Received: 24 January 2024 | Accepted: 23 February 2024

DOI: 10.1111/1759-7714.15275

ORIGINAL ARTICLE

WILEY

METTL3-mediated the m6A modification of SF3B4 facilitates the development of non-small cell lung cancer by enhancing LSM4 expression

Guangsi He[1,2] | Kangsheng Gu[1] | Jie Wei[3] | Jian Zhang[2]

[1]Department of Oncology, the First Affiliated Hospital of Anhui Medical University, Hefei, China

[2]Second Department of Oncology, First People's Hospital of Chuzhou, Chuzhou, China

[3]First Department of Oncology, First People's Hospital of Chuzhou, Chuzhou, China

Correspondence
Kangsheng Gu, Department of Oncology, the First Affiliated Hospital of Anhui Medical University, No. 218 Jixi Road, Hefei, 230022 Anhui, China.
Email: anhui_gukangsheng@163.com

Abstract

Background: Splicing factor B subunit 4 (SF3B4) has been confirmed to participate in the progression of many cancers and is considered to be a potential target for non-small cell lung cancer (NSCLC). Thus, the role and molecular mechanism of SF3B4 in NSCLC progression deserves further study.

Methods: Quantitative real-time PCR and western blot were employed to detect the mRNA and protein levels of SF3B4, Sm-like protein 4 (LSM4) and methyltransferase-like 3 (METTL3). Cell proliferation, apoptosis, invasion, migration and stemness were tested by cell counting kit-8, colony formation, flow cytometry, transwell, wound healing, and sphere formation assays. The interaction between SF3B4 and METTL3 or LSM4 was confirmed by MeRIP, RIP and Co-IP assays. Mice xenograft models were constructed to assess the effects of METTL3 and SF3B4 on NSCLC tumorigenesis.

Results: SF3B4 had high expression in NSCLC tissues and was associated with the shorter overall survival of NSCLC patients. Knockdown of SF3B4 suppressed NSCLC cell proliferation, invasion, migration and stemness, while inducing apoptosis. METTL3 promoted SF3B4 mRNA stability by m6A modification, and its knockdown inhibited NSCLC cell growth, metastasis and stemness by downregulating SF3B4. SF3B4 could interact with LSM4, and sh-SF3B4-mediated the inhibition on NSCLC cell functions could be reversed by LSM4 overexpression. In addition, reduced METTL3 expression restrained NSCLC tumor growth, and this effect was reversed by SF3B4 overexpression.

Conclusion: METTL3-stablized SF3B4 promoted NSCLC cell growth, metastasis and stemness via positively regulating LSM4.

KEYWORDS
LSM4, METTL3, non-small cell lung cancer, SF3B4

INTRODUCTION

Non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancer cases, including lung squamous cell carcinoma, lung adenocarcinoma (LUAD), and large cell lung carcinoma.[1,2] Due to the lack of obvious early symptoms, most patients with NSCLC are initially diagnosed at an advanced stage and often have a poor prognosis.[3,4] Importantly, tumor metastasis is also an important concern for NSCLC patients, which poses a great threat to the survival of patients.[5,6] Therefore, the search for effective molecular targets that affect cancer progression is important for developing new NSCLC therapies.

Splicing factor B subunit 4 (SF3B4), a constituent subunit of the SF3B complex, is an important splicing factor involved in branchpoint recognition, splicing reaction activation and intron removal.[7,8] SF3B4 functions as an oncogene in regulating multiple cancer processes.[9,10] The role of SF3B4 in lung cancer progression has recently been widely revealed. Studies have shown that SF3B4 expression is elevated in LUAD, and its overexpression enhances LUAD cell growth.[11] In addition, SF3B4 knockdown has been confirmed to repress NSCLC cell

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2024 The Authors. *Thoracic Cancer* published by John Wiley & Sons Australia, Ltd.

proliferation and cell cycle.[12] Therefore, SF3B4 has the potential to be a molecular target for NSCLC treatment, and more roles and potential molecular mechanisms of SF3B4 in the progression of NSCLC need to be further revealed. By analyzing the heat map of gene expression profiles in NSCLC cells after SF3B4 knockdown in GSE222598 database, we found that Sm-like protein 4 (LSM4) was significantly reduced by SF3B4 knockdown. However, whether SF3B4 regulates NSCLC progression via mediating LSM4 expression remains unclear.

The development of NSCLC is closely related to epigenetic abnormalities, among which N6-methyladenosine (m6A) is a common and extremely important epigenetic change. m6A modification is the most abundant modification known on mRNA and is responsible for post-translational regulation of mRNA. Methyltransferase-like 3 (METTL3) is a m6A methyltransferase, which mainly recognizes the target RNA for m6A modification, and plays a vital role in embryonic development and tumorigenesis.[13,14] In NSCLC, METTL3 has been suggested to promote cell metastasis via increasing CYP19A1 translation through m6A modification.[15] Also, METTL3 enhanced NSCLC cell proliferation and invasion by m6A modifying SFRP2.[16] In this study, ENCORI database analysis showed that METTL3 could interact with SF3B4, and further analysis revealed that SF3B4 contained abundant m6A methylation levels. However, it is still unclear whether METTL3 regulates SF3B4 expression through m6A modification to regulate NSCLC malignant progression.

The aim of this study was to investigate SF3B4 roles in NSCLC progression and its underlying molecular mechanisms. Based on the above, we propose the hypothesis that METTL3-mediated SF3B4 regulates NSCLC progression by interacting with LSM4.

METHODS

Samples

A total of 34 paired tumor tissues and adjacent normal tissues were collected from 34 NSCLC patients at the First Affiliated Hospital of Anhui Medical University. Informed consent was obtained from each patient. Our experiments were approved by the First Affiliated Hospital of Anhui Medical University.

Cell culture

Human NSCLC cells (A549 and H1299) and bronchial epithelial cells (16HBE) (purchased from Procell, Wuhan, China) were cultured in RPMI-1640 medium (Gibco) containing 10% fetal bovine serum (FBS: Gibco) and 1% penicillin–streptomycin (Invitrogen) under normal conditions.

TABLE 1 Primer sequences used for quantitative real-time polymerase chain reaction (qRT-PCR).

Name		Primers for PCR (5′-3′)
SF3B4	Forward	GAGGCCCTCTCCCTCAGTAA
	Reverse	TTTGCCCCAAGGAGCTACAG
LSM4	Forward	TTGTCACTGCTGAAGACGGC
	Reverse	GCAGATGACTTCTCGCAGGT
GAPDH	Forward	AGAAGGCTGGGGCTCATTTG
	Reverse	AGGGGCCATCCACAGTCTTC

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LSM4, Sm-like protein 4; SF3B4, splicing factor B subunit 4.

RNA interference and overexpression

NSCLC cells were transfected with the shRNA against METTL3 or SF3B4 (sh-METTL3 or sh-SF3B4), and the overexpression vector of SF3B4 or LSM4 (synthesized by RiboBio) with lipofectamine 3000 (Invitrogen).

Quantitative real-time PCR (qRT-PCR)

Total RNAs were extracted and cDNA was synthesized with TRIzol reagent (Invitrogen) and PrimeScript RT reagent (Takara), respectively. Then, qRT-PCR was performed using SYBR Green (Takara), cDNA and specific primers (Table 1). Relative SF3B4 and LSM4 mRNA levels were obtained using the $2^{-\Delta\Delta CT}$ method.

Western blot (WB)

After extraction by radioimmunoprecipitation assay (RIPA) buffer (Beyotime), proteins were separated via SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane. After blockage, membrane was probed with anti-SF3B4 (1:5000, ab157117), anti-METTL3 (1:1000, ab195352), anti-LSM4 (1:500, ab236744), anti-GAPDH (1:2500, ab9485) and goat anti-rabbit immunoglobulin G (IgG) (1:50000, ab205718). Protein bands were exposed by ECL reagent (Beyotime) using ImageQuant LAS 4000.

Cell counting kit-8 (CC-8) assay

The suspension of transfected NSCLC cells were collected and seeded into 96-well plates. After culturing for 48 h, cells were incubated with CCK8 solution (Meilunbio) for 2 h. Cell viability was analyzed using a microplate reader.

Colony formation assay

Transfected NSCLC cells were reseeded in six-well plates. After incubation for 14 days, the colonies were fixed

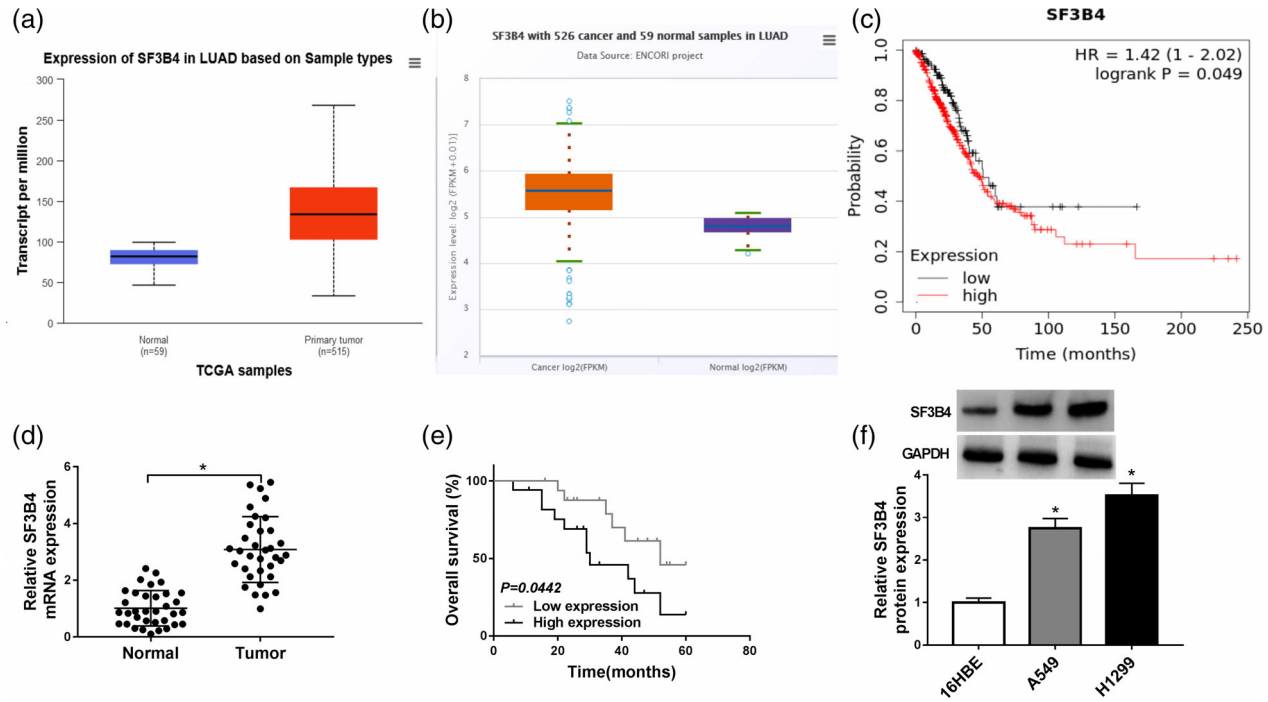

FIGURE 1 Splicing factor B subunit 4 (SF3B4) expression in non-small cell lung cancer (NSCLC) tissues and cells. The TCGA (a) and ENCORI databases (b) were used to analyze SF3B4 expression in lung adenocarcinoma (LUAD) tissues and normal tissues. (c) The Kaplan–Meier Plotter database was used to analyze the relationship between SF3B4 expression and the prognosis of LUAD patients. (d) SF3B4 mRNA expression in NSCLC tumor tissues and adjacent normal tissues was detected by quantitative real-time PCR (qRT-PCR) ($n = 34$). (e) Kaplan–Meier method was used to analyze the relationship between SF3B4 expression and the overall survival of NSCLC patients. (f) SF3B4 protein expression was examined by WB in 16HBE and NSCLC cells ($n = 3$). $^*p < 0.05$.

with polyformaldehyde and stained with crystal violet. The colonies were imaged and counted under a microscope.

Flow cytometry

Transfected NSCLC cells were collected for experiments. According to the manufacturer's protocol, cell apoptosis rate was assessed using an annexin V-FITC apoptosis detection kit (Beyotime) on flow cytometer.

Transwell assay

Transfected NSCLC cells were seeded into the upper chamber of Matrigel-coated tranwell chambers (BD Biosciences) with serum-free medium. Completed medium was filled in the lower chamber. After 24 h, invaded cells were counted under a microscope after fixation and staining.

Wound healing assay

Transfected NSCLC cells were cultured in six-well plates until 90% confluent. Scratches were created using a 200 μL pipette tip, and cells were further cultured for 24 h with serum-free medium. Migration distance was analyzed by calculating the cell wound area under a microscope.

Sphere formation assay

Transfected NSCLC cells were seeded in ultra-low attachment six-well plates (Corning Inc.) in RPMI-1640 medium supplemented with 20 ng/mL β-FGF, 20 ng/mL EGF, 2% B27, and 4 μg/mL insulin (Invitrogen). After 14 days, cell images were captured under a microscope, and sphere formation efficiency was assessed using ImageJ software.

MeRIP assay

Magna MeRIP m6A Kit (Millipore) was used in this experiment. Total RNAs extracted from NSCLC cells transfected with or without sh-NC/sh-METTL3 were sheared into fragments using fragmentation buffer. Then, fragmented RNA was immunoprecipitated with protein A/G magnetic beads precoated with anti-m6A or anti-IgG. The m6A level of SF3B4 in the coprecipitated RNA samples was determined by qRT-PCR.

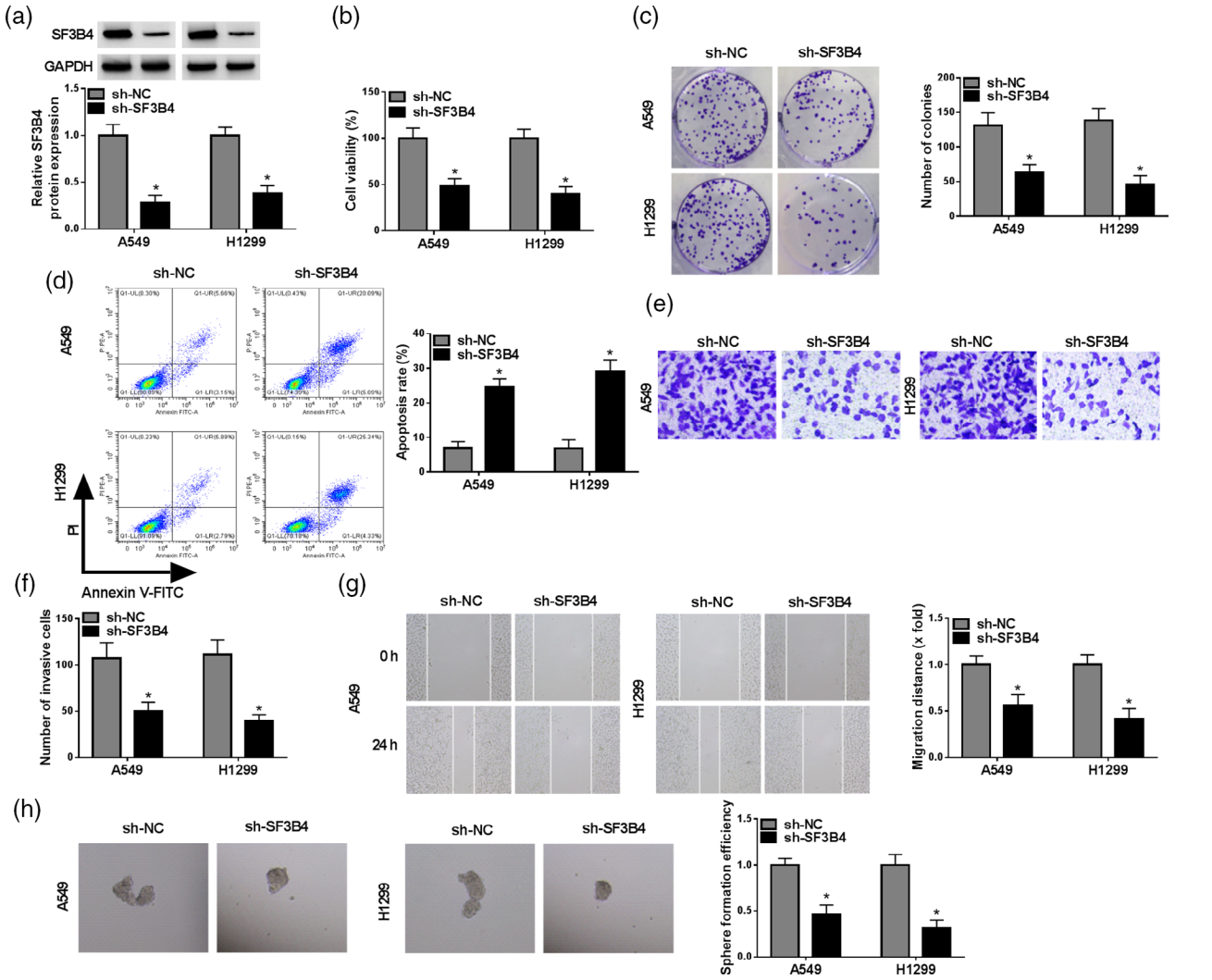

FIGURE 2 Effect of sh-splicing factor B subunit 4 (SF3B4) on non-small cell lung cancer (NSCLC) cell growth, metastasis and stemness. A549 and H1299 cells were transfected with sh-NC or sh-SF3B4 ($n = 3$). (a) SF3B4 protein expression was detected by Western blot. Cell counting kit-8 (CCK-8) assay (b), colony formation assay (c), flow cytometry (d), transwell assay (e, f), wound healing assay (g) and sphere formation assay (H) were used to measure cell proliferation, apoptosis, invasion, migration and stemness. $^*p < 0.05$.

RIP assay

NSCLC cells transfected with sh-NC/sh-METTL3 were lysed in IP lysis buffer (Millipore), and cell lysates were incubated with protein A/G beads pre-coated with anti-METTL3. After purification, SF3B4 enrichment in immunoprecipitated RNAs was analyzed by qRT-PCR.

Actinomycin D (Act D) assay

NSCLC cells transfected with sh-NC/sh-METTL3 were incubated with Actinomycin D solution (5 μg/mL, AAT Bioquest) for 0, 3, 6 and 9 h. qRT-PCR was performed to examine SF3B4 mRNA expression to assess mRNA stability.

Co-IP assay

NSCLC cell lysates were coimmunoprecipitated with Dynabeads protein G (Invitrogen) precoated with anti-SF3B4, anti-LSM4 and anti-IgG. The immunoprecipitate was collected and used for WB to detect LSM4 and SF3B4 protein signals.

Mice xenograft models

Lentivirus encoding sh-METTL3, noncoding shRNA (sh-NC) and SF3B4 overexpression were transduced into A549 cells, and the stable A549 cells were selected using puromycin (2.0 μg/mL, Solarbio). BALB/c mice (Vital River) were injected with stable A549 cells

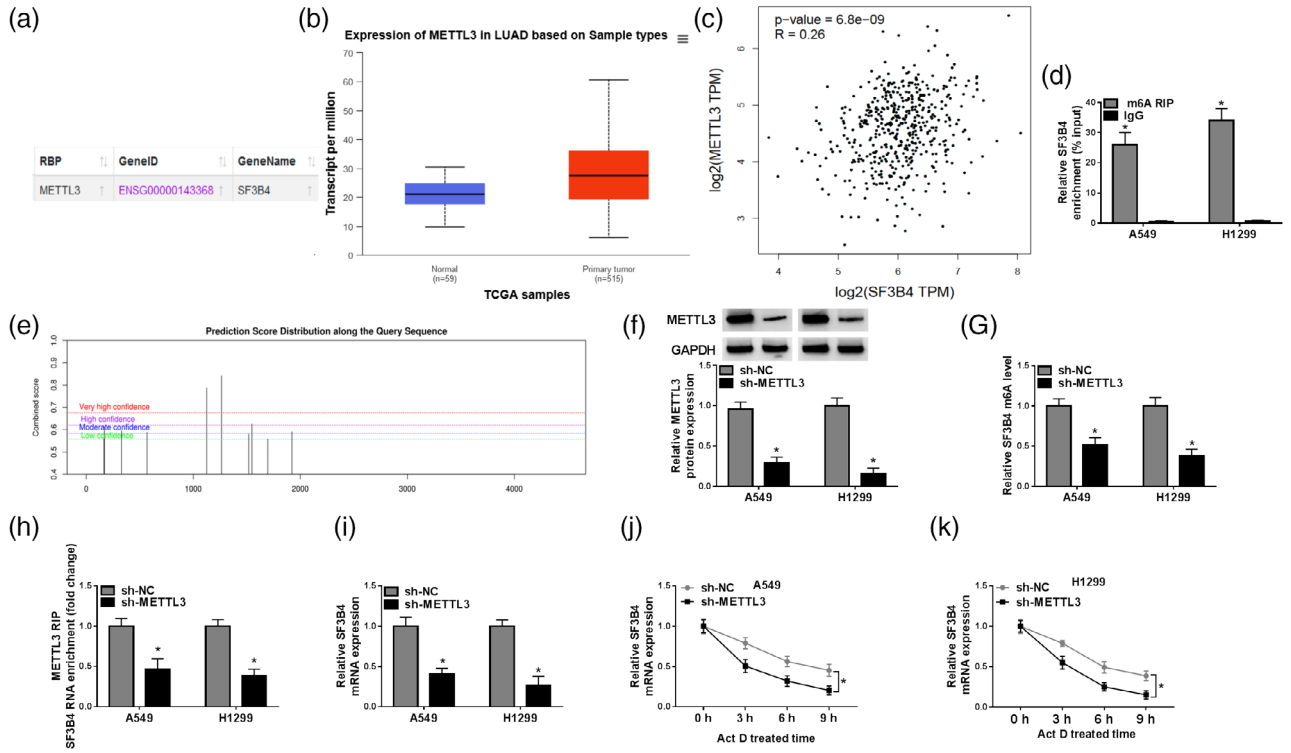

FIGURE 3 METTL3 regulated splicing factor B subunit 4 (SF3B4). (a) The ENCORI database predicted the interaction between SF3B4 and METTL3. (b) The TCGA database analyzed METTL3 expression in lung adenocarcinoma (LUAD) tissues and normal tissues. (c) The GEPIA database predicted the correlation between METTL3 and SF3B4 expression in LUAD tissues. (d) MeRIP assay was performed to detect the m6A level of SF3B4 ($n = 3$). (e) The SRAMP website predicted the methylation modification site in SF3B4 mRNA. (f) The transfection efficiency of sh-METTL3 was confirmed by detecting METTL3 protein expression using Western blot (WB) ($n = 3$). (g) SF3B4 m6A level in A549 and H1299 cells transfected with sh-NC/sh-METTL3 was analyzed by MeRIP assay ($n = 3$). (h) Radioimmunoprecipitation assay confirmed the interaction between METTL3 and SF3B4 ($n = 3$). (i) SF3B4 mRNA level was examined by quantitative real-time PCR (qRT-PCR) in A549 and H1299 cells transfected with sh-NC/sh-METTL3 ($n = 3$). (j, k) Actinomycin D assay was used to assess sh-METTL3 on the mRNA stability of SF3B4 ($n = 3$). $^*p < 0.05$.

subcutaneously. After 8 days, tumor volume was detected every 3 days. Mice were euthanized to collect tumor tissue after 23 days. One part of the tumor tissue was used for WB detection of SF3B4 and LSM4 protein expression, and another part was used to perform immunohistochemical (IHC) staining using SP Kit (Solarbio), anti-SF3B4 (1:100, ab233565), anti-LSM4 (1:100, ab236744) and anti-Ki67 (1:200, ab15580). Our procedures were approved by the First Affiliated Hospital of Anhui Medical University.

Statistical analysis

Data were analyzed as the mean ± SD and student's *t*-test or analysis of variance (ANOVA) was used to determine statistical significance using GraphPad 7.0 software. $p < 0.05$ denoted a statistically significant result.

RESULTS

SF3B4 had increased expression in NSCLC tissues and cells

The TCGA and ENCORI databases highlighted that SF3B4 expression was upregulated in the tissues of LUAD, the main pathological type of NSCLC (Figure 1a,b). The Kaplan–Meier Plotter database showed that high expression of SF3B4 was associated with poor prognosis of LUAD patients (Figure 1c). Through qRT-PCR analysis, we found that SF3B4 mRNA expression was obviously upregulated in NSCLC tumor tissues (Figure 1d). Moreover, NSCLC patients with high SF3B4 expression had a lower overall survival rate, as shown by Kaplan–Meier analysis (Figure 1e). In addition, SF3B4 protein level was confirmed to be elevated in NSCLC cells (A549 and H1299) compared to 16HBE cells (Figure 1f).

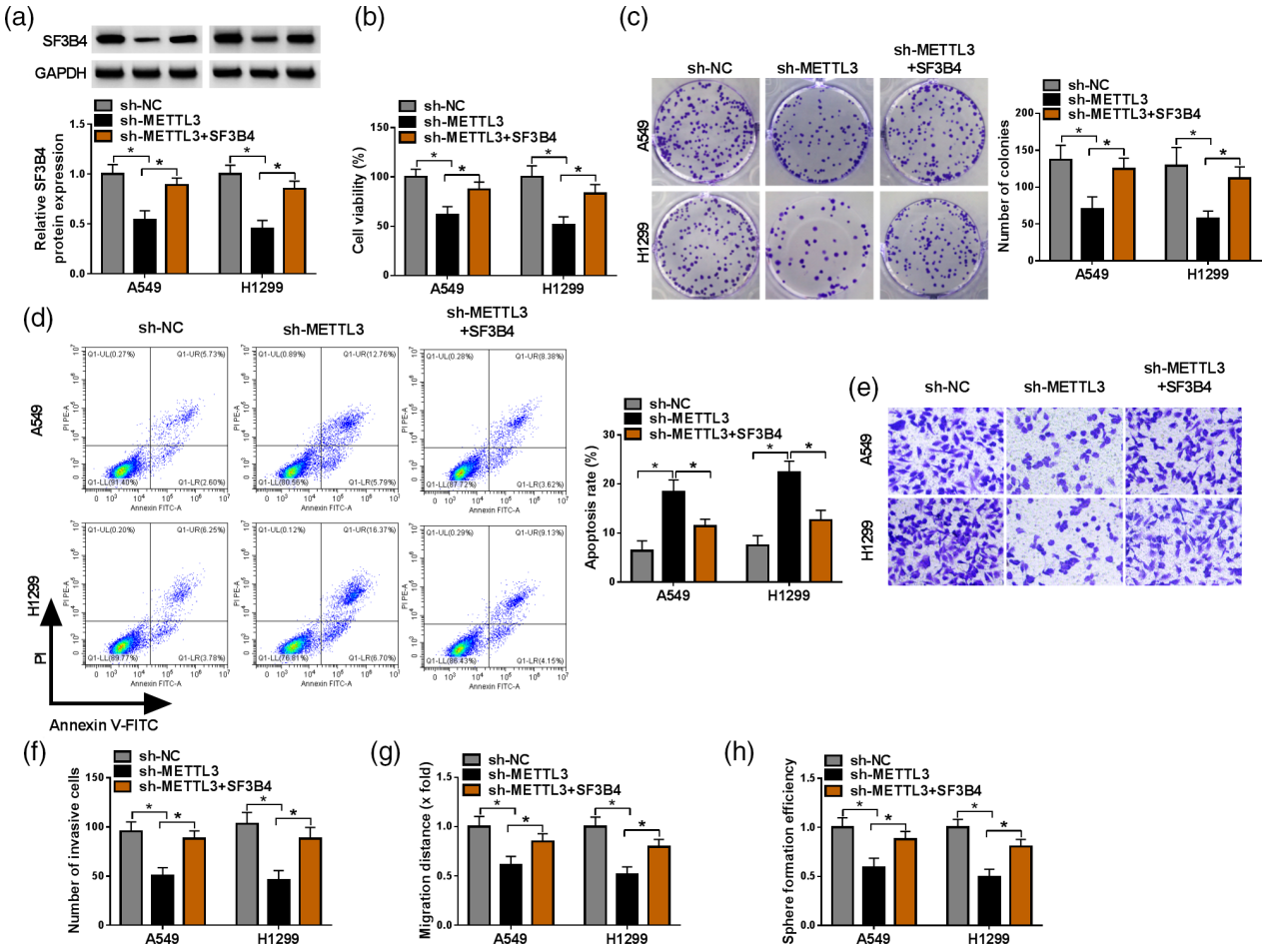

FIGURE 4 Effects of sh-METTL3 and splicing factor B subunit 4 (SF3B4) on non-small cell lung cancer (NSCLC) cell growth, metastasis and stemness. A549 and H1299 cells were transfected with sh-NC/sh-METTL3/SF3B4 ($n = 3$). (a) SF3B4 protein expression was examined using Western blot (WB). Cell proliferation, apoptosis, invasion, migration and stemness were determined using cell counting kit-8 (CCK-8) assay (b), colony formation assay (c), flow cytometry (d), transwell assay (e, f), wound healing assay (g) and sphere formation assay (h). $^*p < 0.05$.

Silencing of SF3B4 repressed NSCLC cell growth, metastasis and stemness

Given the high expression of SF3B4, we further revealed its role in NSCLC progression by loss-of-function experiments. After transfection of sh-SF3B4, SF3B4 protein expression was markedly reduced in A549 and H1299 cells (Figure 2a). SF3B4 knockdown inhibited viability, decreased the number of colonies, and promoted apoptosis rate in A549 and H1299 cells (Figure 2b–d). Meanwhile, invasive cell numbers, migration distance and sphere formation efficiency of A549 and H1299 cells were also significantly suppressed by SF3B4 knockdown (Figure 2e–h). Thus, SF3B4 might contribute to NSCLC cell growth, metastasis and stemness.

METTL3 mediated the m6A methylation of SF3B4

The ENCORI database showed that SF3B4 interacted with METTL3 (Figure 3a). Through database analysis, we revealed that METTL3 was highly expressed in LUAD tissues (Figure 3b), and its expression was positively correlated with SF3B4 expression in LUAD tissues (Figure 3c). We detected that SF3B4 contained abundant m6A levels (Figure 3d), and SRAMP website predicted that SF3B4 had many methylation modification sites (Figure 3e). To further confirm the regulation of METTL3 on SF3B4, we constructed sh-METTL3 to reduce METTL3 protein expression in A549 and H1299 cells (Figure 3f). MeRIP assay suggested that the m6A levels of SF3B4 mRNA in A549 and H1299 cells could be reduced by METTL3 knockdown (Figure 3g), and RIP assay further revealed that SF3B4 mRNA enrichment was significantly reduced by sh-METTL3 (Figure 3h). In addition, downregulation of METTL3 decreased SF3B4 mRNA expression and inhibited its mRNA stability (Figure 3i–k). All results suggested that METTL3 promoted SF3B4 mRNA stability through m6A methylation modification.

METTL3 knockdown inhibited NSCLC cell growth, metastasis and stemness by SF3B4

To study whether METTL3 regulated NSCLC cell functions by regulating SF3B4, NSCLC cells were co-transfected with

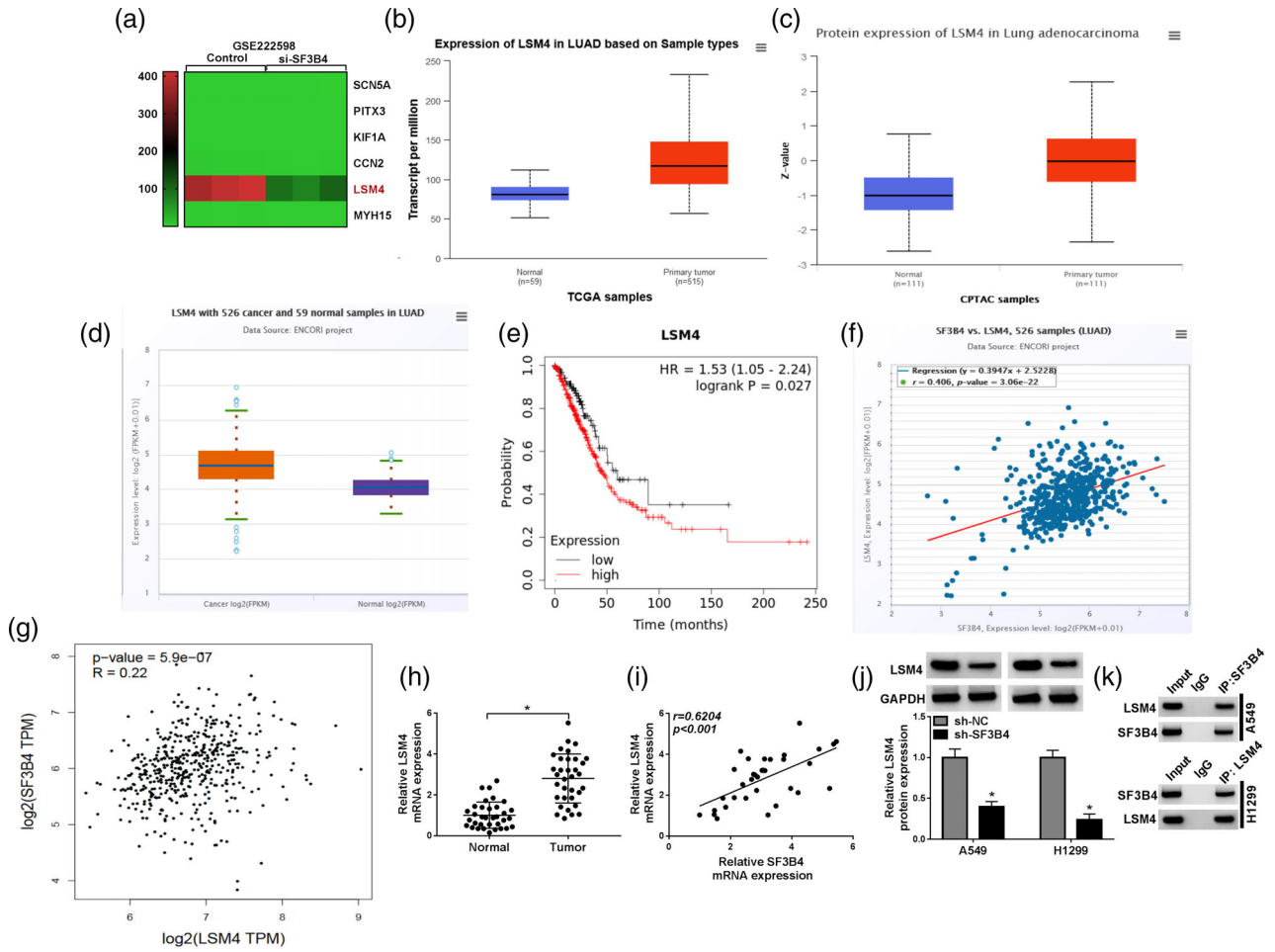

FIGURE 5 Splicing factor B subunit 4 (SF3B4) regulated Sm-like protein 4 (LSM4). (a) Heat map showed the gene expression profiles in non-small cell lung cancer (NSCLC) cells treated with or without si-SF3B4 from GSE222598 database. The TCGA (b), CPTAC (c) and ENCORI databases (d) analyzed LSM4 expression in lung adenocarcinoma (LUAD) tissues and normal tissues. (e) Kaplan–Meier Plotter database analyzed the relationship between LSM4 expression and the prognosis of LUAD patients. ENCORI database (f) and GEPIA database (g) predicted the correlation between SF3B4 and LSM4 expression in LUAD tissues. (h) LSM4 mRNA expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR) in NSCLC tumor tissues and adjacent normal tissues ($n = 34$). (i) Pearson correlation analysis was used to analyze the correlation between LSM4 and SF3B4 expression in NSCLC tissues. (j) LSM4 protein expression was examined by Western blot in A549 and H1299 cells transfected with sh-NC/sh-SF3B4 ($n = 3$). (k) Co-immunoprecipitation assay was performed to confirm the interaction between LSM4 and SF3B4. $^*p < 0.05$.

sh-METTL3 and SF3B4 overexpression vector. SF3B4 protein expression decreased by sh-METTL3 could be abolished using SF3B4 overexpression vector (Figure 4a). In addition, METTL3 knockdown repressed viability, colony numbers, invasive cell numbers, migration distance and sphere formation efficiency, while promoted apoptosis rate in A549 and H1299 cells. However, these effects could be reverted by SF3B4 overexpression (Figure 4b–h). These results found that METTL3 increased SF3B4 expression to enhance NSCLC cell growth, metastasis and stemness.

SF3B4 interacted with LSM4

The heat map of gene expression profiles in NSCLC cells after SF3B4 knockdown was obtained from the GSE222598 database, and LSM4 was significantly decreased in the si-SF3B4 group (Figure 5a). Through TCGA, CPTAC, and ENCORI database analysis, we determined that LSM4 was highly expressed in LUAD tissues (Figure 5b–d). Moreover, the Kaplan–Meier Plotter database predicted that high LSM4 expression was associated with poor prognosis of LUAD patients (Figure 5e). LSM4 expression was also found to be positively correlated with SF3B4 expression in LUAD tissues (Figure 5f,g). In this, we detected LSM4 mRNA expression, and confirmed that it was upregulated in NSCLC tumor tissues and there had positive correlation between LSM4 and SF3B4 expression (Figure 5h,i). After silencing of SF3B4 in A549 and H1299 cells using sh-SF3B4, LSM4 protein expression was remarkably reduced (Figure 5j). Importantly, Co-IP detection results showed that SF3B4 could interact with LSM4 in A549 and H1299 cells (Figure 5k). The above data suggested that SF3B4 positively regulated LSM4 expression.

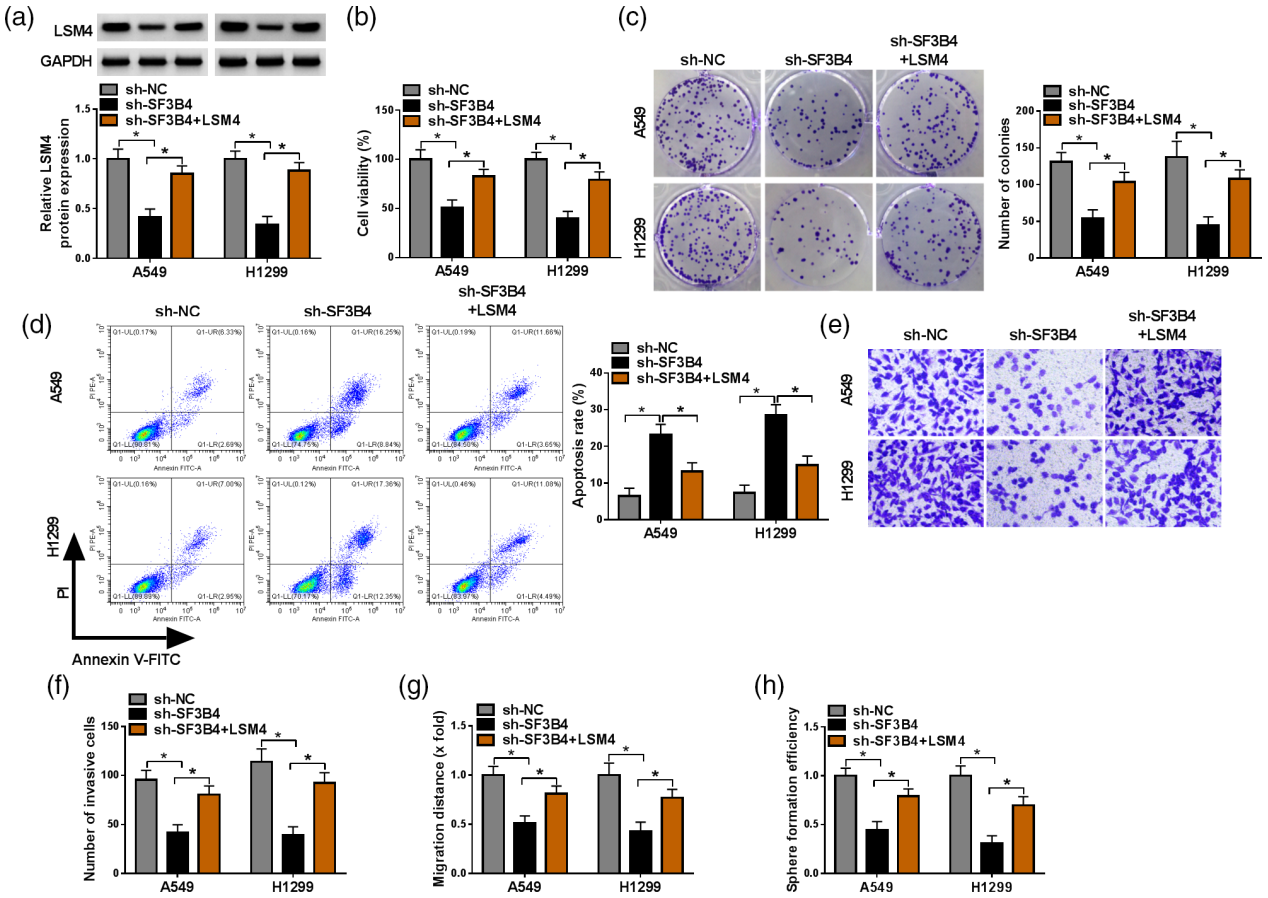

FIGURE 6 Effects of sh-splicing factor B subunit 4 (SF3B4) and Sm-like protein 4 (LSM4) on non-small cell lung cancer (NSCLC) cell progression. A549 and H1299 cells were transfected with sh-NC/sh-SF3B4/LSM4 ($n = 3$). (a) WB detected LSM4 protein expression. Cell counting kit-8 (CCK-8) assay (b), colony formation assay (c), flow cytometry (d), transwell assay (e, f), wound healing assay (g) and sphere formation assay (h) were performed to examine cell proliferation, apoptosis, invasion, migration and stemness. $^*p < 0.05$.

SF3B4 affected LSM4 expression to regulate NSCLC cell progression

In A549 and H1299 cells transfected with sh-SF3B4 and LSM4 overexpression vector, we explored the regulation of SF3B4/LSM4 on NSCLC cell progression. First, sh-SF3B4-mediated the reduction of LSM4 protein expression was abolished by LSM4 overexpression vector (Figure 6a). The proliferation inhibition and apoptosis promotion mediated by SF3B4 knockdown could be partially reversed by LSM4 overexpression, as shown by the increased cell viability, enhanced colony numbers and decreased apoptosis rate in cotransfection group (Figure 6b–d). Also, LSM4 overexpression overturned the inhibitory effect of sh-SF3B4 on invasive cell numbers, migration distance and sphere formation efficiency in A549 and H1299 cells (Figure 6e–h). These results indicated that SF3B4 played an oncogenic role in NSCLC by regulating LSM4 expression.

SF3B4 abolished sh-METTL3-mediated NSCLC tumorigenesis inhibition in vivo

To further demonstrate the roles of METTL3 and SF3B4 in NSCLC tumorigenesis in vivo, we performed animal experiments. As shown in Figure 7a,b, tumor volume and weight were markedly reduced in the sh-METTL3 group, and this effect was eliminated by SF3B4 overexpression. SF3B4 and LSM4 protein levels were confirmed to be decreased in the tumor tissues of sh-METTL3 group, and SF3B4 overexpression reversed these effects (Figure 7c,d). Meanwhile, IHC staining results further revealed that the positive cells of SF3B4, LSM4, and proliferation marker Ki67 were lower in the tumor tissues of sh-METTL3 group, while these effects were eliminated by SF3B4 overexpression (Figure 7e). Thus, METTL3-mediated SF3B4 promoted NSCLC tumor growth by increasing LSM4 expression.

DISCUSSION

As an alternative splicing molecule, SF3B4 has been reported to regulate many human disease processes through alternative splicing. In their study, Diao et al. reported that SF3B4 was abnormally overexpressed in ovarian cancer tissues, and could contribute to cell proliferation and mobility by increasing RAD52 expression via regulating its alternative splicing.[17] Moreover, SF3B4 has been reported to play a pro-tumor role in cervical cancer, which accelerates cancer cell

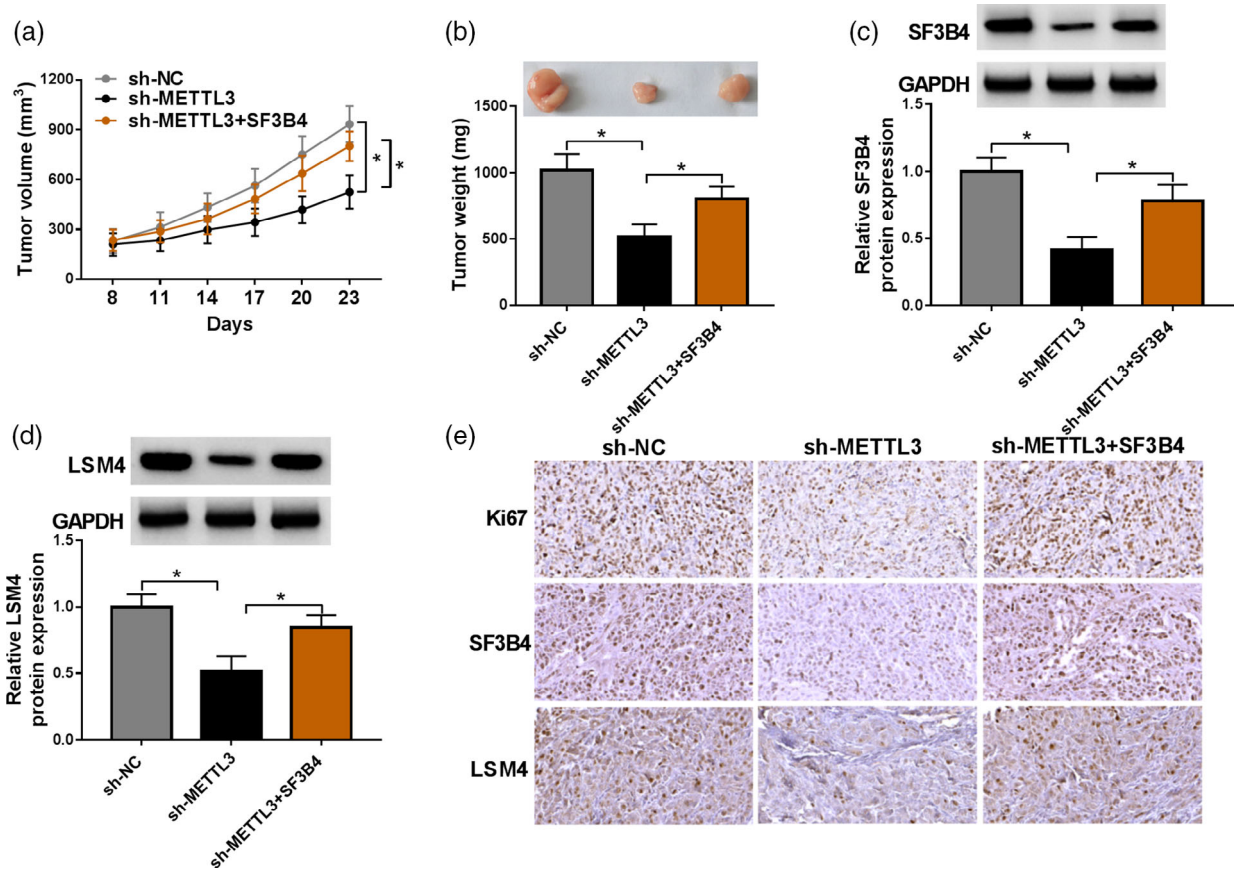

FIGURE 7 Effects of sh-METTL3 and splicing factor B subunit 4 (SF3B4) on non-small cell lung cancer (NSCLC) tumorigenesis inhibition in vivo. (a) Tumor volume and (b) weight were detected. (c, d) SF3B4 and Sm-like protein 4 (LSM4) protein levels in the tumor tissues of each group were examined by Western blot (WB). (e) Immunohistochemical (IHC) staining was performed to assess the positive cells of Ki67, SF3B4 and LSM4 in the tumor tissues of each group. $^*p < 0.05$.

invasion and proliferation through regulating SPAG5 splicing.[9] In addition, SF3B4 might be a diagnostic biomarker of hepatocellular carcinoma,[18,19] and its overexpression has been found to facilitate cell metastasis and tumorigenesis.[20] Although SF3B4 has been confirmed to be highly expressed in NSCLC tissues,[8,12] its role in the progression of NSCLC remains to be revealed. Consistent with the database analysis results, we identified high expression of SF3B4 in NSCLC tissues. In addition to inhibiting proliferation, we discovered that SF3B4 knockdown suppressed NSCLC cell invasion, migration, stemness, and promoted apoptosis. This is a novel finding and reveals a new function of SF3B4 in regulating NSCLC progression.

More and more studies have shown that METTL3 regulates mRNA stability through m6A methylation, thereby mediating cancer progression. For example, METTL3 decreased LATS1 mRNA expression in a m6A-dependent manner, thereby accelerating breast cancer tumorigenesis and glycolysis.[21] Also, METTL3 promoted pancreatic cancer cell proliferation and stemness by increasing ID2 mRNA stability through m6A modification.[22] A recent study revealed that METTL3 had increased expression in NSCLC, which accelerated NSCLC cell growth and metastasis via the targeting inhibition of FDX1.[23] Moreover, METTL3 had been shown to serve as an oncogene in NSCLC via mediating the m6A modification of antiapoptosis marker Bcl-2 mRNA.[24] Through database analysis, we determined that SF3B4 had abundant m6A methylation sites and METTL3 could interact with SF3B4. Further analysis suggested that METTL3 could increase the m6A level of SF3B4 to promote its mRNA stability. Functionally, SF3B4 overexpression reversed sh-METTL3-mediated inhibition in NSCLC cell growth, metastasis, stemness and tumorigenesis, confirming that METTL3-stabilized SF3B4 contributed to NSCLC progression.

LSM4 belongs to the LSM protein family and is a class of RNA-binding proteins involved in mRNA splicing and degradation.[25,26] Yin et al. reported that LSM4 could act as diagnostic and prognostic marker in breast cancer.[27] High LSM4 expression enhanced ovarian cancer cell proliferation, metastasis and glycolysis.[28] LSM4 had increased expression in hepatocellular carcinoma and could serve as a diagnostic marker, which upregulation contributed to cell malignant behaviors.[29,30] Although LSM4 has been shown to be oncogenic in many cancers, its role in NSCLC progression has not been explored. Database analysis revealed that LSM4 expression was high in LUAD tissues and could be decreased by SF3B4 knockdown in NSCLC cells. Furthermore, SF3B4 expression was positively correlated with LSM4 expression, and SF3B4 could interact with LSM4. LSM4 upregulation reversed the NSCLC cell functions inhibited by SF3B4 knockdown, clearly indicating that LSM4 had a carcinogenic role in NSCLC, and SF3B4 promoted NSCLC cell growth,

metastasis, and stemness by elevating LSM4 expression. In vivo, we confirmed the positive regulation of METTL3 on LSM4 expression, which refined our hypothesis of the METTL3/SF3B4/LSM4 axis in NSCLC.

Collectively, our study reveals a novel molecular mechanism of SF3B4 in NSCLC. Our data showed that SF3B4, regulated by METTL3-mediated the m6A modification, accelerated NSCLC cell growth, metastasis, and stemness via upregulating LSM4. These findings offer new evidence that SF3B4 is a potential target for NSCLC treatment, and the proposed METTL3/SF3B4/LSM4 molecular axis provides a new insight into our understanding of the carcinogenic activity of SF3B4.

AUTHOR CONTRIBUTIONS

Kangsheng Gu conceived, designed and revised the current study. Guangsi He wrote the manuscript. Jie Wei and Jian Zhang analyzed the data. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

ORCID

Kangsheng Gu https://orcid.org/0009-0006-6961-1514

REFERENCES

1. Mithoowani H, Febbraro M. Non-small-cell lung cancer in 2022: a review for general practitioners in oncology. Curr Oncol. 2022;29:1828–39.
2. Patel SA, Weiss J. Advances in the treatment of non-small cell lung cancer: immunotherapy. Clin Chest Med. 2020;41:237–47.
3. Hagopian G, Grant C, Nagasaka M. Proteolysis targeting chimeras in non-small cell lung cancer. Cancer Treat Rev. 2023;117:102561.
4. Baran K, Brzezianska-Lasota E. Proteomic biomarkers of non-small cell lung cancer patients. Adv Respir Med. 2021;89:419–26.
5. Sun J, Wu S, Jin Z, Ren S, Cho WC, Zhu C, et al. Lymph node micrometastasis in non-small cell lung cancer. Biomed Pharmacother. 2022;149:112817.
6. Fang C, Wang L, Gong C, Wu W, Yao C, Zhu S. Long non-coding RNAs: how to regulate the metastasis of non-small-cell lung cancer. J Cell Mol Med. 2020;24:3282–91.
7. Sun C. The SF3b complex: splicing and beyond. Cell Mol Life Sci. 2020;77:3583–95.
8. Slusher AL, Kim JJ, Ribick M, Pollens-Voigt J, Bankhead A III, Palmbos PL, et al. Intronic cis-element DR8 in hTERT is bound by splicing factor SF3B4 and regulates hTERT splicing in non-small cell lung cancer. Mol Cancer Res. 2022;20:1574–88.
9. Li Y, Diao Y, Wang Z, Wang S, Peng J, Kong B. The splicing factor SF3B4 drives proliferation and invasion in cervical cancer by regulating SPAG5. Cell Death Discov. 2022;8:326.
10. Yang Z, Wang YX, Wen JK, Gao HT, Han ZW, Qi JC, et al. SF3B4 promotes Twist1 expression and clear cell renal cell carcinoma progression by facilitating the export of KLF 16 mRNA from the nucleus to the cytoplasm. Cell Death Dis. 2023;14:26.
11. Qu A, Han B, Hua M, Wang C, Li T. SF3B4 downregulation restrains lung adenocarcinoma tumorigenesis via 5′ alternative splicing of KAT2A. Sci Rep. 2024;14:30.
12. Kim H, Lee J, Jung SY, Yun HH, Ko JH, Lee JH. SF3B4 depletion retards the growth of A549 non-small cell lung cancer cells via UBE4B-mediated regulation of p53/p21 and p27 expression. Mol Cells. 2022;45:718–28.
13. Wei X, Huo Y, Pi J, Gao Y, Rao S, He M, et al. METTL3 preferentially enhances non-m(6)a translation of epigenetic factors and promotes tumourigenesis. Nat Cell Biol. 2022;24:1278–90.
14. Kwon J, Jo YJ, Namgoong S, Kim NH. Functional roles of hnRNPA2/B1 regulated by METTL3 in mammalian embryonic development. Sci Rep. 2019;9:8640.
15. Meng W, Xiao H, Zhao R, Chen J, Wang Y, Mei P, et al. METTL3 drives NSCLC metastasis by enhancing CYP19A1 translation and oestrogen synthesis. Cell Biosci. 2024;14:10.
16. Zhao S, Song P, Zhou G, Zhang D, Hu Y. METTL3 promotes the malignancy of non-small cell lung cancer by N6-methyladenosine modifying SFRP2. Cancer Gene Ther. 2023;30:1094–104.
17. Diao Y, Li Y, Wang Z, Wang S, Li P, Kong B. SF3B4 promotes ovarian cancer progression by regulating alternative splicing of RAD52. Cell Death Dis. 2022;13:179.
18. Son JA, Weon JH, Baek GO, Ahn HR, Choi JY, Yoon MG, et al. Circulating small extracellular vesicle-derived splicing factor 3b subunit 4 as a non-invasive diagnostic biomarker of early hepatocellular carcinoma. J Exp Clin Cancer Res. 2023;42:288.
19. Shen Q, Nam SW. SF3B4 as an early-stage diagnostic marker and driver of hepatocellular carcinoma. BMB Rep. 2018;51:57–8.
20. Liu Z, Li W, Pang Y, Zhou Z, Liu S, Cheng K, et al. SF3B4 is regulated by microRNA-133b and promotes cell proliferation and metastasis in hepatocellular carcinoma. EBioMedicine. 2018;38:57–68.
21. Xu Y, Song M, Hong Z, Chen W, Zhang Q, Zhou J, et al. The N6-methyladenosine METTL3 regulates tumorigenesis and glycolysis by mediating m6A methylation of the tumor suppressor LATS1 in breast cancer. J Exp Clin Cancer Res. 2023;42:10.
22. Chen J, Zhang H, Xiu C, Gao C, Wu S, Bai J, et al. METTL3 promotes pancreatic cancer proliferation and stemness by increasing stability of ID2 mRNA in a m6A-dependent manner. Cancer Lett. 2023;565:216222.
23. Qian S, Liu J, Liao W, Wang F. METTL3 promotes non-small-cell lung cancer growth and metastasis by inhibiting FDX1 through copper death-associated pri-miR-21-5p maturation. Epigenomics. 2023;15:1237–55.
24. Zhang Y, Liu S, Zhao T, Dang C. METTL3-mediated m6A modification of Bcl-2 mRNA promotes non-small cell lung cancer progression. Oncol Rep. 2021;46:163.
25. Lyons SM, Ricciardi AS, Guo AY, Kambach C, Marzluff WF. The C-terminal extension of Lsm4 interacts directly with the 3′ end of the histone mRNP and is required for efficient histone mRNA degradation. RNA. 2014;20:88–102.
26. Wang S, Xue M, He C, Shen D, Jiang C, Zhao H, et al. AtMC1 associates with LSM4 to regulate plant immunity through modulating pre-mRNA splicing. Mol Plant Microbe Interact. 2021;34:1423–32.
27. Yin J, Lin C, Jiang M, Tang X, Xie D, Chen J, et al. CENPL, ISG20L2, LSM4, MRPL3 are four novel hub genes and may serve as diagnostic and prognostic markers in breast cancer. Sci Rep. 2021;11:15610.
28. Hou W, Zhang Y. Circ_0025033 promotes the progression of ovarian cancer by activating the expression of LSM4 via targeting miR-184. Pathol Res Pract. 2021;217:153275.
29. Sun ZP, Tan ZG, Peng C. Long noncoding RNA LINC01419 promotes hepatocellular carcinoma malignancy by mediating miR-485-5p/LSM4 axis. Kaohsiung J Med Sci. 2022;38:826–38.
30. Chen L, Lin YH, Liu GQ, Huang JE, Wei W, Yang ZH, et al. Clinical significance and potential role of LSM4 overexpression in hepatocellular carcinoma: an integrated analysis based on multiple databases. Front Genet. 2021;12:804916.

How to cite this article: He G, Gu K, Wei J, Zhang J. METTL3-mediated the m6A modification of SF3B4 facilitates the development of non-small cell lung cancer by enhancing LSM4 expression. Thorac Cancer. 2024;15(11):919–28. https://doi.org/10.1111/1759-7714.15275