

# Nicotinamide N-methyltransferase enhances the progression of prostate cancer by stabilizing sirtuin 1

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**Abstract.** A previous study demonstrated that nicotinamide N-methyltransferase (NNMT) is upregulated in the tissues of patients with prostate cancer (PCa); however, the specific underlying mechanism of this remains unclear. To begin with, the expression of NNMT was investigated in the peripheral blood of patients with PCa and of healthy control subjects. The results indicated that the expression level of NNMT was elevated in the peripheral blood and tissues of patients with PCa. Furthermore, the overexpression of NNMT enhanced PC-3 cell viability, invasion and migration capacity. Additionally, the overexpression of NNMT significantly increased the mRNA level of sirtuin 1 (SIRT1) in PC-3 cells. In addition, nicotinamide treatment significantly suppressed the expression of SIRT1 even in PC-3 cells transfected with adeno-associated virus-NNMT. Furthermore, the PC-3 cell invasion capacity was notably decreased by the nicotinamide treatment; however, such effects were largely abolished by the overexpression of NNMT in PC-3 cells. These data indicated that NNMT enhanced PC-3 cell migration and invasion mainly by regulating SIRT1 expression. In summary, the present study indicated that NNMT is an important regulator of SIRT1 expression in PC-3 cells and may be a potential therapeutic target for PCa.

## Introduction

Prostate cancer (PCa) is the second most commonly diagnosed form of cancer and the sixth leading cause of cancer-associated mortality among males globally (1). It has been reported that the 5-year survival rate is ~100% for patients with localized cancer but is only 31% for those with distant metastases (2). Therefore, it is of great importance to identify an effective treatment method for PCa therapy.

Nicotinamide N-methyltransferase (NNMT) was identified as an S-adenosyl-L-methionine-dependent cytoplasmic enzyme (3). Previous studies have indicated its critical role in the biotransformation and detoxification of multiple drugs and xenobiotic compounds (3,4). Abnormal upregulation of NNMT has been extensively identified in various tumor types. For instance, in the progression of PCa, overexpression of NNMT has been frequently determined (4). Furthermore, NNMT has been frequently reported to be a non-invasive biomarker of cancer in body fluids, including serum (5), saliva (6) and urine (7). It was originally defined as the enzyme responsible for nicotinamide methylation, which is an important form of vitamin B3 (8). A current study has demonstrated that the role of NNMT is far from the clearance of excess vitamin B3 (9). It has demonstrated that the global epigenetic histone profiles are regulated by NNMT, and it mainly exerts the acetylation modifications through regulating the expression of sirtuin 1 (SIRT1) (9). Furthermore, enhanced NNMT expression in the liver was reported to stabilize SIRT1 protein, thereby maintaining hepatic nutrient metabolism (9); however, whether NNMT may upregulate SIRT1 in PCa has yet to be elucidated.

Elevated SIRT1 expression has been extensively identified in multiple types of human malignancy, including ovarian, liver, stomach, ductal and pancreatic cancer (10,11). In addition, various molecules are indicated to be regulated by SIRT1, including p53, forkhead box O1-4, nuclear factor- $\kappa$ B and E2F1 (12-14); however, the regulatory control of SIRT1 remains poorly understood in PCa. In the present study, it was demonstrated that increased NNMT expression elevated the expression level of SIRT1, thereby prompting the malignant progression of PCa.

## Materials and methods

**Tissue samples.** Patient-matched PCa and adjacent non-cancerous prostate tissues (15 pairs) were obtained from patients (mean age, 68.7±13.7 years; age range 45-83 years) who had undergone radical prostatectomy at the Department of Oncology, 202 Hospital of Chinese People's Liberation Army (Shenyang, China) between January 2015 to Feb 2016. The adjacent non-cancerous prostate tissues from the patients with PCa were used as controls. PCa tissue specimens (n=15) were identified as prostatic adenocarcinoma. None of the cases had received any previous cancer-associated treatment or had

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a history of any other type of cancer. Tumors were staged according to the 2010 revised TNM system (15).

The whole blood samples (5 ml) from the aforementioned patients with PCa and healthy donors (69.2±14.3) were collected in tubes containing EDTA between January 2015 and February 2016 at the Department of Oncology, 202 Hospital of Chinese People's Liberation Army. Total RNA was extracted using RNazol LS (Vigorous Biotechnology Beijing Co., Ltd., Beijing, China, <http://www.vigorousbiol.com/>), according to the manufacturer's protocols. The concentration and purity of the RNA samples were determined by OD260/OD280.

The clinicopathological characteristics are presented in Table I. All the samples were snap-frozen in liquid nitrogen immediately and were stored at -80°C following surgery until RNA extraction. The study protocols were approved by the Local Ethics Committees of 202 Hospital of the Chinese People's Liberation Army and written informed consent was obtained from all patients and healthy donors prior to tissue or blood collection.

**Cell culture.** The PCa PC-3 cell line was purchased from the Chinese Academy of Sciences Cell Bank and cells were cultured in RPMI-1640/F12 medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.), streptomycin (100 mg/ml) and penicillin (100 U/ml) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

**Construction of adeno-associated virus (AAV) vectors.** AAV-NNMT or AAV-NC was constructed by Shanghai GeneChem Co., Ltd. (Shanghai, China). The PCa PC-3 cells were seeded at the density of 10<sup>6</sup> cells/well. At 70% confluency, AAV-NNMT or AAV-NC was transfected without transfection reagent into PC-3 cells at 30 multiplicity of infection (MOI) for 48 h. Following 48 h transfection, the cells were collected for subsequent experimentation.

**Small interfering RNA (siRNA) transfection.** siRNA was synthesized based on the human NNMT target sequence (3'-GCTCAAGAGCAGCTACTACAT-5'; Shanghai Genchem Co., Ltd., Shanghai, China). In brief, PC-3 cells were seeded onto a 6-well plate and were transfected with 20 μM siRNA using Lipofectamine<sup>®</sup> 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Following transfection for 24 h, cells were collected for further experiments.

**MTT assay.** To investigate the effect of NNMT on PC-3 cell viability, PC-3 cells were seeded in 96-well tissue culture plates at a density of 5x10<sup>4</sup> cells per well in DMEM medium. When the confluence reached 70%, AAV-NNMT was transfected each well at 37°C for 24, 48 and 72 h. When the confluence reached 70%, siRNA targeting NNMT was transfected into each well for 24, 48 and 72 h, respectively. Cell viability was examined with MTT assay kits (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The blue formazan products in the cells were dissolved in 150 μl dimethyl sulfoxide (Sigma-Aldrich; Merck KGaA) and spectrophotometrically measured at a wavelength of 550 nm. All experiments were performed in triplicate.

**Scratch assay.** PC-3 cells were seeded onto a 6-well plate at a density of 5x10<sup>5</sup> cells/well until 90% confluence. Next, a scratch was produced in each well using 10-μl pipette tips. Following AAV-NNMT or AAV-NC transfection for 48 h at 37°C, the cells were washed with phosphate-buffered saline (PBS) three times and the width of the scratch was measured under an inverted microscope (IX83, Olympus Corporation, Tokyo Japan) (x10 magnification) at different time points following transfection. All the experiments were repeated three times.

**Transwell assay.** Migration and invasion assays were performed using transwell chambers with membrane pore size of 8.0 μm (Corning Incorporated, Corning, NY, USA). Membranes were uncoated for the migration assays and coated with 25 μg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) for the invasion assays. They were incubated with PBS (migration) or Matrigel during 1 h at 37°C, 5% CO<sub>2</sub> atmosphere. PC-3 cells at log-phase were prepared and fasted for 12 h in serum-free RPMI-1640/F12 as aforementioned. The cells were collected and rinsed with PBS three times. Next, cells were re-suspended at 6x10<sup>5</sup> cells/ml. Additionally, 0.1 ml cell suspension was added into 24-well Transwell chambers, with the lower chamber containing 0.5 ml RPMI-1640/F12 medium, supplemented with FBS. Following 24 h of incubation, cells were stained with 0.1% crystal violet for 10 min at room temperature and were rinsed with PBS, followed by rinsing with 33% acetic acid. Subsequently, cells were visualized under an inverted microscope (magnification, x200) to examine the microporous membrane lower cell. Subsequently, 10 randomly selected fields were used to calculate the average.

**Western blot analysis.** Total protein was extracted from PC-3 cells or PCa tissues or adjacent non-cancerous prostate tissues using radioimmunoprecipitation assay buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). A BCA protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) was used to determine the protein concentration. A total of 20 μg protein in each lane was separated by 12% SDS-PAGE, prior to being transferred onto a polyvinylidene fluoride membrane. Following blocking with 5% skimmed milk powder for 2 h at room temperature, monoclonal antibodies against NNMT (cat. no., ab58743), SIRT1 (cat. no., ab32441) and GAPDH (cat. no., ab9485) (all dilution, 1:1,000; Abcam, Cambridge, MA, USA) were applied for overnight incubation at 4°C. Non-specific binding was blocked using 8% (w/v) milk in Tris-buffered saline with 1% Tween-20 (TBST; Beijing SolarBio Science & Technology Co., Ltd.) for 2 h at room temperature. Following several washes with TBST, the membranes were incubated with horseradish-peroxidase (HRP)-conjugated goat anti-rabbit or anti-mouse IgG, or HRP-conjugated mouse anti-goat IgG (all dilution, 1:5,000; OriGene Technologies, Inc., Beijing, China) for 2 h at room temperature, prior to being washed with TBST. GAPDH was used as the internal control. Signals were detected using enhanced chemiluminescence, according to the manufacturer's protocols (EMD Millipore, Billerica, MA, USA). ImageJ 1.8.0 software (National Institutes of Health, Bethesda, MD, USA) was used for densitometry.

Table I. Clinicopathological characteristics of patients with PCa and healthy controls.

Characteristic	Healthy control	PCa	P-value
Age, years (range)	69.2 (46-85)	68.7 (48-86)	
PSA±SD, ng/ml	2.4±1.6	124.3±250.6	<0.001
Gleason score, no. (%)			
≤7		7 (46.7)	
≥8		8 (53.3)	
Stage, no. (%)			
T1-4N0M0		9 (60.0)	
Metastasis (any T, N>0, M>0)		6 (40.0)	

TNM, tumor-node-metastasis; SD, standard deviation; PSA, prostate specific antigen; PCa, prostate cancer.

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).* Total RNA was extracted from PC-3 cells or PCa tissues or adjacent non-cancerous prostate tissues using TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and was used as the template to synthesize cDNA using a reverse transcription kit (Takara Bio, Inc., Otsu, Japan) according to the instructions. Briefly, 1  $\mu$ l random primer, 1  $\mu$ l dNTP mixture and 1  $\mu$ l RNA were mixed at 65°C for 5 min. Then, 2  $\mu$ l 5X PrimeScript<sup>™</sup> Buffer, 1  $\mu$ l PrimeScript RTase, and 4  $\mu$ l DEPC H<sub>2</sub>O were added at 30°C for 10 min, 42°C for 20 min, and 95°C for 4 min. Then, the cDNA were collected. RT-qPCR was performed using a fluorescent qPCR kit (Qiagen GmbH, Hilden, Germany) and specific primers. Subsequently, PCR amplification was performed. A total of 1  $\mu$ g cDNA was used for qPCR using the SYBR<sup>®</sup> green Master mix (Roche Diagnostics, Basel, Switzerland) and a Roche Lightcycler 480 (Roche Diagnostics) at 95°C for 10 min followed by 50 cycles of: 95°C for 10 sec; 55°C for 10 sec; 72°C for 5 sec; 99°C for 1 sec; 59°C for 15 sec; 95°C for 1 sec; and cooling to 40°C. All experiments were performed in triplicate against a GAPDH housekeeping gene. Relative expression was normalized against the endogenous control, GAPDH, using the 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method (16). The primers for NNMT, SIRT1, and GAPDH were listed as follows: NNMT-forward (f), 5'-CTGCCTAGACGGTGT GAAGG-3'; NNMT-reverse (r), 5'-CTTGACCGCCTGTCTCAA CT-3'; SIRT1-f, 5'-CCTGCCTGGATCCCCTTAGT-3'; SIRT1-r, 5'-GGCCTGTTGCTCTCCTCATT-3'; GAPDH-f, GAGAAG GCTGGGGCTCATTT; GAPDH-r, AGTGATGGCATGGAC TGTGG.

*Nicotinamide treatment.* 10  $\mu$ g/ $\mu$ l Nicotinamide (72340, Sigma-Aldrich; Merck KGaA) was dissolved in ddH<sub>2</sub>O. To evaluate the effects of nicotinamide on the expression of SIRT1, nicotinamide was added in the RPMI-1640/F12 culture at the final concentration of 20 ng/ $\mu$ l in the presence or absence of AAV-NNMT.

*Statistical analysis.* SPSS 19.0 software (IBM Corp., Armonk, NY, USA) was used to process all of the collected data,

and those that fit a normal distribution are presented as the mean  $\pm$  standard deviation. Multiple-group comparisons were performed using analysis of variance, followed by the least significant difference post hoc test. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Elevated levels of NNMT in the peripheral blood and tissues of patients with PCa.* In the present study, the NNMT mRNA level in the peripheral blood of healthy donors was used as a negative control (NC) when comparing with the NNMT mRNA level of NNMT in the peripheral blood of patients with PCa. In order to compare the expression of NNMT in prostate tissues, the PCa tissues and adjacent non-cancerous prostate tissues were collected from patients with PCa. To begin with, the expression of NNMT in the peripheral blood and tissues of patients with PCa was evaluated. As depicted in Fig. 1A, the mRNA level of NNMT was notably elevated in the peripheral blood of patients with PCa, compared with that in the NC samples. The expression of NNMT in the tissues of patients with PCa was also evaluated and the data demonstrated that NNMT expression was notably increased, compared with that in the NC adjacent non-cancerous prostate tissues (Fig. 1B). Furthermore, the protein expression level of NNMT was determined to be increased in the tissues of patients with metastases, compared with that of the patients without metastases (Fig. 1C).

*NNMT enhances the expression of SIRT1 in PC-3 cells.* Next, the possible association between NNMT and SIRT expression was evaluated. Overexpression of NNMT significantly increased the mRNA expression of SIRT1 in PC-3 cells (P<0.05; Fig. 2A). Furthermore, western blot analysis indicated that the protein expression of SIRT1 was elevated in the tissues of patients with PCa, compared with that in the tissues of NC subjects (Fig. 2B).

*NNMT promotes PC-3 cell viability, migration and colony formation.* Following this, the effect of NNMT on the malignant phenotype of PC-3 cells was investigated. An MTT assay demonstrated that the overexpression of NNMT increased the cell viability by 34.5, 78.6 and 123.2% at 24 (P<0.05), 48 (P<0.05) and 72 h (P<0.01), respectively (Fig. 3A). By contrast, the knockdown of NNMT notably suppressed PC-3 cell viability by 12.3, 22.1 and 34.5% at 24, 48 (P<0.05) and 72 h (P<0.05), respectively (Fig. 3B). Furthermore, the upregulation of NNMT elevated the cell migration and colony formation capacity (Fig. 3C and D).

*NNMT exerts its oncogenic role primarily by upregulating SIRT1.* Additionally, the underlying mechanism through which NNMT promotes the malignancy of PCa was investigated. Therefore, an SIRT1 inhibitor, nicotinamide, was selected. As depicted in Fig. 4A, treatment with nicotinamide notably suppressed the expression of SIRT1 even in cells transfected with adeno-associated virus (AAV)-NNMT (Fig. 4A). At the same time, treatment with nicotinamide suppressed PC-3 cell migration (Fig. 4B). By contrast, the upregulation of NNMT notably abolished the nicotinamide-reduced cell migration

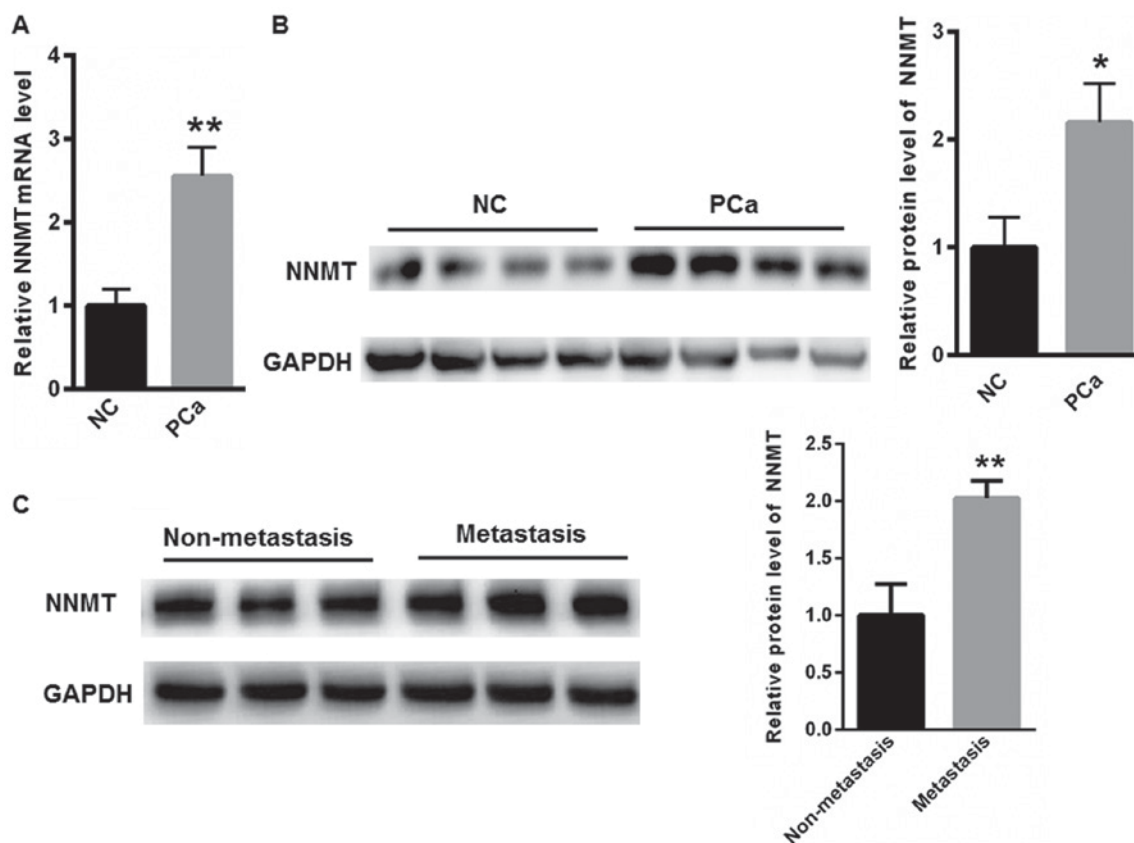


Figure 1. Elevated expression of NNMT in the peripheral blood and tissues of patients with PCa. (A) The mRNA expression level of NNMT was notably elevated in the peripheral blood of patients with PCa, compared with that in the blood of the healthy donors. (B) Western blot analysis demonstrated that NNMT expression was markedly increased in the tissues of patients with PCa, compared with expression in the NC adjacent non-cancerous prostate tissues. (C) The protein expression of NNMT was increased in the tissues of patients with metastases, compared with that in the tissues of patients without metastases. \* $P < 0.05$ , \*\* $P < 0.01$  vs. control. NNMT, nicotinamide N-methyltransferase; NC, negative control; PCa, prostate cancer.

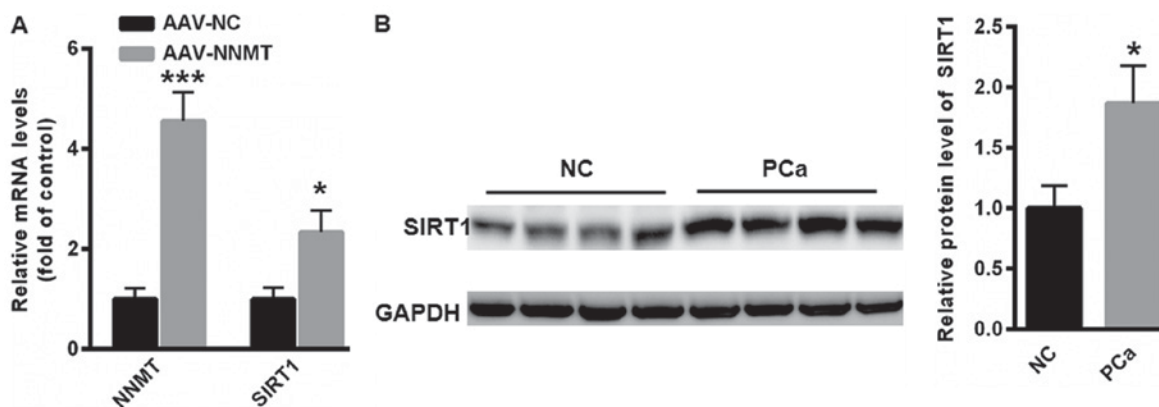


Figure 2. NNMT enhances the expression of SIRT1 in PC-3 cells. (A) NNMT significantly increased the mRNA expression level of SIRT1 in PC-3 cells. (B) Western blot analysis indicated that the protein expression of SIRT1 was elevated in the tissues of patients with PCa, compared with that in the adjacent non-cancerous tissues. \* $P < 0.05$ , \*\*\* $P < 0.001$  vs. control. NNMT, nicotinamide N-methyltransferase; NC, negative control; PCa, prostate cancer; SIRT1, sirtuin 1; AAV, adeno-associated virus.

capacity (Fig. 4B). These data indicated that NNMT enhanced PC-3 cell malignancy primarily through the upregulation of NNMT.

## Discussion

PCa is the most common neoplasia among males globally (17). Therefore, it is of great importance to investigate

the molecular mechanisms underlying PCa development and progression. In the present study, the primary focus was on NNMT, a phase II metabolizing enzyme that primarily catalyzes the methylation of nicotinamide and other pyridines (9). The data indicated that the level of NNMT was elevated in the peripheral blood and tissues of patients with PCa, indicating a potential oncogenic role of NNMT in the progression of PCa.

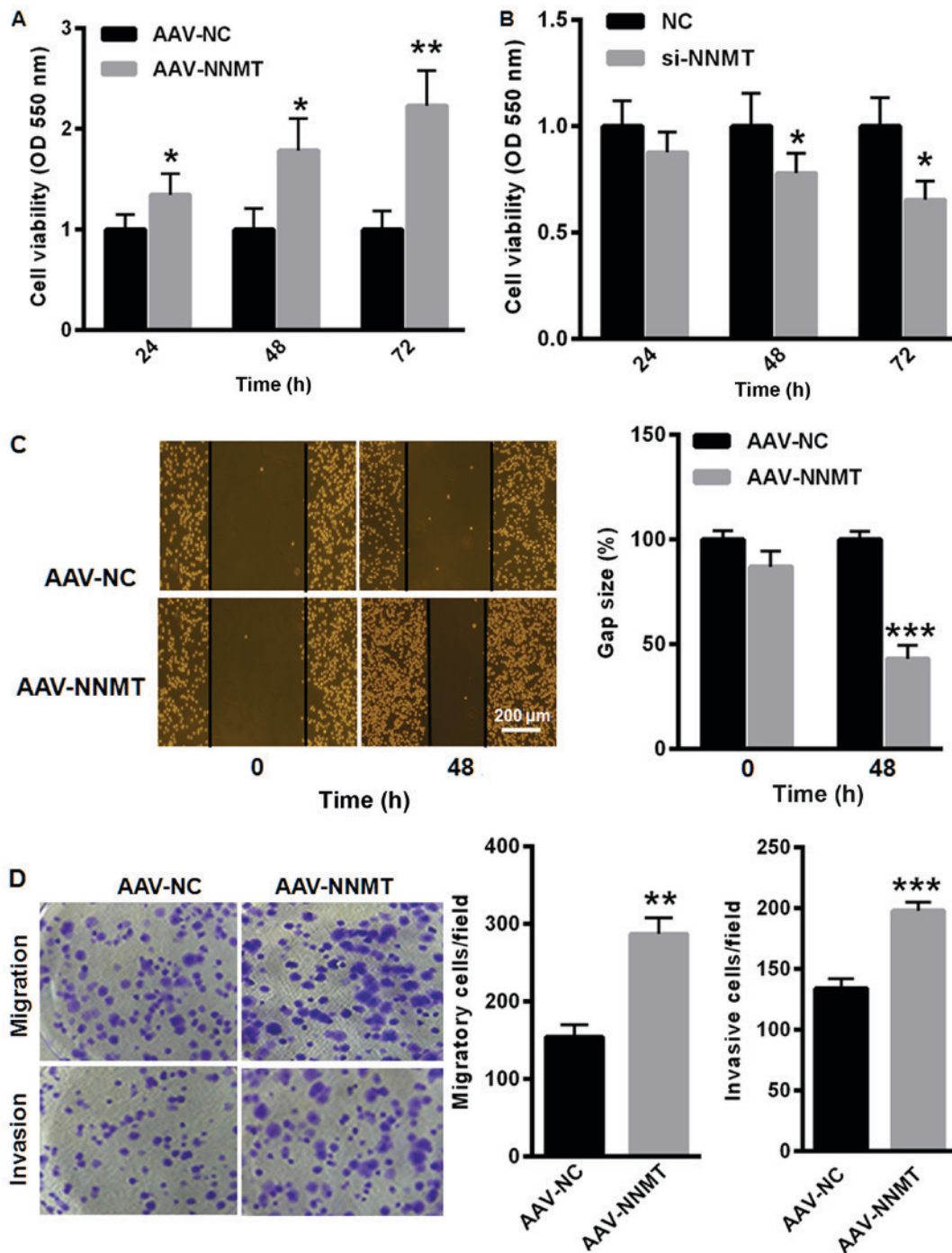


Figure 3. NNMT promotes PC-3 cell viability, migration and colony formation. (A) Overexpression of NNMT elevated the viability of PC-3 cells by 34.5, 78.6 and 123.2% at 24, 48 and 72 h, respectively. (B) Knockdown of NNMT markedly suppressed PC-3 cell viability by 12.3, 22.1 and 34.5% at 24, 48 and 72 h, respectively. Upregulation of NNMT elevated the cell migration and invasion capacity as analyzed by the (C) scratch assay, and (D) invasion and migration assays. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. control. NNMT, nicotinamide N-methyltransferase; NC, negative control; AAV, adeno-associated virus; si, small interfering; OD, optical density.

Previous studies have been focused on the functional roles of NNMT in metabolic diseases, including diabetes and obesity (9,18). Other studies reported its involvement in the development and progression of carcinoma *in vivo* (19,20). Multiple subsequent data have indicated that the upregulation of NNMT in various cancer types is associated with a poor prognosis (19,21). Furthermore, NNMT was also demonstrated to enhance the proliferative, migratory, invasive and differentiation capacities of different types of tumor (20,22).

For instance, the overexpression of NNMT promoted renal carcinoma cell proliferation and invasion mainly by activating the phosphoinositide 3-kinase/Akt pathway in nasopharyngeal carcinoma (21). In the present study, NNMT expression was demonstrated to be increased in PCa, but the biological function and underlying mechanisms of this remain unclear.

The *in vitro* experiments of the present study revealed that the overexpression of NNMT significantly enhanced PC-3 cell viability, cell migration and invasive capacity. These oncogenic

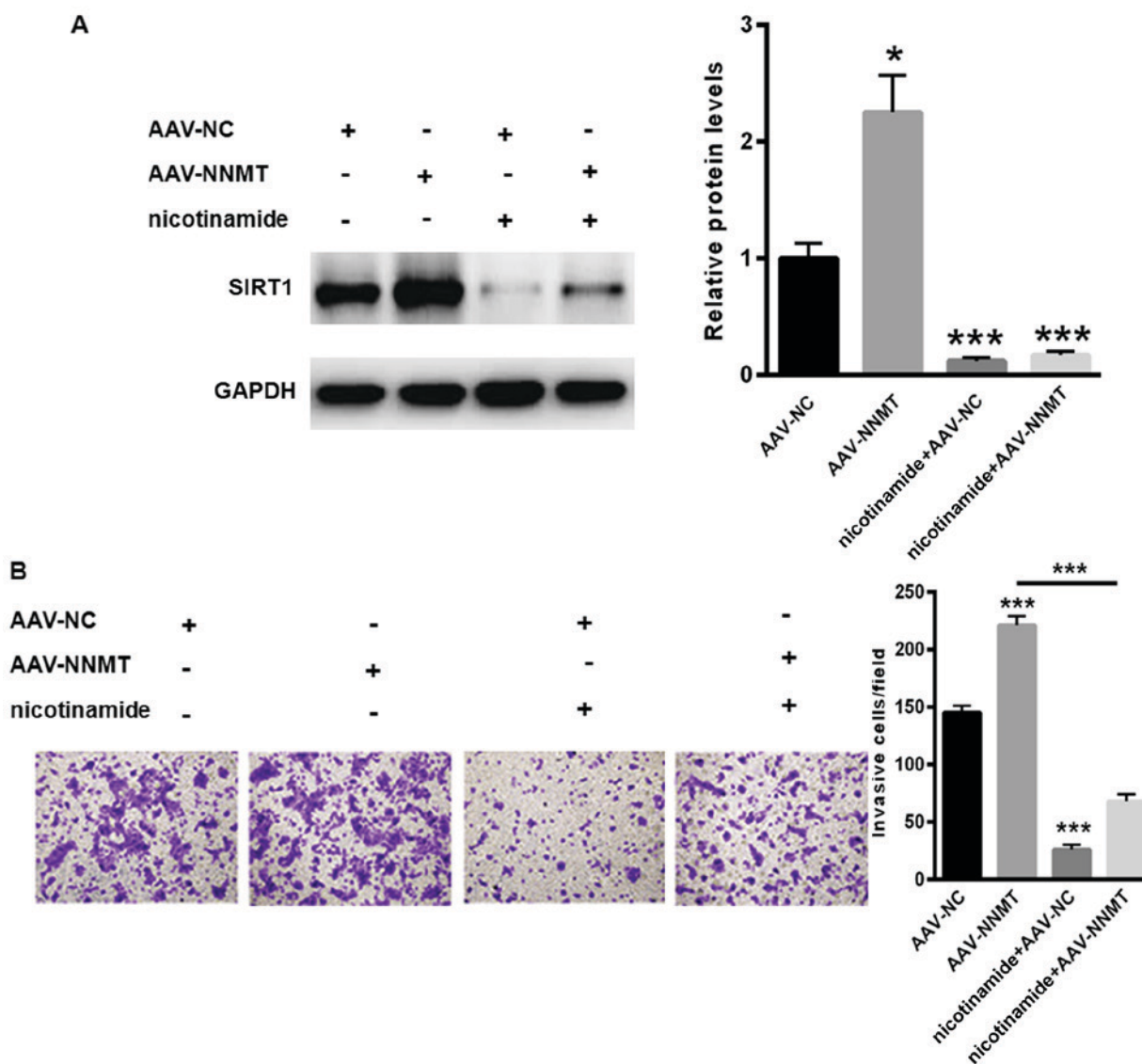


Figure 4. NNMT exerts its oncogenic role primarily by upregulating SIRT1. (A) Treatment with nicotinamide notably suppressed the expression of SIRT1. (B) The Transwell assay demonstrated that the upregulation of NNMT notably abolished the nicotinamide-induced reduction in the cell migration capacity. \* $P < 0.05$ , \*\*\* $P < 0.001$  vs. control. NNMT, nicotinamide N methyltransferase; NC, negative control; AAV, adeno-associated virus; SIRT1, sirtuin 1.

results prompted additional investigation into the underlying mechanism of NNMT in the development of PCa. Recently, NNMT was demonstrated to stabilize the mRNA and protein expression levels of SIRT1, thereby maintaining lipid homeostasis in the liver (9); however, whether NNMT functions through SIRT1 in PCa has yet to be investigated. Previous studies have indicated that SIRT1 serves an important role in multiple physiological processes, including aging, metabolism, neurogenesis and cell survival, based on its capacity to deacetylate histone and non-histone substrates (23,24). For instance, SIRT1 was indicated to increase the expression of matrix metalloproteinase-2, thereby enhancing PCa cell invasion (25). In order to validate the role of SIRT1 in PCa malignancies in the present study, a specific inhibitor, nicotinamide, was selected. It was determined that the PC-3 cell invasion capacity was notably decreased by nicotinamide treatment, even in PC-3 cells transfected with AAV-NNMT. These data indicated that NNMT enhanced PC-3 cell migration and invasion primarily through regulating SIRT1 expression.

In summary, the present study indicated that NNMT is an important regulator of SIRT1 expression in PC-3 cells and may be a potential therapeutic target for PCa.

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#### Availability of data and materials

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

### Authors' contributions

ZY performed the experiments and analyzed the data. YL performed the western blotting. XL conceived the idea, supervised the study and wrote the manuscript.

### Ethics approval and consent to participate

The study protocols were approved by the Local Ethics Committees of 202 Hospital of the Chinese People's Liberation Army and written informed consent was obtained from all patients and healthy donors prior to tissue or blood collection.

### Consent for publication

The patients and healthy control provided written informed consent for the publication of any associated data and accompanying images.

### Competing interests

The authors declare that they have no competing interests.

### References

- Wang X, Yang B and Ma B: The UCA1/miR-204/Sirt1 axis modulates docetaxel sensitivity of prostate cancer cells. *Cancer Chemother Pharmacol* 78: 1025-1031, 2016.
- Byles V, Zhu L, Lovaas JD, Chmielewski LK, Wang J, Faller DV and Dai Y: SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis. *Oncogene* 31: 4619-4629, 2012.
- Xu Y, Liu P, Zheng DH, Wu N, Zhu L, Xing C and Zhu J: Expression profile and prognostic value of NNMT in patients with pancreatic cancer. *Oncotarget* 7: 19975-19981, 2016.
- Zhou W, Gui M, Zhu M, Long Z, Huang L, Zhou J, He L and Zhong K: Nicotinamide N-methyltransferase is overexpressed in prostate cancer and correlates with prolonged progression-free and overall survival times. *Oncol Lett* 8: 1175-1180, 2014.
- Chlopicki S, Kurdziel M, Sternak M, Szafarz M, Szymura-Oleksiak J, Kamiński K and Żołądź JA: Single bout of endurance exercise increases NNMT activity in the liver and MNA concentration in plasma; the role of IL-6. *Pharmacol Rep* 64: 369-376, 2012.
- Sartini D, Pozzi V, Renzi E, Morganti S, Rocchetti R, Rubini C, Santarelli A, Lo Muzio L and Emanuelli M: Analysis of tissue and salivary nicotinamide N-methyltransferase in oral squamous cell carcinoma: Basis for the development of a noninvasive diagnostic test for early-stage disease. *Biol Chem* 393: 505-511, 2012.
- Sartini D, Muzzonigro G, Milanese G, Pozzi V, Vici A, Morganti S, Rossi V, Mazzucchelli R, Montironi R and Emanuelli M: Upregulation of tissue and urinary nicotinamide N-methyltransferase in bladder cancer: Potential for the development of a urine-based diagnostic test. *Cell Biochem Biophys* 65: 473-483, 2013.
- Pissios P: Nicotinamide N-Methyltransferase: More than a vitamin B3 clearance enzyme. *Trends Endocrinol Metab* 28: 340-353, 2017.
- Hong S, Moreno-Navarrete JM, Wei X, Kikukawa Y, Tzamelis I, Prasad D, Lee Y, Asara JM, Fernandez-Real JM, Maratos-Flier E and Pissios P: Nicotinamide N-methyltransferase regulates hepatic nutrient metabolism through Sirt1 protein stabilization. *Nat Med* 21: 887-894, 2015.
- Cui Y, Li J, Zheng F, Ouyang Y, Chen X, Zhang L, Chen Y, Wang L, Mu S and Zhang H: Effect of SIRT1 gene on Epithelial-mesenchymal transition of human prostate cancer PC-3 cells. *Med Sci Monit* 22: 380-386, 2016.
- Duan K, Ge YC, Zhang XP, Wu SY, Feng JS, Chen SL, Zhang LI, Yuan ZH and Fu CH: miR-34a inhibits cell proliferation in prostate cancer by downregulation of SIRT1 expression. *Oncol Lett* 10: 3223-3227, 2015.
- Hoffmann MJ, Engers R, Florl AR, Otte AP, Muller M and Schulz WA: Expression changes in EZH2, but not in BMI-1, SIRT1, DNMT1 or DNMT3B are associated with DNA methylation changes in prostate cancer. *Cancer Biol Ther* 6: 1403-1412, 2007.
- Jung-Hynes B and Ahmad N: Role of p53 in the anti-proliferative effects of Sirt1 inhibition in prostate cancer cells. *Cell Cycle* 8: 1478-1483, 2009.
- Nakane K, Fujita Y, Terazawa R, Atsumi Y, Kato T, Nozawa Y, Deguchi T and Ito M: Inhibition of cortactin and SIRT1 expression attenuates migration and invasion of prostate cancer DU145 cells. *Int J Urol* 19: 71-79, 2012.
- Cheng L, Montironi R, Bostwick DG, Lopez-Beltran A and Berney DM: Staging of prostate cancer. *Histopathology* 60: 87-117, 2012.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup>(Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Wang BD, Ceniccola K, Hwang S, Andrawis R, Horvath A, Freedman JA, Olender J, Knapp S, Ching T, Garmire L, *et al*: Alternative splicing promotes tumour aggressiveness and drug resistance in African American prostate cancer. *Nat Commun* 8: 15921, 2017.
- Trammell SA and Brenner C: NNMT: A bad actor in fat makes good in liver. *Cell Metab* 22: 200-201, 2015.
- Palanichamy K, Kanji S, Gordon N, Thirumoorthy K, Jacob JR, Litzenberg KT, Patel D and Chakravarti A: NNMT silencing activates tumor suppressor PP2A, inactivates oncogenic STKs, and inhibits tumor forming ability. *Clin Cancer Res* 23: 2325-2334, 2017.
- Xie X, Liu H, Wang Y, Zhou Y, Yu H, Li G, Ruan Z, Li F, Wang X and Zhang J: Nicotinamide N-methyltransferase enhances resistance to 5-fluorouracil in colorectal cancer cells through inhibition of the ASK1-p38 MAPK pathway. *Oncotarget* 7: 45837-45848, 2016.
- Win KT, Lee SW, Huang HY, Lin LC, Lin CY, Hsing CH, Chen LT and Li CF: Nicotinamide N-methyltransferase overexpression is associated with Akt phosphorylation and indicates worse prognosis in patients with nasopharyngeal carcinoma. *Tumour Biol* 34: 3923-3931, 2013.
- Yu T, Wang YT, Chen P, Li YH, Chen YX, Zeng H, Yu AM, Huang M and Bi HC: Effects of nicotinamide N-methyltransferase on PANC-1 cells proliferation, metastatic potential and survival under metabolic stress. *Cell Physiol Biochem* 35: 710-721, 2015.
- Liu T, Liu PY and Marshall GM: The critical role of the class III histone deacetylase SIRT1 in cancer. *Cancer Res* 69: 1702-1705, 2009.
- Yamamoto H, Schoonjans K and Auwerx J: Sirtuin functions in health and disease. *Mol Endocrinol* 21: 1745-1755, 2007.
- Lovaas JD, Zhu L, Chiao CY, Byles V, Faller DV and Dai Y: SIRT1 enhances matrix metalloproteinase-2 expression and tumor cell invasion in prostate cancer cells. *Prostate* 73: 522-530, 2013.



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