Medical Principles and Practice

Original Paper

Med Princ Pract
DOI: 10.1159/000535479

Received: June 20, 2023
Accepted: November 23, 2023
Published online: November 29, 2023

NSUN6 Regulates NM23-H1 Expression in an m5C Manner to Affect Epithelial-Mesenchymal Transition in Lung Cancer

Zhansheng Lu Bo Liu Demiao Kong Xiaojiang Zhou Dengke Pei Di Liu

Department of Thoracic Surgery, Guizhou Provincial People's Hospital, Guiyang City, PR China

Highlights of the Study

- Levels of NSUN6 are low in lung cancer and may play a role in promoting cancer.
- NM23-H1 was downregulated in lung cancer cells.
- Over-expression of NSUN6 inhibits lung cancer cell proliferation, migration, and EMT by regulating NM23-H1.
- NSUN6 regulated NM23-H1 expression by modifying its mRNA 3′-UTR through m5C.

Keywords
Lung cancer · NSun family member 6 · NM23-H1 · 5-Methylcytosine · Epithelial-mesenchymal transition

Abstract
Purpose: The expression and regulatory mechanism of NSUN6 in lung cancer are still unclear. Our study explored whether NSUN6 mediates progression of lung cancer by affecting NM23-H1 expression in an m5C-dependent manner. ***Methods:*** qRT-PCR, CCK-8, colony formation, transwell, and Western blot analysis were employed to probe the impact of NSUN6 on lung cancer cell proliferation, migration, and epithelial-mesenchymal transition (EMT). RMVar database was utilized to forecast the downstream genes of NSUN6. The mode of interaction between NSUN6 and NM23-H1 was determined by dot blot, luciferase assay, m5C RIP, and cell function assays. The effect of NSUN6 expression on tumor growth was verified in vivo. ***Results:*** Expression of NSUN6 was reduced in lung cancer cells, and over-expression of NSUN6 restricted the proliferation of lung cancer cells, migration, and EMT. NSUN6 regulated NM23-H1 expression by modifying the 3′-UTR of NM23-H1 mRNA through m5C and inhibited lung cancer cell proliferation, migration, and EMT. In vivo experiments also showed that over-expression of NSUN6 inhibited the occurrence of lung cancer. ***Conclusion:*** NSUN6 regulates NM23-H1 expression in an m5C-dependent manner to affect EMT in lung cancer. Thus, NSUN6 may be considered as a potential therapeutic target for lung cancer.

© 2023 The Author(s).
Published by S. Karger AG, Basel

Introduction

Lung cancer is the most prevalent neoplasm in the world [1]. In China, it accounts for 37% of the global incidence and has a mortality rate of about 40% [2]. It is insidious and usually has no obvious manifestations in the early stage, resulting in most patients being diagnosed

karger@karger.com
www.karger.com/mpp

© 2023 The Author(s).
Published by S. Karger AG, Basel

This article is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC) (http://www.karger.com/Services/OpenAccessLicense). Usage and distribution for commercial purposes requires written permission.

Karger
OPEN ACCESS

Correspondence to:
Zhansheng Lu, luzhansheng0155@163.com

at an advanced stage [3, 4]. It is managed by a combination of chemotherapy, radiotherapy, and surgery [5]. Although treatment has greatly improved, drug resistance, tumor plasticity, and heterogeneity have led to challenging treatment problems, and the overall 5-year survival rate remains less than 20% [6, 7].

RNA post-transcriptional modification regulates cell biological behavior by adding a chemical group to the nucleotide strand of RNA, generally termed the epitranscriptome [8, 9]. It is well known that epigenetic alterations may lead to a variety of diseases, including cancer [10]. Modification of cytosine by methylation (5-methylcytosine [m5C]) is a major epigenetic RNA modification, and increasing evidence shows that it is associated with development of many cancers [11, 12]. NSun family member 6 (NSUN6) is an RNA m5C transferase, which can stabilize mRNA by binding to particular sites in the 3′-UTR and is thought to participate in the regulation of cancer progression [13]. NSUN6 inhibits the development of pancreatic cancer by regulating cell proliferation [14], but its expression in lung cancer and its regulatory function are still unclear.

Non-metastatic protein 23 H1 (NM23-H1), a housekeeping enzyme, was originally thought to be a tumor suppressor gene [15, 16]. The downregulation and heterogeneous deficiency of this gene are associated with the high metastasis and poor prognosis of lung cancer [17, 18], but the mechanism remains unclear. Previous studies have found that NM23-H1 mutation is usually accompanied by changes in tumor metastasis-related genes [19]. In addition, the RMVar database shows that the 3′-UTR of NM23-H1 includes m5C modification sites, suggesting that NSUN6 may mediate the progression of lung cancer by affecting the expression of NM23-H1 in an m5C-dependent manner.

We investigated the function of NSUN6 in regulating lung cancer progression both in vitro and in vivo. We explored the effect of the interaction between NSUN6 and NM23-H1 on the progression of lung cancer. Our findings suggest that NSUN6 mediates the expression of NM23-H1 by m5C modification, which affects the epithelial-mesenchymal transition (EMT) observed in lung cancer.

Materials and Methods

Cell Culture and Transfection

Human bronchial epithelial cells (BEAS-2B) and lung cancer cell lines (A549, PC9, H1299, H1975) were obtained from Shanghai Cell Resource Center. The cells were cultured in DMEM complete medium supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. Experiments were performed using cells that were cultured to the logarithmic growth phase. H1299 cells (1×10^5) were spread into a 6-well plate, and transfected, when about 80% confluent, with NSUN6 over-expression plasmid (pc-NSUN6), NM23-H1 knockdown plasmid (sh-NM23-H1-wild type [WT], sh-NM23-H1-mutant [MUT]), or their NC controls (Sigma-Aldrich, USA) using Lipofectamine 2000 transfection reagent as described by the manufacturer (Thermo Fisher Scientific, USA). The stably transfected cell line was prepared with puromycin (2 μg/mL) and hygromycin B (500 μg/mL) for 2–3 days for selection, according to the manufacturer's instructions.

Quantitative Real-Time PCR

Total RNA was extracted using the standard TRIzol method, and yields/purity were measured using the Nanodrop 2000 (Thermo Fisher Scientific, USA). Total RNA was reverse transcribed into cDNA using PrimeScript™ Reverse Transcription Master Mix (code No. RR036A; Takara) and subsequently used for PCR amplification of NSUN6 and NM23-H1 (and GAPDH as internal control) using an ABI StepOne Plus System (Applied Biosystems, NJ, USA). There were 40 cycles of 95°C for 5 min, 95°C for 20 s, and 59°C for 15 s. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for amplification were NSUN6 sense, 5′-ATC TGC GTC CGT TTC ACC-3′, and antisense, 5′-GCT TCC ACC ACA CCT CAT C-3′; NM23-H1 sense, 5′-GCA GCC GGA GTT CAA ACC TA-3′, and antisense, 5′-TGC ACA CCA GGC TGA CTT AG-3′; GAPDH sense, 5′-TAT GAT GAT ATC AAG AGG GTA GT-3′, and antisense, 5′-TGT ATC CAA ACT CAT TGT CAT AC-3′.

CCK-8 Proliferation Assay

Single-cell suspensions of H1299 cells grown to logarithmic phase before harvesting were seeded into 96-well plates at 2×10^4 mL per well. CCK-8 reagent (Dojindo, Shanghai, China) was added at 0, 24, 48, and 72 h. The absorbance value (at 450 nm) was detected on a microplate reader (BIOTEK, VT, USA).

Clone Formation Assay

H1299 cells (1×10^5 cells/well) were seeded into 6-well plates, and the culture medium was replaced every 48 h and discarded after 10–14 days of culture. Cells were then fixed with 4% paraformaldehyde for 30 min after washing in PBS. The cells were then stained with 4% crystal violet for 15 min, and the number of cell clones was counted under a microscope (Zeiss Axio Vert.A1, Jena, Germany).

Transwell Assay

At 24 h after transfection, H1299 cells were harvested by trypsinization and spread at a density of 1.5×10^5 into transwell chambers with a pore size of 0.8 μm inside 24-well plates. Chambers were washed once with PBS after 24 h of plating. They were then fixed with methanol for 20 min and stained with crystal violet for 20 min. Cells in the upper chamber were removed by using a cotton swab; migrated cells were photographed under an inverted microscope and then counted in three randomly selected fields per well. The experiment was repeated three times.

Western Blot

RIPA lysis buffer (PC101, EpiZyme, Shanghai, China) contains protease inhibitors and protease phosphorylation inhibitors to extract total cell lysates. Supernatants were collected after centrifugation. Total protein was detected by BCA protein quantification. Protein samples (20 μg) were separated by 10% SDS-PAGE protein electrophoresis (120 V for 60 min) and then transferred onto the membrane. The membrane was blocked with 5% nonfat milk at 25°C for 2 h, Then, primary antibodies against NSUN6 (Abcam, ab307430, 1:1,000), NM23-H1 (Abcam, ab92327, 1: 1,000), E-cadherin (Abcam, ab76055, 1:1,000), N-cadherin (Abcam, ab245117, 1:1,000), vimentin (Abcam, ab92547, 1:1,000), and GAPDH (Abcam, ab245355, 1:5,000) were added, and membranes were incubated overnight. The secondary antibody was then added, and cells were incubated at 25°C for 2 h. Protein expression was detected using chemiluminescence ECL reagent visualization. Image ProPlus software was utilized to quantify the relative expression of target proteins, GAPDH as an internal control.

Dot Blot Assay

After RNA extraction, diluted RNA (100 ng/μL) was added to the Hybond-N+ membranes (GE HealthCare, USA). RNA on the spot membrane was then cross-linked with ultraviolet light, and blocked and incubated with m5C antibody (Abcam, ab186830). Subsequently, HRP-coupled anti-rabbit IgG secondary antibody was added, and the dot blot was observed using the ECL Western blot (WB) detection kit (Thermo Fisher Scientific).

Dual-Luciferase Reporter Assay

We used the RMVar database (https://rmvar.renlab.org/) to predict the downstream targets of NSUN6. The 3′-UTR sequence of NSUN6-predicted target gene NM23-H1 was downloaded. The NM23-H1 binding region was inserted into the pMIR reporter luciferase vector and named pMIR-NM23-H1-WT. The binding site of NM23-H1 in the 3′-UTR was mutated and cloned into the plasmid pMIR-NM23-H1-MUT. H1299 cells were passaged into a 24-well plate with 80% confluence. pc-NSUN6, pc-NC, and luciferase plasmid pMIR-NM23-H1-WT/MUT were transfected into H1299 cells using Lipofectamine 2000. After 48 h of culture, luciferase activity was measured by the dual luciferase reporter system.

m5C RNA Immunoprecipitation

The Magna RNA Immunoprecipitation (RIP) Kit (Millipore, MA, USA) combined with quantitative real-time PCR (qRT-PCR) method was utilized to verify the binding of NSUN6 to NM23-H1. H1299 cells were lysed using RIP lysates. Then, agarose beads containing NM23-H1 or HDGF (as positive control), and GAPDH or ACTIN (as negative control) were incubated with the cell lysates at 4°C for 6 h. Beads were treated with proteinase K buffer to remove proteins. Then, immunoprecipitated RNA was obtained using TRIzol reagent, and the enrichment of NM23-H1 was detected by qRT-PCR.

Subcutaneous Xenograft Nude Mice Model

Four-week-old male BALB/c nude mice from the Experimental Animal Center, Cancer Hospital, Chinese Academy of Medical Sciences, were adaptively housed for 7 days before use. Transfected H1299 cell monolayers in the logarithmic growth phase were washed with 1×PBS and resuspended in DMEM medium; cell concentration was adjusted to 5×10^7 cells/mL. The skin of left forelimb costal abdomen of mice was sterilized, and cell suspension (0.1 mL) was injected subcutaneously to establish the xenograft models. The nude mice were randomly divided into two groups: pc-NC group and pc-NSUN6 group. Six mice were inoculated in each group. Tumor volume ($0.5\times$ length $\times$ width^2) was measured every 7 days with calipers after inoculation and observed continuously. After 4 weeks, the mice were anesthetized and sacrificed, and the tumor was removed for further analysis, including weight measurement.

Immunohistochemistry

After the tumors were removed from the nude mice, they were fixed with 4% formaldehyde for 24 h, and the tumor tissue was sectioned into 4 μm slices, fixed again for 30 min, rinsed with running water for 1 h, and put into an automatic dehydrator to gradually remove water. Paraffin sections were deparaffinized with xylene, hydrated with gradient ethanol, rinsed with distilled water and PBS, and blocked with peroxidase for 10 min. Then, primary antibody (Abcam; Ki-67, ab16667, 1:200; NSUN6, ab214227, 1:200; NM23-H1, ab92327, 1:200) and secondary antibody (1:400) were added successively, and DAB color solution was dropped. After dropping hematoxylin nuclei for counterstaining and dehydration with gradient ethanol, the slides were covered with coverslips and allowed to stand at 25°C, and the sections were photographed under a microscope after drying.

Statistical Analysis

SPSS software (v 22.0) was utilized for data analysis. The data are presented as mean ± standard deviation; *t* test was utilized for comparison between two groups. Analysis of variance was utilized for comparison among multiple groups, and LSD *t* test was utilized for further pairwise comparison. $p < 0.05$ indicates a statistically significant difference.

Results

Expression of NSUN6 Is Decreased in Lung Cancer

We used the Cancer Genome Atlas (TCGA) dataset analysis (https://genome-cancer.ucsc.edu/), qRT-PCR, and WB to explore NSUN6 expression in lung cancer tissues and cells. The TCGA dataset assay showed that expression of NSUN6 was decreased in lung cancer (Fig. 1a). WB and qRT-PCR assays indicated that NSUN6 expression was markedly reduced in lung cancer cells in comparison with BEAS-2B cells (Fig. 1b, c). As the expression of NSUN6 was lowest in H1299 cells, this cell line was selected for subsequent experiments.

Ectopic Over-Expression of NSUN6 Results in Reduced Lung Cancer Cell Proliferation, Migration, and EMT

To explore the relationship between NSUN6 and the cytological behavior of lung cancer cells, we constructed NSUN6 overexpressing vectors. qRT-PCR and

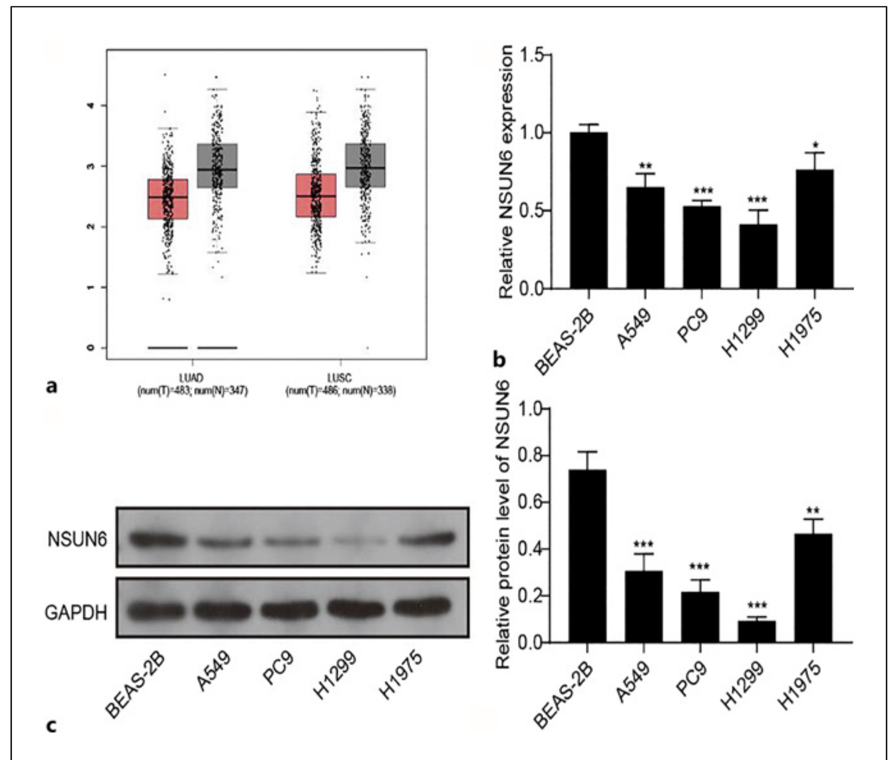


Fig. 1. Expression of NSUN6 in lung cancer. **a** TCGA database was used to study NSUN6 expression in lung cancer. **b**, **c** The qRT-PCR and WB assays were conducted to evaluate NSUN6 expression in lung cancer cell lines (A549, PC9, H1299, H1975) and human bronchial epithelial cells (BEAS-2B). Cell experiments were repeated three times. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

WB analysis indicated that NSUN6 expression was upregulated after over-expression of NSUN6, confirming the successful construction of the vector (Fig. 2a, b). Over-expression of NSUN6 markedly reduced the proliferation and migration of lung cancer cells as detected by CCK-8, colony formation, and transwell assay (Fig. 2c–e). As EMT is associated with tumor metastasis, we subsequently tested the expression levels of EMT-related proteins. As anticipated, expression of E-cad was significantly augmented when NSUN6 was overexpressed, while N-cad and vimentin were both reduced (Fig. 2f).

Over-Expression of NSUN6 Inhibits Lung Cancer Cell Proliferation, Migration, and EMT by Regulating NM23-H1

NM23-H1 is related to tumor invasiveness and prognosis of lung cancer patients [18]. The qRT-PCR analysis demonstrated that NM23-H1 expression was decreased in lung cancer cells compared to BEAS-2B cells (Fig. 3a). To investigate whether NSUN6 influences lung cancer progression by regulating NM23-H1 expression, rescue experiments were conducted. qRT-PCR and WB analysis showed that expression of NM23-H1 was markedly elevated after over-expression of NSUN6 and reduced significantly after knockdown of NM23-H1 (Fig. 3b, c). CCK-8, colony formation, and transwell analysis showed that knockdown of NM23-H1 led to the partial blocking of the restriction effect of NSUN6 over-expression on lung cancer cell proliferation and migration (Fig. 3d–f). WB results also showed that knockdown of expression of NM23-H1 partially reversed the regulatory effect of over-expression of NSUN6 on EMT-related proteins (Fig. 3g).

NSUN6 Regulates Expression of NM23-H1 by Modifying Its mRNA 3′-UTR through m5C

To further clarify the mechanism by which NSUN6 regulates NM23-H1, dot blot assay, RMVar database, luciferase assay, and RIP assays were performed to explore the binding of NSUN6 and NM23-H1. Dot blot assay showed that over-expression of NSUN6 and sh-NM23-H1-MUT decreased the m5C level, but there was no difference in m5C levels between sh-NM23-H1-WT and pc-NC groups (Fig. 4a). The RMVar database showed that the 3′-UTR of NM23-H1 contained an m5C modification site, and the plasmid was designed based on the 3′-UTR mutation site of NM23-H1 (Fig. 4b). Luciferase assay showed that NM23-H1-WT reporter gene luciferase activity in pc-NSUN6 group

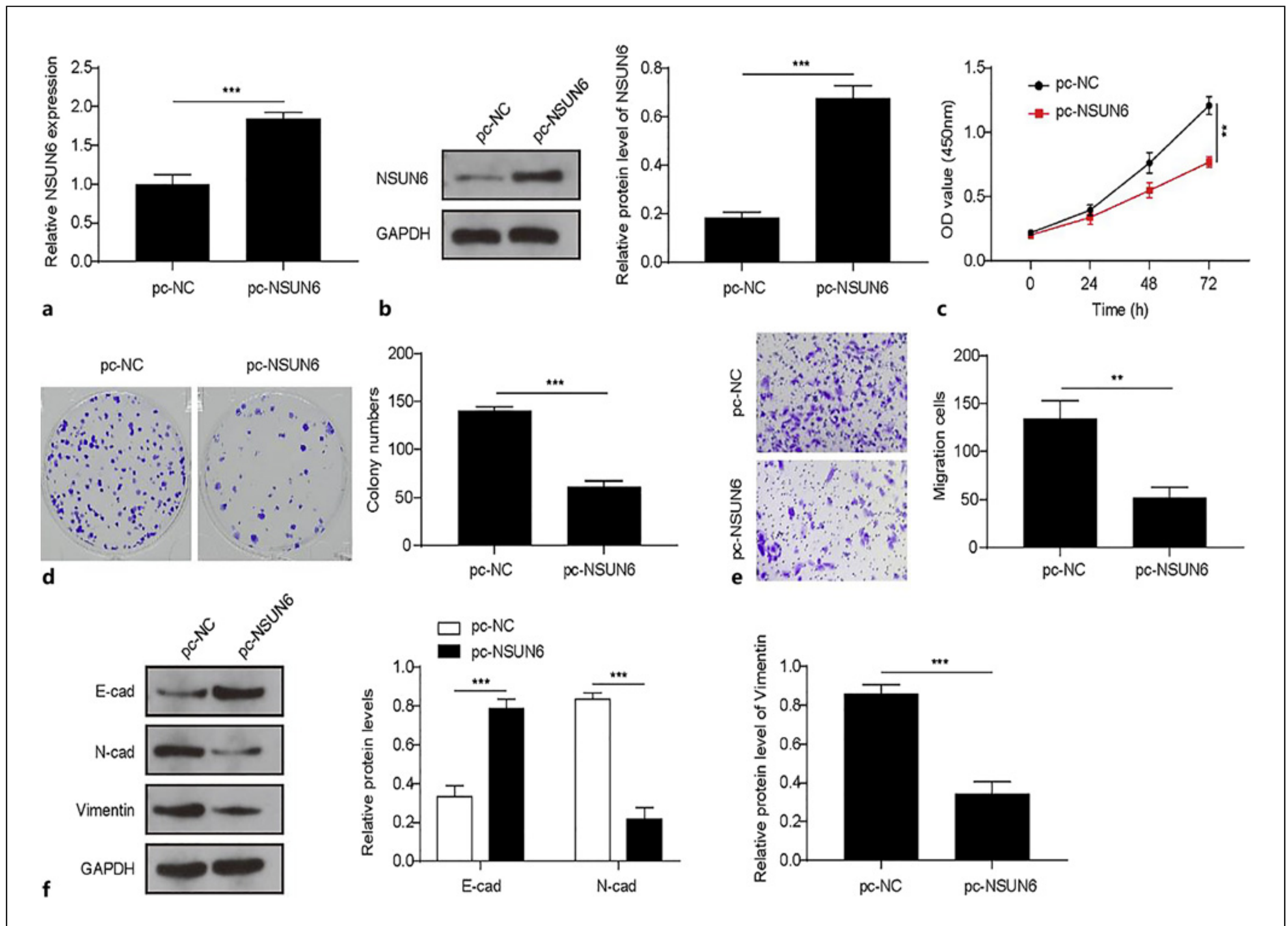


Fig. 2. Effects of over-expression of NSUN6 on proliferation, migration, and EMT of lung cancer cells. **a**, **b** The qRT-PCR and WB tests were carried out to detect NSUN6 expression. **c–e** CCK-8, colony formation, and transwell methods were utilized to evaluate the proliferation and migration of lung cancer cells. **f** The expression levels of EMT-related proteins (E-cad, N-cad, and vimentin) were detected by WB. Experiments were repeated three times. $^{**}p < 0.01$, $^{***}p < 0.001$.

was markedly lower than that in the pc-NC group (Fig. 4c). The m5C RIP assay showed that the pc-NC group m5C antibody was significantly polymerized in the 3′-UTR of NM23-H1 mRNA, whereas the pc-NSUN6 group had significantly reduced m5C levels (Fig. 4d). The positive control group (HDGF) also showed consistent results. GAPDH and ACTIN were used as negative controls, and there was no difference between the pc-NC and pc-NSUN6 groups. Analysis by CCK-8, colony formation, and transwell assays indicated that the cell proliferation and migration ability of pc-NSUN6+sh-NM23-H1-MUT group were similar to those of the pc-NSUN6 group, but less than those of the pc-NSUN6+sh-NM23-H1-WT and pc-NC group (Fig. 4e–g). WB assay showed that sh-NM23-H1-WT partially reversed the regulatory effect of NSUN6 over-expression on the expression of EMT-regulated proteins (E-cad, N-cad, and vimentin) (Fig. 4h).

Over-Expression of NSUN6 Inhibits Progression of Lung Tumor in vivo

To explore the effect of NSUN6 on lung cancer in vivo, we constructed transplanted tumor mouse models. Compared to the control group, the tumor growth and weight of the pc-NSUN6 group were decreased (Fig. 5a–c). Immunohistochemistry showed that the expression of Ki-67 in the NSUN6-overexpressed group was markedly lower than that in the pc-NC group, while

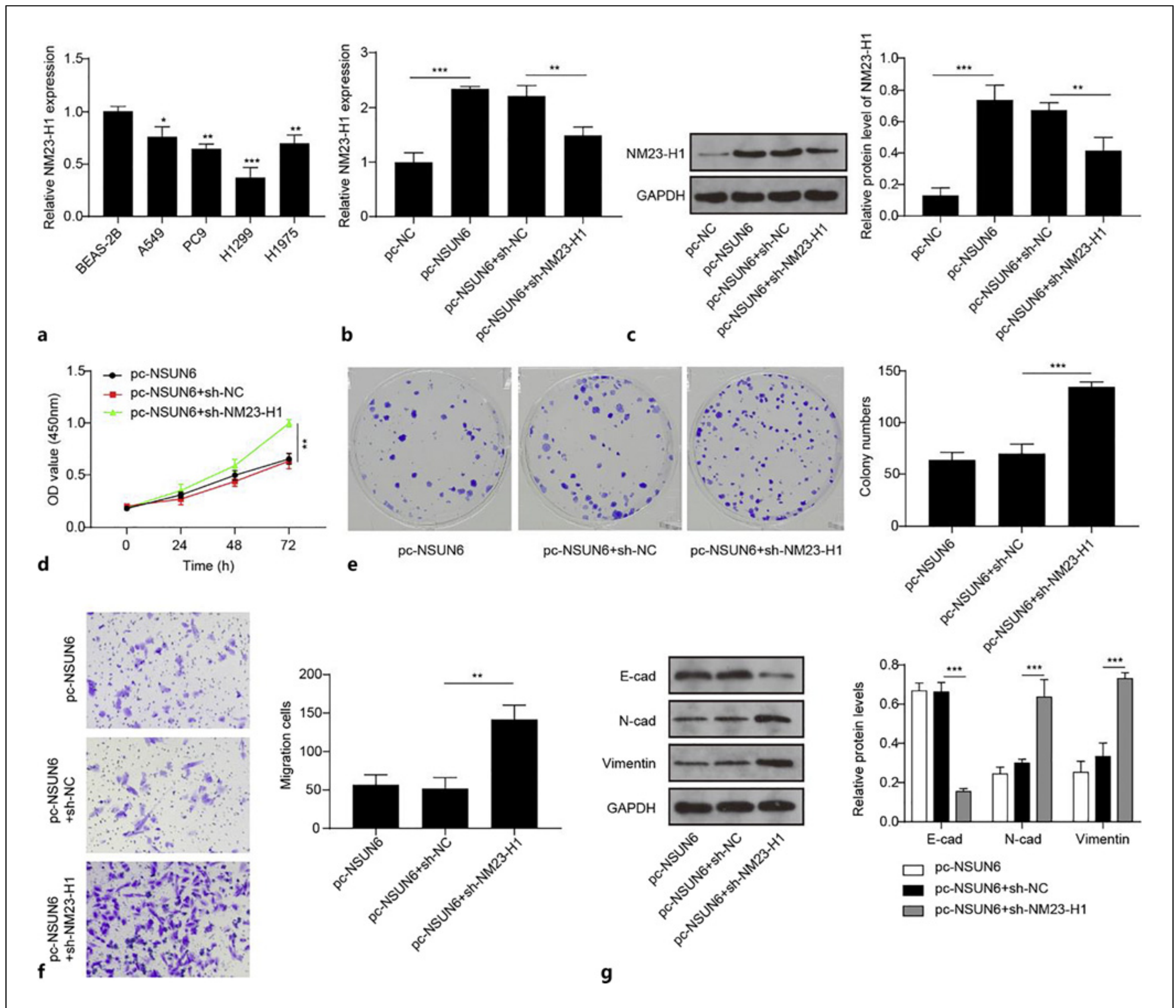
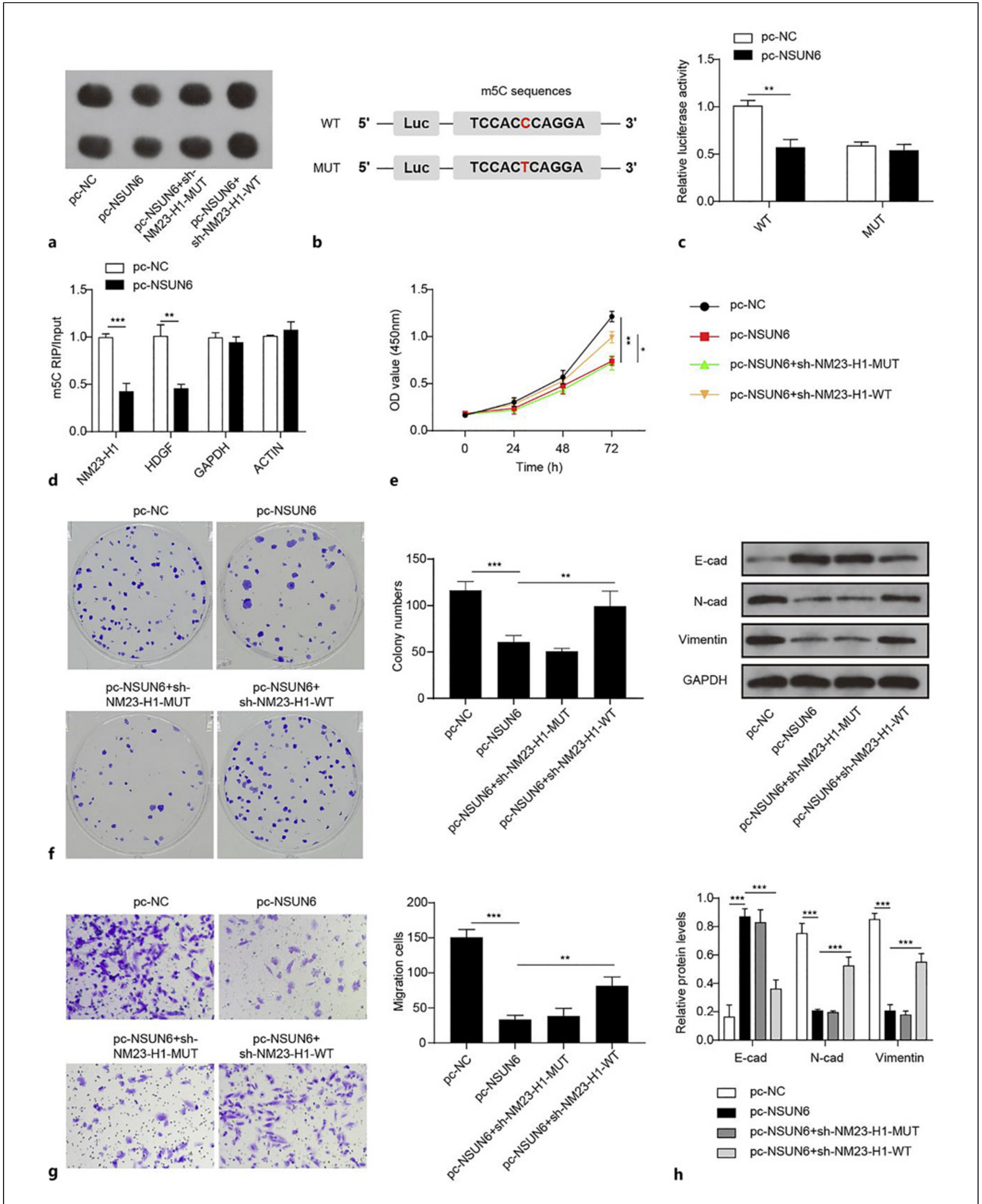


Fig. 3. Over-expression of NSUN6 restrains lung cancer cell proliferation, migration, and EMT by regulating NM23-H1. **a** qRT-PCR assay was carried out to detect NM23-H1 expression in lung cancer cells. **b, c** qRT-PCR and WB assays were utilized to detect NM23-H1 expression after NSUN6 over-expression. **d–g** The effects of NM23-H1 deficiency on the proliferation, migration, and EMT of NSUN6-overexpressed lung cancer cells were evaluated by CCK-8, colony formation, transwell, and WB assays. Cell experiments were repeated three times. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

the expression of NM23-H1 was significantly higher than that in the pc-NC group (Fig. 5d). WB results also indicated that compared to the pc-NC group, E-cad expression was increased, while the expression of N-cad and vimentin was reduced in the NSUN6 over-expression group (Fig. 5e). These results demonstrated that over-expression of NSUN6 could restrain lung tumorigenesis.

Discussion

The morbidity and mortality of lung cancer are gradually increasing in China, threatening people's health and life [20]. Although significant advances have been made in recent years, lung cancer is still a thorny issue [21]. With the increasing incidence of lung cancer, it is essential that we identify new markers for regulating the



4

(For legend see next page.)

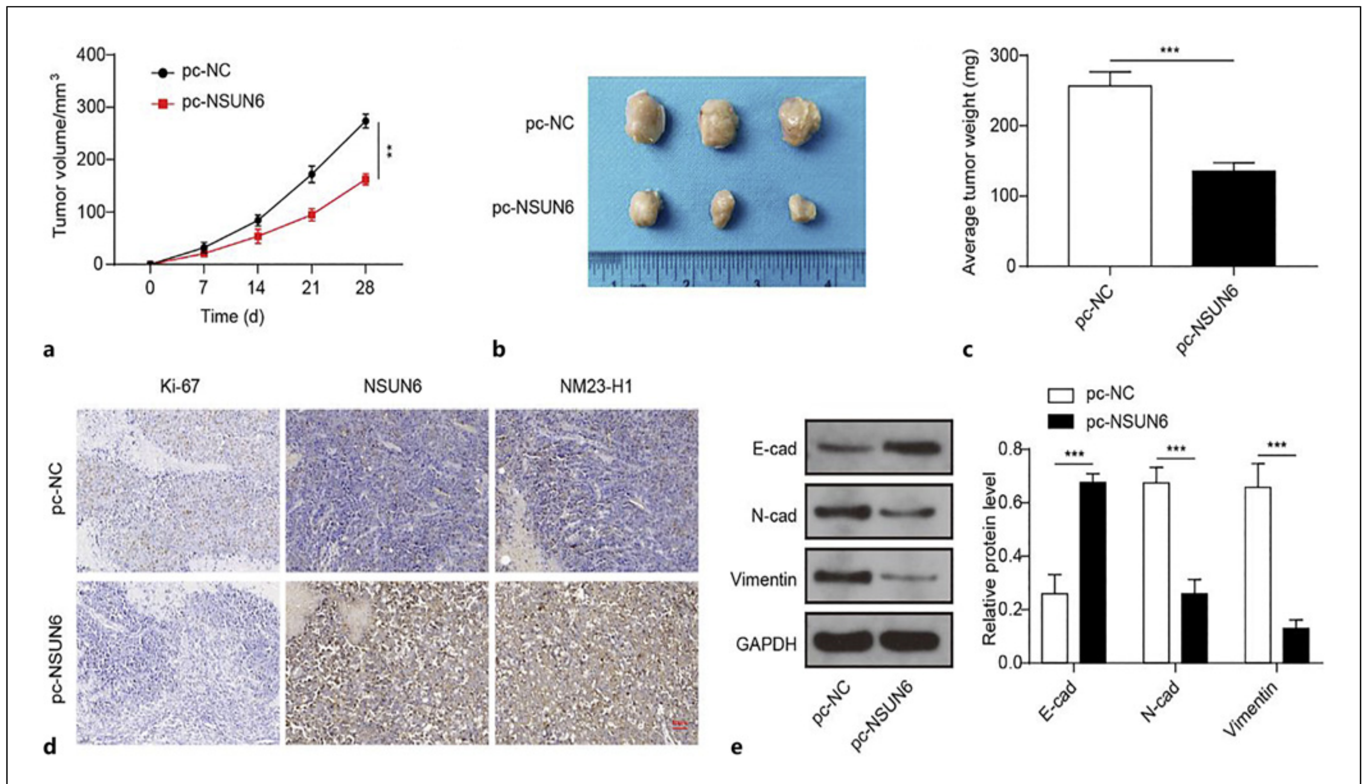


Fig. 5. Effect of over-expression of NSUN6 on lung tumorigenesis in vivo. **a–c** The growth and weight changes of transplanted tumors in mice were detected (n = 6). **d** IHC was utilized to evaluate the expression of Ki-67, NSUN6, and NM23-H1 in transplanted tumors. **e** WB was carried out to analyze the expression of E-cad, N-cad, and vimentin in transplanted tumors. IHC, immunohistochemistry. $^{**}p < 0.01$, $^{***}p < 0.001$.

occurrence and development of lung cancer. In this study, we found that the expression of NSUN6 is decreased in lung cancer, and that over-expression of NSUN6 increases the expression of NM23-H1 in an m5C manner to inhibit the migration, invasion, and EMT of lung cancer, which may provide potential targets for the treatment of lung cancer.

Accumulating evidence suggests that m5C-related regulators may be predictive biomarkers for a variety of cancers [22, 23]. However, little is known about the correlation between m5C-related genes and lung cancer. NSUN6 is an RNA m5C methyltransferase found to be associated with the prognosis of prostate, pancreatic, colorectal, and other cancers [14, 24, 25]. However, the relationship between NSUN6 and lung cancer progression has remained unclear. Our findings indicate that NSUN6 was downregulated in lung cancer, and over-expression of NSUN6 inhibits the proliferation and migration of lung cancer cells. Yang et al. [14] reported that NSUN6 restrains the progression of pancreatic cancer by mediating cell proliferation. EMT is known to play a crucial role in cancer progression, with cancer cells acquiring more invasive characteristics [26]. Many studies have confirmed that EMT plays a key role in the initiation and completion of each step of the metastasis of epithelial tumors (such as breast cancer, pancreatic cancer, lung cancer, colorectal cancer, etc.) (reviewed in [27]). The present study shows that over-expression of

Fig. 4. NSUN6 regulates expression of NM23-H1 by modifying its mRNA 3′-UTR through m5C. **a** Dot blot was used to detect m5C modification level. **b** RMVar database was used to predict the m5C modification site of NM23-H1 3′-UTR. **c** Detection of luciferase activity in lung cancer cells modified with WT or MUT NM23-H1. **d** Assessment of the m5C modification of NM23-H1 mRNA by m5C-RIP qPCR in lung cells. **e–h** The effects of NM23-MUT or NM23-H1-WT on the proliferation, migration, and EMT of NSUN6-overexpressed lung cancer cells were detected by CCK-8, colony formation, transwell, and WB assays. Cell experiments were repeated three times. EMT, epithelial-mesenchymal transition; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

NSUN6 elevates the expression of E-cad and decreases the expression of N-cad and vimentin. Taken together, we suggest that over-expression of NSUN6 could inhibit the progression of lung cancer.

NM23-H1 transcriptional and protein levels have been reported to be significantly reduced in various malignant tumor cell lines and animal models, and negatively correlated with tumor metastasis potential [28]. Tumor cells have evolved several ways to regulate the expression and function of NM23-H1 during tumorigenesis and metastasis [29]. NM23-H1 can reverse hypoxia-induced EMT and stemness of lung cancer cells by restricting the Wnt/β-catenin signaling pathway [30]. However, the upstream genes that regulate NM23-H1 expression in lung cancer are not well understood [31]. Our findings suggest that over-expression of NSUN6 attenuates cell proliferation, migration, and EMT of lung cancer by regulating NM23-H1 by binding to its 3′-UTR via m5C modification, similar to other studies which have demonstrated the binding of NSUN6 to target genes through modification of m5C [32].

Conclusion

Expression of NSUN6 is downregulated in lung cancer cells and tissues, and restrains the proliferation, migration of lung cells by targeting NM23-H1. NSUN6 regulates the expression of NM23-H1 in an m5C-dependent manner to affect EMT in lung cancer, providing a possible molecular basis for the treatment of lung cancer.

Statement of Ethics

This study was approved by the Ethics Committee of Guizhou Provincial People's Hospital. Procedures involving animals and their care were conducted according to the guidelines of the Animal Research Committee of Guizhou Provincial People's Hospital.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Funding Sources

This work is supported by Guizhou Provincial Science and Technology Projects (2019.1202).

Author Contributions

Zhansheng Lu, Bo Liu, Demiao Kong, and Xiaojiang Zhou conceived and designed the project. Dengke Pei and Di Liu generated the figures and tables. Zhansheng Lu and Bo Liu performed the expression analysis. All authors read and approved the final manuscript.

Data Availability Statement

The datasets used or analyzed during this study can be made available from the corresponding author upon reasonable request.

References

1 Doumat G, Daher D, Zerdan MB, Nasra N, Bahmad HF, Recine M, et al. Drug repurposing in non-small cell lung carcinoma: old solutions for new problems. Curr Oncol. 2023;30(1):704–19.

2 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.

3 Zeng H, Ran X, An L, Zheng R, Zhang S, Ji JS, et al. Disparities in stage at diagnosis for five common cancers in China: a multicentre, hospital-based, observational study. Lancet Public Health. 2021;6(12):e877–7.

4 Zhao H, Zhang X, Guo L, Shi S, Lu C. A robust seven-gene signature associated with tumor microenvironment to predict survival outcomes of patients with stage III-IV lung adenocarcinoma. Front Genet. 2021;12:684281.

5 Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL, et al. Lung cancer: current therapies and new targeted treatments. Lancet. 2017;389(10066):299–311.

6 Hilzenrat RA, Yip S, Melosky B, Ho C, Laskin J, Sun S, et al. Disparate time-to-treatment and varied incidence of actionable non-small cell lung cancer molecular alterations in British Columbia: a historical cohort study. Curr Oncol. 2022;30(1):145–56.

7 Xu M, Zhang L, Guo Y, Bai L, Luo Y, Wang B, et al. Nanoemulsion co-loaded with XIAP siRNA and gambogic acid for inhalation therapy of lung cancer. Int J Mol Sci. 2022;23(22):14294.

8 Barbieri I, Kouzarides T. Role of RNA modifications in cancer. Nat Rev Cancer. 2020;20(6):303–22.

9 Nombela P, Miguel-López B, Blanco S. The role of m(6)A, m(5)C and Ψ RNA modifications in cancer: novel therapeutic opportunities. Mol Cancer. 2021;20(1):18.

10 Xu G, Li C, Di Z, Yang Y, Liang L, Yuan Q, et al. Development of the expression and prognostic significance of m(5) C-related LncRNAs in breast cancer. Cancer Med. 2023;12(6):7667–81.

11 Chen B, Xi Y, Zhao J, Hong Y, Tian S, Zhai X, et al. m5C regulator-mediated modification patterns and tumor microenvironment infiltration characterization in colorectal cancer: one step closer to precision medicine. Front Immunol. 2022;13:1049435.

12 Wu S, Li M, Su R, Shen H, He Y, Zhou Y. Modification of m5C regulators in sarcoma can guide different immune infiltrations as well as immunotherapy. Front Surg. 2022;9:948371.

13 Awah CU, Winter J, Mazdoom CM, Ogunwobi OO. NSUN6, an RNA methyltransferase of 5-mC controls glioblastoma response to temozolomide (TMZ) via NELFB and RPS6KB2 interaction. Cancer Biol Ther. 2021;22(10–12):587–97.
14 Yang R, Liang X, Wang H, Guo M, Shen H, Shi Y, et al. The RNA methyltransferase NSUN6 suppresses pancreatic cancer development by regulating cell proliferation. EBioMedicine. 2021;63:103195.
15 Kim B, Lee KJ. Activation of Nm23-H1 to suppress breast cancer metastasis via redox regulation. Exp Mol Med. 2021;53(3):346–57.
16 Vargas-Accarino E, Herrera-Montávez C, Ramón Y Cajal S, Aasen T. Spontaneous cell detachment and reattachment in cancer cell lines: an in vitro model of metastasis and malignancy. Int J Mol Sci. 2021;22(9):4929.
17 Ma W, Chen J, Xue X, Wang Z, Liu H, Wang T, et al. Alteration in gene expression profile and biological behavior in human lung cancer cell line NL9980 by nm23-H1 gene silencing. Biochem Biophys Res Commun. 2008;371(3):425–30.
18 Li D, Li W, Tian C, Pang Y, Xu L, Wang Y, et al. Prognostic and clinicopathological significance of nm23-H1 expression in non-small cell lung cancer: a meta-analysis. Medicine. 2022;101(39):e30815.
19 Ai C, Ma G, Deng Y, Zheng Q, Gen Y, Li W, et al. Nm23-H1 inhibits lung cancer bone-specific metastasis by upregulating miR-660-5p targeted SMARCA5. Thorac Cancer. 2020;11(3):640–50.
20 Cao M, Chen W. Epidemiology of lung cancer in China. Thorac Cancer. 2019;10(1):3–7.
21 Tan J, Chen F, Ouyang B, Li X, Zhang W, Gao X. CDCA4 as a novel molecular biomarker of poor prognosis in patients with lung adenocarcinoma. Front Oncol. 2022;12:865756.
22 Huang Z, Pan J, Wang H, Du X, Xu Y, Wang Z, et al. Prognostic significance and tumor immune microenvironment heterogenicity of m5C RNA methylation regulators in triple-negative breast cancer. Front Cell Dev Biol. 2021;9:657547.
23 Pan J, Huang Z, Xu Y. m5C RNA Methylation regulators predict prognosis and regulate the immune microenvironment in lung squamous cell carcinoma. Front Oncol. 2021;11:657466.
24 Huang Y, Huang C, Jiang X, Yan Y, Zhuang K, Liu F, et al. Exploration of potential roles of m5C-related regulators in colon adenocarcinoma prognosis. Front Genet. 2022;13:816173.
25 Yu G, Bao J, Zhan M, Wang J, Li X, Gu X, et al. Comprehensive analysis of m5C methylation regulatory genes and tumor microenvironment in prostate cancer. Front Immunol. 2022;13:914577.
26 Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. Cell. 2016;166(1):21–45.
27 Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871–90.
28 Chen J, Jiang Q, Jiang XQ, Li DQ, Jiang XC, Wu XB, et al. miR-146a promoted breast cancer proliferation and invasion by regulating NM23-H1. J Biochem. 2020;167(1):41–8.
29 Yu L, Wang X, Zhang W, Khan E, Lin C, Guo C. The multiple regulation of metastasis suppressor NM23-H1 in cancer. Life Sci. 2021;268:118995.
30 Wu CE, Zhuang YW, Zhou JY, Liu SL, Zou X, Wu J, et al. Nm23-H1 inhibits hypoxia induced epithelial-mesenchymal transition and stemness in non-small cell lung cancer cells. Biol Chem. 2019;400(6):765–76.
31 Sheng Y, Xu M, Li C, Xiong Y, Yang Y, Kuang X, et al. Nm23-H1 is involved in the repair of ionizing radiation-induced DNA double-strand breaks in the A549 lung cancer cell line. BMC Cancer. 2018;18(1):710.
32 Wang W, Huang H, Jiang H, Tian C, Tang Y, Gan D, et al. A cross-tissue investigation of molecular targets and physiological functions of Nsun6 using knockout mice. Int J Mol Sci. 2022;23(12):6584.