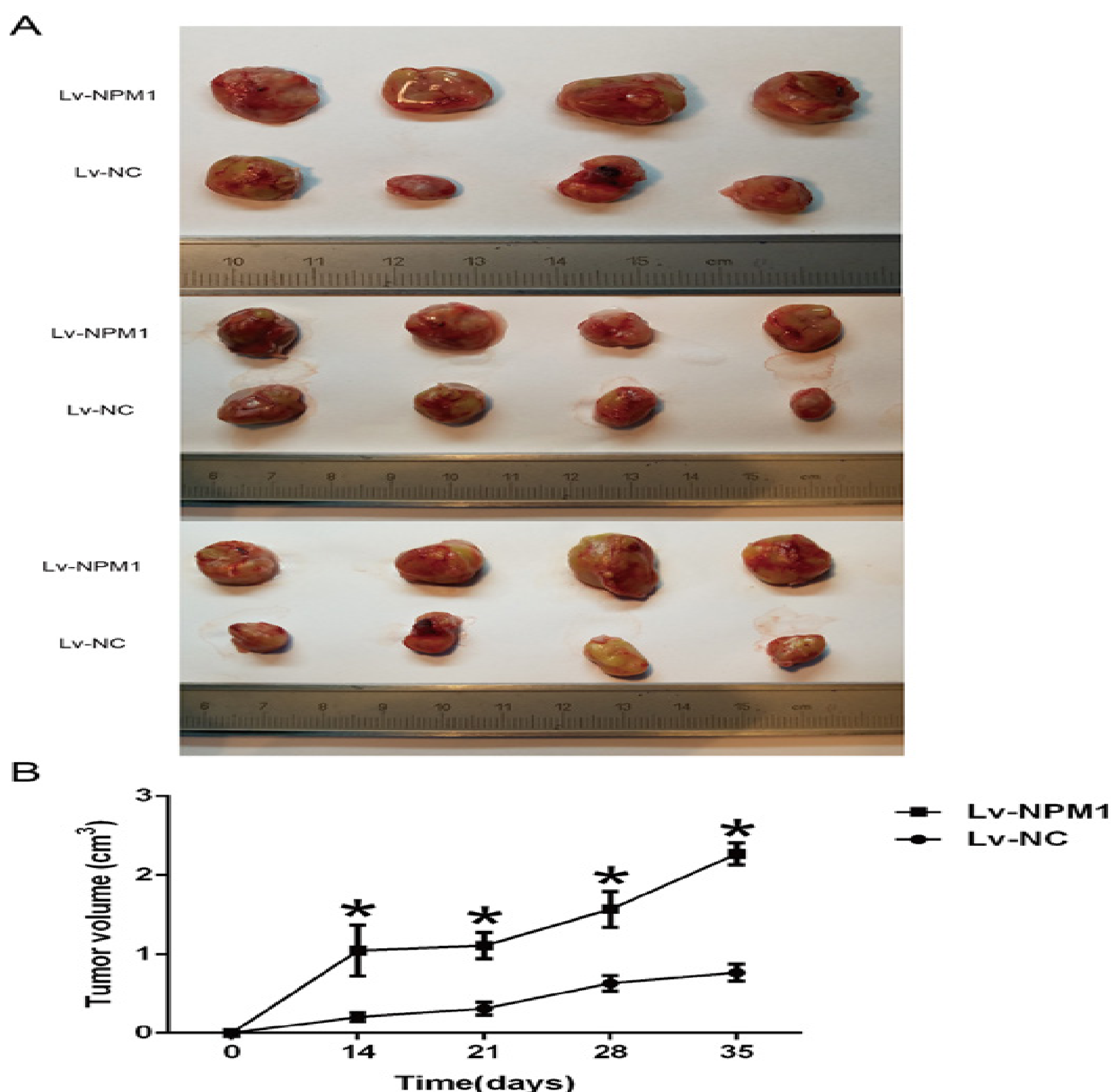


**Figure 4.** Tumorigenicity comparison between Lv-NPM1 and Lv-NC cells in nude mice.
35 days after the experiment, nude mice injected with NPM1 silencing cells had more pronounced tumor masses than the negative control nude mice.

were obtained by lentivirus infection, and stable cell lines were screened by limited dilution method. Lentivirus infection: At the logarithmic growth stage of cultured cells, 96 well plates were inoculated with 1 × 103 cells per well. The cells were incubated at 37 ° C for 24 hours. After the cells grew to 50% confluence, they were infected with NPM1 silencing lentivirus or NPM1 over-expressing lentivirus for 24 hours. Meanwhile, the negative control groups were inoculated with corresponding negative control lentivirus. MOI value was used to analyze the virus concentration. Finally, 106pfu/mL virus concentration was used to infect cells to ensure the maximum infection efficiency and cell survival rate. After virus infection, the cells were cultured in a complete medium. When the cells reached 80% confluence, the cells were transferred to the culture flask for expanded culture. Establishment of stable infected cells: the infected cells were screened by limited dilution method. The infected cells were diluted to 0.5-1 cells per 96 well plate. After 3-4 weeks, the cells grew into a visible cell set. The cells expressing GFP fluorescence were further cultured. Silencing effect of NPM1: Western blot was used to confirm the silencing effect of NPM1.

### *Wound-healing assay*

In order to evaluate the migration ability of bladder cancer cells, wound-healing assay was performed. The migration ability of cells in each group can be judged by the difference of repair ability of cells in different groups for wound area. The plate was irradiated with ultraviolet for 30 minutes before operation. Approximately $3 \times 10^4$ infected cells were incubated at 24 holes. Wounds were made according to the instructions. After 24 hours low serum concentration medium was used to incubate the cells. Rinsed the plate gently with PBS for 2 times after 24 or 48 hours. After that the photos were taken under microscopy (CKX41, Olympus, USA). According to the pictures, the rate of cell migration in each group was calculated. The migration rate was the ratio

of wound width to 0 hour wound area width.

***Transwell invasion assay***

In order to evaluate the invasion ability of bladder cancer cells, transwell invasion assay was performed. The invasion ability of bladder cancer cells was evaluated by counting the amount of cells which can digest matrigel. Matrigel invasion assay was performed using transwell chambers. $1\times10^4$ Lv-NPM1 cells, $1\times10^4$ Lv5-NPM1 and the cells were seeded in the upper chamber of a 24-well plate, which was coated with growth factor reduced matrigel. The upper chamber was filled with 500µL serum-free medium. The lower chamber was filled with 500µL medium containing 10% FBS to induce cell invasion. The chamber was incubated at 37°C for 24 hours. At the end of incubation, cells in the upper surface of the membrane were removed with a cotton swab. Migrated cells were stained with crystal violet. The images were obtained by microscope and the cells were counted in ten different view fields. The experiment was conducted in triplicate.

***Xenograft growth in nude mice***

To evaluate the tumorigenicity of Lv-NPM1 cells, xenograft model was made by subcutaneous injection of Lv-NPM1 cells in athymic nude mice. Male mice (6-8 week old) were randomized into two groups. The mice were inoculated subcutaneously in the left armpit with 100µL serum-free RPMI medium containing $5\times10^7$ Lv-NPM1 cells. They were also inoculated subcutaneously in the right armpit with 100 µL serum-free RPMI medium containing 5×107 Lv-NC cells (as the negative control). All the mice were kept in standard laboratory conditions and provided with ad libitum food and water. General health of these animals was daily observed and tumor growth at the injection site was monitored by palpation. Tumor volume was measured outside of body by vernier caliper and calculated using the formula: length × (width) 2 × 0.5. After 35 days of the experiment, all nude mice were sacrificed by cervical dislocation and tumors were removed and stored in liquid nitrogen. The experiment was conducted in triplicate.

***Statistical analysis***

All independent experimental data were expressed as mean values. Statistical analysis was performed using Graphpad Prism 6.0 statistical software. The P-values were calculated by analysis of variance (ANOVA) for two groups. Pre-assumptions: H0: there was no significant difference between the experimental group and the control group H1: there was statistical difference between the experimental group and the control group. *P*-value < .05 indicates a statistically significant result.

## RESULTS

The establishment of NPM1 silencing cell lines and NPM1 over-expressing cell lines

The NPM1 silencing cell lines and NPM1 over-expressing cell lines were obtained by lentivirus infection and limited dilution method. The results of Q-PCR and Western blot showed that the selected cells by limited dilution method had more than 80% GFP fluorescence efficiency and more than 90% gene efficiency (**Figure 1A**). NPM1 silencing stable infection cell line NPM1 can be effectively inhibited, and can be long-term inhibited screened. (**Figures 1B, 1C**). The level of NPM1 protein in T24/DDP Lv-NPM1 cell line was only 0.15 times that of the negative control. The level of NPM1 protein in PUMC-91/DDP Lv-NPM1 cell line was only 0.03 times that of the negative control. Compared with the negative cells, the NPM1 protein of the over-expressing cell lines also increased significantly (**Figures 1B, 1C**). The level of NPM1 protein in T24/DDP Lv5-NPM1 cell line was 1.5 times that of the negative control. The level of NPM1 protein in PUMC-91/DDP Lv5-NPM1 cell line was 1.35 times that of the negative control.

The effect of NPM1 silencing and NPM1 overexpressing on the cell mobility of cisplatin resistant bladder cancer cells

Compared with the negative control group, NPM1 silencing cell line T24/DDP Lv-NPM1 showed strong migration ability in 24 hours (73.33% vs 13.83%) and 48 hours (86.53% vs 44.83%). At the same time, there was no significant difference between the overexpressing cell lines (T24/DDP Lv5-NPM1) and the corresponding negative control cell lines in 24 hours and 48 hours. In the comparison of NPM1 silencing cell line PUMC-91/DDP Lv-NPM1 and its negative control (PUMC-91/DDP Lv-NC), PUMC-91/DDP Lv-NPM1 also showed stronger cell migration ability in 24 hours (20.13% vs 8.47%) and 48 hours (90.43% vs 9.76%). Besides, there was no significant difference between the overexpressing cell line and the corresponding negative control cell lines in 24 hours and 48 hours. The results are shown in **Figure 2**.

The effect of NPM1 silencing and NPM1 overexpressing on the cell invasion of cisplatin resistant bladder cancer cells

Compared with the negative control group, the invasive cells proportion of T24/DDP Lv-NPM1 cells was higher than Lv-NC cells (1983 cells/lp vs 788 cells/lp). At the same time, there was no significant difference in the proportion of cells in the invasive cells proportion between NPM1 overexpressing cell line and related negative control group. Compared with the negative control group, the invasive cells proportion of PUMC-91/DDP Lv-NPM1 cells was higher than Lv-NC cells (755 cells/lp vs 133 cells/lp). At the same time, there was no significant difference in the proportion of cells in the invasive cells proportion between NPM1 overexpressing cell line (PUMC-91/DDP Lv5-NPM1) and related negative control group (PUMC-91/DDP Lv5-NC). The results are shown in **Figure 3**.

The effect of NPM1 silencing on the tumorigenicity of cisplatin resistant bladder cancer cells in vivo

After 35 days of the experiment, greater masses were observed in animals injected with Lv-NPM1 cells compared with Lv-NC cells. Meanwhile, the tumor mass of the nude mice injected NPM1 silenced cells was more obvious than that of the negative control nude mice.at 14-35 days ($P$ <.001), as shown in Figure 4.

## DISCUSSION

Drug resistance is the major obstacle to cancer chemotherapy. There is much research about monitoring and avoidance of drug resistance. The accurate judgment of invasion and migration of drug resistant bladder cancer can effectively guide the treatment of bladder cancer and improve the treatment effect.

Nucleophosmin (NPM1) is a nucleolar protein which can provide clinical information for the development of cancers.[13,14] NPM1 plays important roles in p53, MDM2 and other signal pathways,[15,16] which means

NPM1 can affect tumor characteristics and provide important information for drug resistance. The upregulation of NPM1 expression will significantly affect the relapse rate and the sensitivity of chemotherapy drugs of leukemia.[17,18] However, the contrary effect of NPM1 on therapeutic effect has also been reported. Jian et al. found that the abnormal high expression of NPM1 may inhibit the growth of tumor cells,[19] which suggested that NPM1 might have a positive correlation with the anti-tumor activity. Some studies showed that NPM1 mutation can change genome stability, which has been described as having both oncogenic and tumor suppressive functions.[20] NPM1 overexpression is linked to a poor prognosis.[21] However, in gastric cancer and breast cancer, NPM1 is associated with poor prognosis.[19,22] These studies show that the value of NPM1 still needs to be further evaluated for different tumors.

In general, NPM1 is of great value in the progression of non-urinary tumors.[23] Myc is one of the protein related to the occurrence and prognosis of tumors.[24] The lack of a functional NPM1 was previously associated with increased levels of Myc.[25] NPM1 regulates c-Myc protein stability through its effect on the γ-isoform of the F-box E3 ubiquitin ligase Fbw7. This effect of NPM1 on Fbw7γ is relevant for f its substrates, c-Myc, which accumulates in the absence of NPM1. In the study of bladder cancer, the activation of Myc can go through β-catenin/c-Myc signaling pathway and AFF4/NF-κB/Myc signaling pathway to increase the ability of cell migration and proliferation.[26]

In this experiment, NPM1 silencing and NPM1 overexpressing bladder cancer cell lines were constructed by lentivirus infection and limited dilution method to evaluate the value of NPM1 in drug resistant bladder cancer. In this study, the results showed that the loss of NPM1 was correlated with high cell mobility in bladder cancer cells. Compared with negative control group, NPM1 silencing cell line T24/DDP Lv-NPM1 showed strong migration ability in 24 hours and 48 hours. In the comparison of NPM1 silencing cell line PUMC-91/DDP Lv-NPM1 and its negative control, PUMC-91/DDP Lv-NPM1 showed stronger cell migration ability in 24 hours and 48 hours, as shown in **Table 2**.

Besides, NPM1 silencing could make cisplatin resistant bladder cancer aggressive. Compared with the negative control, the proportion of the invasive cells of T24/DDP Lv-NPM1 cells was higher than Lv-NC cells. Compared with negative control group, the invasive cells proportion of PUMC-91/DDP Lv-NPM1 cells was higher than Lv-NC cells.

Compared with the above results in vitro, the tumorigenesis experiment also showed similar results in vivo. Greater masses were observed in animals injected with Lv-NPM1 cells compared with Lv-NC cells. Based on the above experimental results, we confirmed that in the absence of NPM1, bladder cancer cells have higher invasiveness and mobility, and may point to poor prognosis and higher risk of recurrence. The experimental results may be related to the explanation of NPM1 mechanism. Loss of NPM1 function has been shown to be associated with increased genome instability. Combined with our experimental results, we speculate that the loss of NPM1 might promote the activation of Myc signal pathway in bladder cancer cells to increase the invasion and migration ability of bladder cancer. These ideas will be further confirmed in future experiments.

NPM1 shows an important role in the clinical application of tumor markers. NPM1 has high sensitivity and specificity, which can monitor the progress of tumor timely and effectively. At the same time, as a tumor related marker, it is associated with a variety of tumor signal pathways, which is of great significance to the study of tumor progression mechanism.[27] Unlike many bladder cancer markers, which are susceptible to infection and other factors, NPM1 has less interference and can more accurately reflect the changes of tumor in vivo.[28] Combined with our results of this study, its clinical value in bladder cancer is worthy of further exploration. As a protein existing in cells, is NPM1 easily detected in the urine of patients with bladder cancer? What is the sensitivity of NPM1 in clinical noninvasive monitoring of bladder cancer? These questions need to be further confirmed in the clinical research of bladder cancer.

## CONCLUSIONS

Down-regulated expression of NPM1 may indicate the poor outcome of bladder cancer, which means that the tumor may be more malignant. Monitoring the changes of NPM1 in time can effectively adjust the treatment strategy of bladder cancer and treat bladder cancer.

## ACKNOWLEDGEMENT

This work was supported by Enhancement Funding of Beijing Key Laboratory of Urinary Cellular Molecular Diagnostics (grant number: 2020-JS02).

## CONFLICT OF INTEREST

The authors report no conflict of interest.

## REFERENCES

1. Bhanvadia SK. Bladder Cancer Survivorship. Curr Urol Rep. 2018;19:111.
2. Martinez Rodriguez RH, Buisan Rueda O, Ibarz L. Bladder cancer: Present and future. Med Clin (Barc). 2017;149:449-455.
3. Duff D, Long A. Roles for RACK1 in cancer cell migration and invasion. Cell Signal. 2017;35:250-255.
4. Kim JY, Cho YE, An YM, et al. GLTSCR2 is an upstream negative regulator of nucleophosmin in cervical cancer. J Cell Mol Med. 2015;19:1245-52.
5. Endo A, Matsumoto M, Inada T, et al. Nucleolar structure and function are regulated by the deubiquitylating enzyme USP36. J Cell Sci. 2009;122:678-86.
6. Forghieri F, Riva G, Lagreca I, et al. Characterization and dynamics of specific T cells against nucleophosmin-1 (NPM1)-mutated peptides in patients with NPM1-mutated acute myeloid leukemia. Oncotarget. 2019;10:869-882.
7. Liu Y, Zhang F, Zhang XF, et al. Expression of nucleophosmin/NPM1 correlates with migration and invasiveness of colon cancer cells. J Biomed Sci. 2012;19:53.
8. He J, Xiang Z, Xiao J, Xiao H, Liu L. [The poor chemotherapeutic efficacy in lung adenocarcinoma overexpressing c-Src and nucleophosmin/B23(NPM1)]. J Exp Hematol. 2016;32:1378-1381.
9. Li S, Zhang X, Zhou Z, et al. Downregulation

of nucleophosmin expression inhibited proliferation and induced apoptosis in salivary gland adenoid cystic carcinoma. J Oral Pathol Med. 2017;46:175-181.
10. Heath EM, Chan SM, Minden MD, et al. Biological and clinical consequences of NPM1 mutations in AML. Leukemia. 2017;31:798-807.
11. Lei T, Zhao X, Jin S, et al. Discovery of potential bladder cancer biomarkers by comparative urine proteomics and analysis. Clin Genitourin Cancer. 2013;11:56-62.
12. Hu H, Meng Q, Lei T, Zhang M. Nucleophosmin1 associated with drug resistance and recurrence of bladder cancer. Clin Exp Med. 2015;15:361-9.
13. Li TT, Li J, Geng YH, et al. [NRAS Gene Expression and Its Clinical Significance in Patients with Acute Myeloid Leukemia]. J Exp Hematol. 2020;28:76-81.
14. Jain P, Kantarjian H, Patel K, et al. Mutated NPM1 in patients with acute myeloid leukemia in remission and relapse. Leuk Lymphoma. 2014;55:1337-44.
15. Kurki S, Peltonen K, Latonen L, et al. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer Cell. 2004;5:465-75.
16. Jin A, Itahana K, O'Keefe K, Zhang Y. Inhibition of HDM2 and activation of p53 by ribosomal protein L23. Mol Cell Biol. 2004;24:7669-80.
17. Bertoli S, Tavitian S, Berard E, et al. More than ten percent of relapses occur after five years in AML patients with NPM1 mutation. Leuk Lymphoma. 2020:1-4.
18. Chen S, Li X, Ma S, et al. Chemogenomics analysis of drug targets for the treatment of acute promyelocytic leukemia. Ann Hematol. 2020.
19. Jian Y, Gao Z, Sun J, et al. RNA aptamers interfering with nucleophosmin oligomerization induce apoptosis of cancer cells. Oncogene. 2009;28:4201-11.
20. Wang W, Budhu A, Forgues M, Wang XW. Temporal and spatial control of nucleophosmin by the Ran-Crm1 complex in centrosome duplication. Nat Cell Biol. 2005;7:823-30.
21. Coutinho-Camillo CM, Lourenco SV, Nishimoto IN, Kowalski LP, Soares FA. Nucleophosmin, p53, and Ki-67 expression patterns on an oral squamous cell carcinoma tissue microarray. Hum Pathol. 2010;41:1079-86.
22. Leal MF, Mazzotti TK, Calcagno DQ, et al. Deregulated expression of Nucleophosmin 1 in gastric cancer and its clinicopathological implications. BMC Gastroenterol. 2014;14:9.
23. Chang S, Yim S, Park H. The cancer driver genes IDH1/2, JARID1C/ KDM5C, and UTX/ KDM6A: crosstalk between histone demethylation and hypoxic reprogramming in cancer metabolism. Exp Mol Med. 2019;51:66.
24. Trop-Steinberg S, Azar Y. Is Myc an Important Biomarker? Myc Expression in Immune Disorders and Cancer. Am J Med Sci. 2018;355:67-75.
25. Bonetti P, Davoli T, Sironi C, et al. Nucleophosmin and its AML-associated mutant regulate c-Myc turnover through Fbw7 gamma. J Cell Biol. 2008;182:19-26.
26. Chen JB, Zhang M, Zhang XL, et al. Glucocorticoid-Inducible Kinase 2 Promotes Bladder Cancer Cell Proliferation, Migration and Invasion by Enhancing beta-catenin/c-Myc Signaling Pathway. J Cancer. 2018;9:4774-4782.
27. Box JK, Paquet N, Adams MN, et al. Nucleophosmin: from structure and function to disease development. BMC Mol Biol. 2016;17:19.
28. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet. 2009;374:239-49.