METTL3 promotes proliferation and migration of colorectal cancer cells by increasing SNHG1 stability

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Abstract. N^6-methyladenosine (m6A) serves an essential role in RNA modulation and is implicated in multiple malignancies, including colorectal cancer (CRC). Methyltransferase-like 3 (METTL3) is an important writer in m6A modification, however its role in CRC in modifying small nucleolar RNA host gene 1 (SNHG1), an oncogenic long noncoding RNA, remains unclear. In the present study, METTL3 expression in CRC was assessed using online bioinformatics analysis, immunohistochemistry staining, western blotting, reverse transcription (RT)-quantitative PCR (qPCR) and cell transfections. Cell proliferation, migration and invasion were determined using functional Cell Counting Kit-8 (CCK-8) and Transwell assays. SNHG1 expression in CRC was evaluated using online bioinformatics analysis and RT-qPCR. Methylated RNA immunoprecipitation qPCR was performed to assess m6A modification changes of SNHG1 mRNA. The present study demonstrated that METTL3 is upregulated in CRC tissues and cell lines. Moreover, METTL3 expression was associated with several unfavourable clinical features in patients with CRC, including the stage of lymph node metastases and overall survival. Functional Transwell and CCK-8 assays demonstrated that knockdown of METTL3 suppressed CRC cell proliferation and migration. Furthermore, METTL3 was positively correlated with SNHG1 in CRC tissue, as indicated by analysis of data from The Cancer Genome Atlas. Mechanistically, SNHG1 contains 18 m6A modification sites. Through cell transfections and actinomycin D assays, the present study found that METTL3-mediated m6A modification at these sites enhances the stability of SNHG1 in CRC cells. Finally, it was demonstrated that SNHG1 knockdown partially diminished the facilitative effect of METTL3 on CRC cell migration and proliferation. The present study concluded that METTL3, a potential biomarker for assessing overall survival and metastasis in CRC, may serve as an oncogene, promote SNHG1 m6A modification, improve the stability of SNHG1 and enhance SNHG1-mediated oncogenic function in CRC.

Introduction

Colorectal cancer (CRC) is a common type of intestinal tumour with ~2 million new cases and 1 million estimated associated deaths reported in 2020, representing 10.7 and 9.5% of all new cancer cases and deaths globally, respectively (1). Moreover, cancer statistics from 2018 show that CRC resulted in the deaths of ~27,390 male and 23,240 female patients in the United States, which is the highest death toll among digestive tract tumours (2). However, despite the advances in the treatment of CRC, such as surgical removal and systemic chemotherapy, the mortality rate remains high due to recurrence and distant organ metastases (3). Distant metastasis to the liver is responsible for CRC-related death, with a 5-year survival rate of <10% (4). Therefore, exploring new biomarkers that indicate the promotion of tumour initiation and progression, and assessing the underlying molecular mechanisms, are of foremost importance to develop targeted treatments of CRC malignancies.

N^6-methyladenosine (m6A), a common RNA modification in epigenetic regulation, affects multiple aspects of RNA metabolism, such as pre-mRNA processing, translation efficiency, transcript stability and microRNA biogenesis (5-8). Methyltransferase-like 3 (METTL3), also called MT-A70, belongs to the class I methyltransferase (MTase) family and is an important catalytic enzyme of m6A MTase systems (9). METTL3 is widely involved in numerous cancer types, such as gastric and bladder cancer, by acting as an oncogene or as a tumour suppressor (10). Yue *et al* (11) reported that METTL3 was upregulated in gastric cancer and enhanced the stability of zinc finger MYM-type containing 1 mRNA, thereby facilitating the epithelial-mesenchymal transition (EMT) program and metastasis. In another study, Han *et al* (12) reported that METTL3 was upregulated in bladder cancer and reduced the

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expression of phosphatase and tensin homolog by accelerating the maturation of pri-miR221/222, which ultimately promoted the proliferation of bladder cancer. Li *et al* (13) reported that, as an oncogene, METTL3 promoted CRC progression by maintaining sex determining region Y-Box 2 (SOX2) expression in a m6A-insulin-like growth factor 2 mRNA binding protein 2-dependent manner. However, it remains unclear as to whether METTL3-mediated m6A modification affects any long noncoding (lnc) RNAs in CRC.

Small nucleolar RNA host gene 1 (SNHG1) is an oncogenic lncRNA that serves a key role in CRC progression, such as in proliferation, metastasis, EMT and oncogenesis (14-18). In the present study, the association between METTL3-mediated m6A and SNHG1 in CRC was evaluated and the function of the METTL3/SNHG1 axis in CRC was assessed.

Materials and methods

Patients and tissue samples. Pairs of CRC and adjacent tissue specimens (n=74) were obtained from Liaoning Cancer Hospital and Institute (Shenyang, China) during surgical resection from January 2015 to September 2016. All tissue specimens were preserved in liquid nitrogen. The Medical Ethics Committee of Liaoning Cancer Hospital Research Institute approved the study (approval no. LNCANHOS-2018-012) and all patients signed written informed consent forms.

Cell culture. The normal human colonic epithelial NCM460 cell line and four human CRC cell lines (LOVO, RKO, SW480 and HT29) were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. All cells were cultured using RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc.) and supplemented with 10% foetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin (Shanghai Baoman Biotechnology Co., Ltd.) and 100 mg/ml streptomycin (Shanghai Baoman Biotechnology Co., Ltd.). All cells were maintained in a cell incubator with 5% CO_2 at 37˚C.

Bioinformatics analysis of data from the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases. Data from two CR dsxdC-related GEO datasets, GSE41258 (19) and GSE41328 (20) were analysed using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r) to analyse the expression of METTL3 in CRC (21). Probe nos. 209265_s_at and 213653_at represent METTL3 in datasets GSE41258 and GSE41328, respectively. The expression and relationship of METTL3 and SNHG1 using data from TCGA (https://portal.gdc.cancer.gov/) were analysed using the University of ALabama at Birmingham CANcer data analysis portal (UALCAN; http://ualcan.path.uab.edu/index.html), according to the website's instructions (22). Potential m6A modification sites of SNHG1 were predicted using RMBase (V2.025; http://rna.sysu.edu.cn/rmbase/index.php), according to the website's instructions (23).

Immunohistochemistry (IHC) staining. IHC staining was performed according to a previously described method (24). CRC tissue were fixed with 10% formalin at room temperature for 48 h, dehydrated via gradient alcohol, paraffin-embedded, sliced (4-μm thick), dewaxed in xylene (for 10 min, repeated three times) and then rehydrated using a descending alcohol series, followed by antigen retrieval using Target Retrieval Solution (Dako; Agilent Technologies, Inc.), according to the manufacturer's instructions. Hydrogen peroxide (3%) was applied for 15 min to block endogenous peroxidase activity at room temperature. Sections were sealed using 10% Rangoat serum (Wuhan Servicebio Technology Co., Ltd.) for 5 min at room temperature. Incubation with anti-METTL3 rabbit monoclonal primary antibodies (1:500; cat. no. ab195352; Abcam) was performed overnight at 4˚C in the refrigerator and then the next day with biotinylated HRP-conjugated goat anti-rabbit immunoglobulin G secondary antibodies at 37˚C for 30 min (1:2,000; cat. no. ab205718; Abcam). This was followed by incubation with 2 μg/ml streptavidin maleate peroxidase for 30 min at 37˚C (LSAB kit; Dako; Agilent Technologies, Inc.), staining with 3,3-diaminobenzidine color development kit for 20 min in the dark and then counterstaining with haematoxylin at room temperature for 2 min. The sections were then dehydrated and finally mounted. Control sections of tissue were processed under the same conditions but did not contain primary antibodies. Images were observed using a fluorescence microscope.

RNA extraction and reverse transcription (RT)-quantitative PCR (qPCR). Total RNA was extracted from CRC tissues and 5×10^5 cells of each cell line using TRIzol® reagent according to the manufacturer's instructions (Invitrogen; Thermo Fisher Scientific, Inc.). Reverse transcription was performed using PrimeScript RT Master Mix at 37˚C for 15 min and 85˚C for 5 sec (Takara Biotechnology Co., Ltd.). Takara Biotechnology TB green premix Ex Taq™ II (cat. no. RR820A) was used for the qPCR, and the thermocycling conditions were as follows: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec, and then 95˚C for 15 sec and 60˚C for 60 sec. The relative expression of METTL3 or SNHG1 was quantified using the $2^{-\Delta\Delta Cq}$ method (25) and β-actin was used as an internal control (Takara Biotechnology Co., Ltd.). Primer sequences are listed in Table IA.

Western blotting. Western blotting was conducted as described previously (26). Total proteins were extracted from NCM460, HT29, LOVO and PKO cell lines using RIPA lysis buffer (Sigma-Aldrich; Merck KGaA) and protein concentrations were then semi-quantified using a BCA protein assay kit (Santa Cruz Biotechnology, Inc.) Proteins (10 μl/lane) were separated by 10% SDS-PAGE and then transferred to PVDF membranes (Amresco, LLC). The membranes were blocked with 5% BSA (Sigma-Aldrich; Merck KGaA) for 1 h at room temperature, after which the blocking solution was removed and anti-METTL3 rabbit monoclonal (1:1,000; cat. no. ab195352; Abcam) and anti-GAPDH mouse monoclonal (1:500; cat. no. ab8245; Abcam) primary antibodies were refrigerated at 4˚C and incubated with the membranes overnight. Primary antibodies were then washed away with TBST (0.1% Tween) and goat anti-mouse immunoglobulin G HRP-conjugated secondary antibodies (1:2,000; cat. no. ab205719; Abcam) were added at room temperature for 1 h. The ECL Western Blotting substrate kit (cat. no. ab65623; Abcam) was utilized to visualise the target proteins and ImageJ software (v2; National Institutes of Health) was used to analyse the protein bands.

Table I. Primer and oligonucleotide sequences used in the present study.

A, Primer sequences used in the present study

Name	Sequence (5'-3')
METTL3	F: TTGTCTCCAACCTTCCGTAGT
	R: CCAGATCAGAGAGGTGGTGTAG
SNHG1	F: GCACGTTGGAACCGAAGAGA
	R: GCAGCTGAATTCCCCAGGATA
β-actin	F: CTTCTACAATGAGCTGCGTG
	R: TCATGAGGTAGTCAGTCAGG

B, Oligonucleotide sequences used in the present study

Name	Sequence (5'-3')
METTL3	F: CTTGGTACCGAGCTCGGATCCATGTCGGACACGTGGAGCTC
	R: TGCTGGATATCTGCAGAATTCGCTCTGTAAGGAAGTGCTTC
shMETTL3#1	F: CCGGGCAAGAATTCTGTGACTATGGCTCGAGCCATAGTCACAGAATTCTTGCTTTTTG
	R: AATTCAAAAAGCAAGAATTCTGTGACTATGGCTCGAGCCATAGTCACAGAATTCTTGC
shMETTL3#2	F: CCGGGCTGCACTTCAGACGAATTATCTCGAGATAATTCGTCTGAAGTGCAGCTTTTTG
	R: AATTCAAAAAGCTGCACTTCAGACGAATTATCTCGAGATAATTCGTCTGAAGTGCAGC
shNC	TTCTCCGAACGTGTCACGT
siSNHG1-1	GAAACAGCAGTTGAGGGTTTG
siSNHG1-2	GGTTTGCTGTGTATCACATTT
siSNHG1-3	GCCAATTGTTGATTGAACTTC
siNC	UUCUCCGAACGUGUCACGUTT

METTL3, methyltransferase-like 3; SNHG1, small nucleolar RNA host gene 1; sh, short hairpin RNA; NC, negative control; si, small interfering RNA; F, forward; R, reverse.

Cell Counting Kit-8 (CCK-8) assay. HT29 and LOVO cells were seeded in 96-well plates at a density of $2x10^3$ cells/well and incubated at 37˚C with 5% CO_2. On days 1-5, 10 μl CCK-8 solution (Dojindo Molecular Technologies, Inc.) was added to each well and incubated at 37˚C. After 2 h, the 96-well plates were removed and a microplate reader (Bio-Rad Laboratories, Inc.) was used to measure the absorbance at 450 nm. Experiments were performed in triplicate.

Transwell assay. The assay was performed as previously published (27). LOVO and HT29 cells ($5x10^4$ for migration assays and $1x10^5$ for invasion assays) were seeded into uncoated or Matrigel-precoated (BD Biosciences; 4 h at 37˚C) upper chambers (Corning, Inc.). Serum-free medium was added to both upper chambers and medium containing 10% FBS was added to the lower cell chambers. The cells were incubated at 37˚C with 5% CO_2 for 12 h, after which the cells remaining in the upper chamber were removed and those in the lower chamber were fixed using anhydrous ethanol at room temperature for 30 min, stained with 1% crystal violet for 1 h and counted using an inverted microscope (Leica Microsystems GmbH).

Plasmid and oligonucleotide transfection. Specific lentivirus short hairpin (sh) RNA targeting METTL3, negative control shRNA (shNC) and METTL3 overexpression plasmids were synthesized by Shanghai GenePharma Co., Ltd. Specific small interfering (si) RNAs targeting SNHG1 and negative control (NC) siRNA were chemically synthesized by Guangzhou RiboBio Co., Ltd. To obtain cells with stable knockdown or overexpression of METTL3, $3x10^5$ HT29 and LOVO cells were transfected with the aforementioned constructed plasmids (140 μg/ml; 2 μl; siSHNG1) and selected with Geneticin (G418; 800 μg/ml; Procell Life Science & Technology Co., Ltd.) for 4 weeks. METTL3 expression levels in selected cell clones were confirmed using RT-qPCR (as per the aforementioned method) and the constructed cell clones with stable overexpressed or knocked down METTL3 were used for further functional assays. To determine the effect of SNHG1 on METTL3, HT29 and LOVO cells with stable overexpression of METTL3 were transfected with SNHG1 siRNAs at 37˚C for 24 husing the RiboFECT™ Transfection Kit (166T; Guangzhou RiboBio Co., Ltd.), performed according to the manufacturer's protocols. RT-qRCR assay was performed 24 h after transfection to determine knockdown efficiency, and then follow-up experiments were performed. The sequences of siRNAs are listed in Table IB.

Methylated RNA immunoprecipitation (MeRIP) qPCR. MeRIP-qPCR assays were performed to determine the m6A modification level of SNHG1 as previously reported (13).
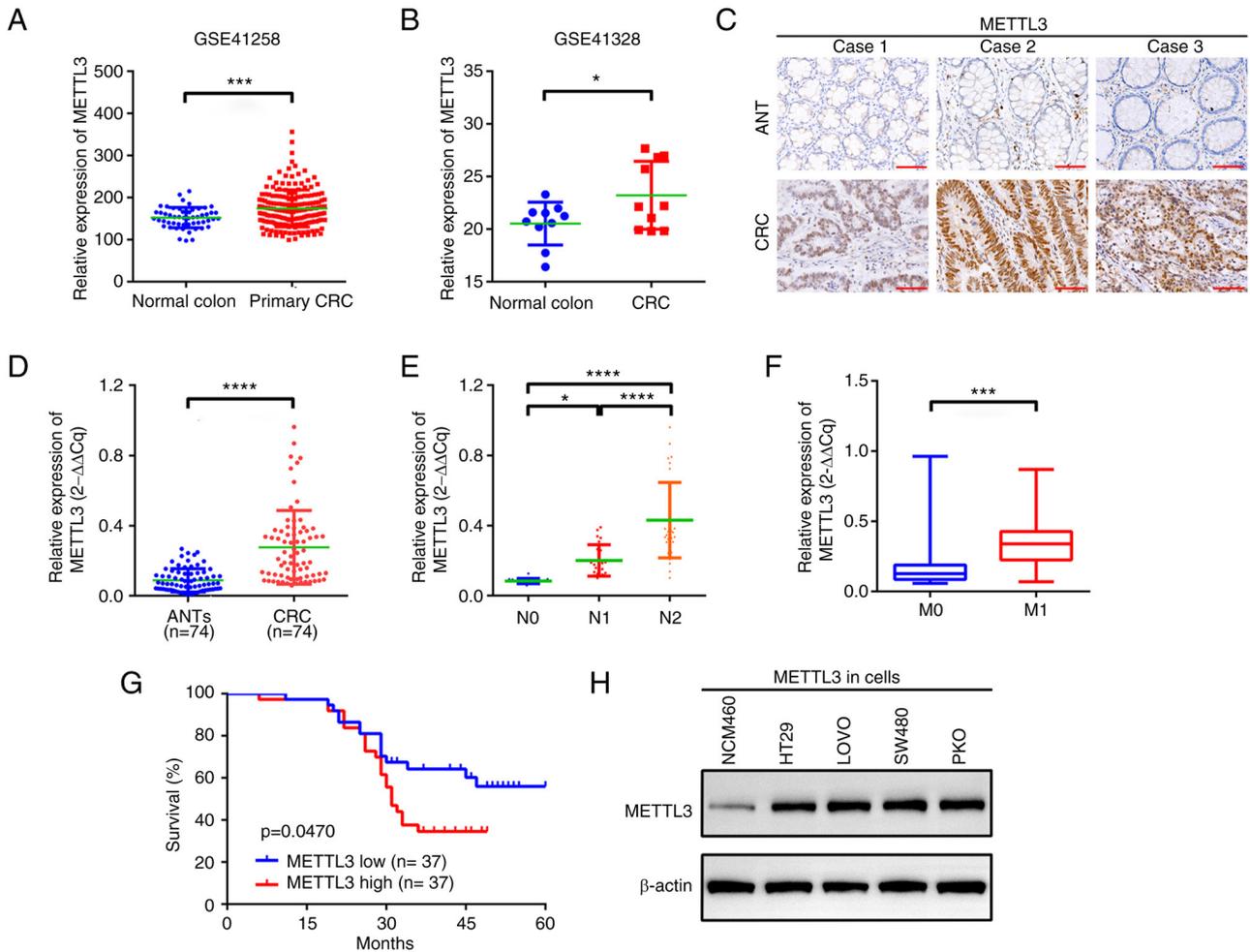
Figure 1. METTL3 is upregulated in patients with CRC. METTL3 expression in CRC, demonstrated by analysis of datasets from two genome-wide studies, (A) GSE41258 and (B) GSE41328. (C) METTL3 expression in CRC and in paired ANTs, assessed using immunohistochemistry. Scale bar, 20 µm. (D) Differential expression of METTL3 in CRC tissues and ANTs, evaluated using reverse transcription-quantitative PCR. (E) The expression of METTL3 in patients with advanced N stage compared with those with low N stage. (F) METTL3 expression in patients with M_1 stage compared with those with M_0 stage. (G) Kaplan-Meier analysis indicating overall survival of patients with high METTL3 expression compared with that of patients with low METTL3 expression. n=37 for each group. (H) Expression of METTL3 in NCM460, LOVO, RKO, SW480 and HT29 cells, measured using western blotting. $^*P<0.05$; $^{***}P<0.001$; $^{****}P<0.0001$. METTL3, methyltransferase-like 3; CRC, colorectal cancer; ANT, adjacent nontumor tissue; N stage, lymph node stage; M stage; metastasis stage.

All procedures were carried out using the Magna MeRIP™ m6A Kit (cat. no. 1710499; Merck KGaA), according to the manufacturer's instructions. In brief, total RNA was isolated as described above and fragmented using 2 µl RNA fragmentation buffer. The input control was one-tenth of the isolated RNA saved. The magnetic bead A/G blend was washed three times, resuspended and incubated with 500 µl MeRIP reaction fluid (containing fragmental RNAs, RNase inhibitor and IP buffer) at 4°C for 2 h. The beads were then harvested by The beads were then harvested by Magnetic rack adsorption to remove superessence and eluted with elution buffer (containing IP buffer, 20 mM m6A and RNase inhibitor; from the MeRIP kit) in a vertical mixer (4°C, 5 min, 10 rpm/min). Eluted RNA fragments were harvested and purified using the A&D Pure TRIzol Total RNA Purification Kit (A&D Co,Ltd). Enrichment levels were determined using qPCR (as per the aforementioned method) and the corresponding m6A enrichment level of each sample was calculated by normalizing the input data (RNA from target sample without m6A antibodies).

Statistical analysis. Data were analysed and evaluated using GraphPad Prism V7.0 (GraphPad Software; Dotmatics) software and SPSS 19.0 statistical software (IBM Corp.). Differences between groups were analysed using unpaired Student's t test or one-way ANOVA followed by a post hoc LSD or Tukey's test. Kaplan-Meier analysis was used to estimate overall survival using the log-rank test. P<0.05 was considered to indicate a statistically significant difference. All data were from three independent repeated experiments and are expressed as the mean ± standard deviation.

Results

METTL3 is upregulated in patients with CRC. METTL3 mRNA expression was initially analysed using the GEO datasets GSE41258 and GSE41328. CRC tissues demonstrated significantly higher METTL3 protein expression compared with that in normal colonic tissues (Fig. 1A and B). Additionally, METTL3 expression was significantly increased in CRC tissue
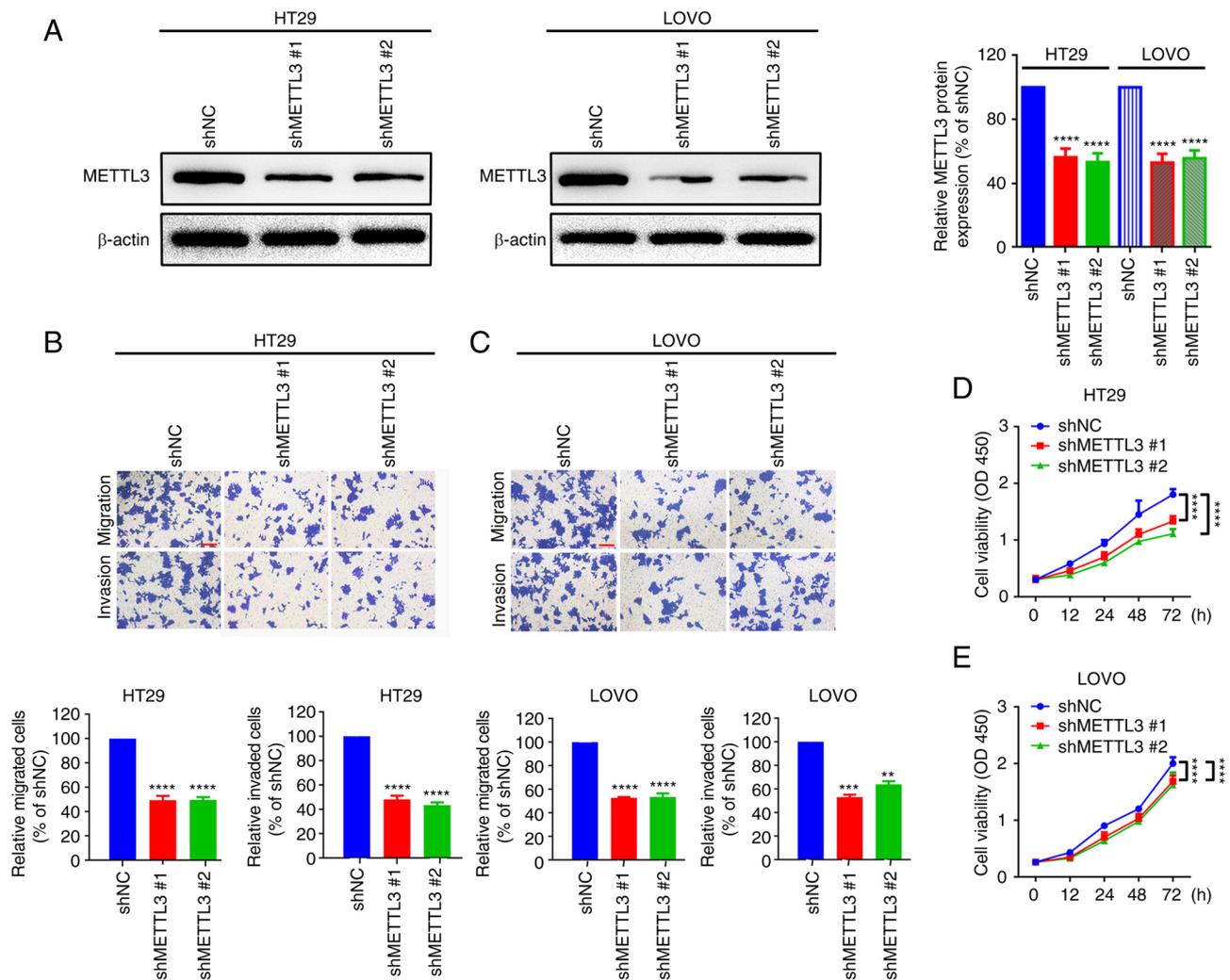
Figure 2. Knockdown of METTL3 suppresses the migration and proliferation of HT29 and LOVO cells. (A) METTL3 protein expression after transfection of specific METTL3 short hairpin RNAs, measured using western blotting. ****P<0.0001. Cell motility changes in (B) HT29 and (C) LOVO cells after knockdown of METTL3, assessed using Transwell assays. Scale bar, 200 μm. Changes in the proliferation of (D) HT29 and (E) LOVO cells after knockdown of METTL3, evaluated using Cell Counting Kit-8 assays. **P<0.01 vs. shNC; ***P<0.001 vs. shNC; ****P<0.0001 vs. shNC. METTL3, methyltransferase-like 3; NC, negative control; sh, short hairpin RNA; OD, optical density.

samples compared with that in paired adjacent non-tumour tissue samples (Fig. 1C and D). METTL3 protein expression was also demonstrated to be upregulated in CRC cells (Fig. 1H). High METTL3 expression was significantly associated with lymph node and metastasis stages and a significantly shorter overall survival in patients with CRC, compared with those with low METTL3 expression (Fig. 1E-G). However, other factors were not considered. These findings indicated that METTL3 may be a promising biomarker of CRC.

The data above showed that METTL3 knockdown inhibits CRC migration and proliferation. METTL3 shRNAs were transfected into two CRC cell lines, HT29 and LOVO, to determine the functional role of METTL3 in CRC cells. Western blotting demonstrated that METTL3 was successfully knocked down in HT29 and LOVO cells (Fig. 2A). The migration of HT29 and LOVO cells was then assessed and was demonstrated to have significantly decreased after METTL3 knockdown, compared with that in the shNC groups (Fig. 2B and C). Finally, a CCK-8 assay was performed, and

compared with that of the shNC group, the proliferation of HT29 and LOVO cells was significantly decreased when METTL3 was knocked down (Fig. 2D and E). The data showed that METTL3 knockdown inhibits CRC cell migration and proliferation.

METTL3-mediated m6A modification is associated with SNHG1 stability in HT29 and LOVO cells. Previous studies reported that METTL3 is closely implicated in certain lncRNA m6A modifications (28-30). SNHG1 is an oncogenic lncRNA in certain cancers, including CRC (16,31,32). Analysis of METTL3 and SNHG1 expression data from TCGA using the online software UALCAN (22), both METTL3 and SNHG1 were demonstrated to be notably upregulated in CRC primary tumour samples, compared with that in normal samples (Fig. 3A-D). Additionally, a significant positive correlation was demonstrated between METTL3 and SNHG1 expression in CRC (Fig. 3E and F). Moreover, SNHG1 contained 18 m6A modification sites, demonstrated using RMBase 2.0 (23) (Table SI) and METTL3 provided six m6A binding sites for
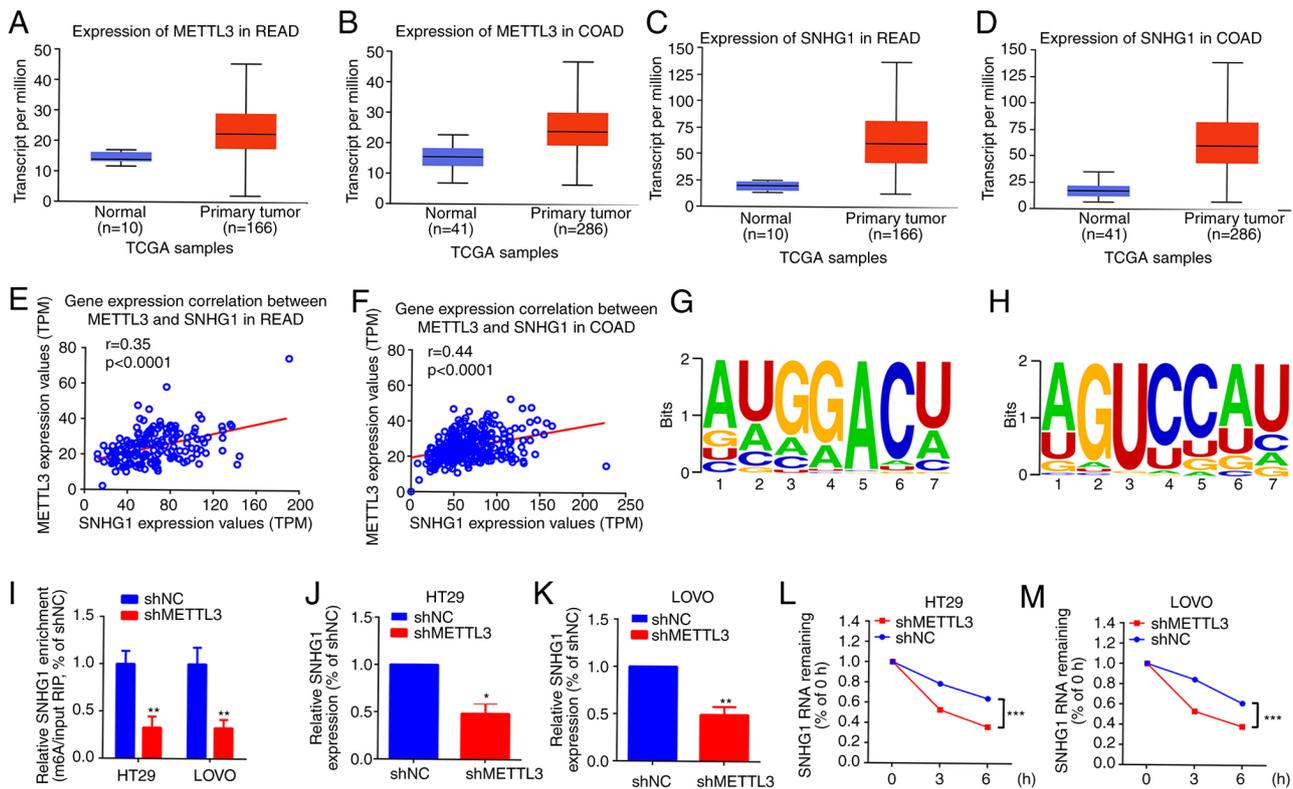
Figure 3. METTL3-mediated m6A modification is associated with SNHG1 upregulation in HT29 and LOVO cells. The expression of METTL3 in (A) READ and (B) COAD and the expression of SNHG1 in (C) READ and (D) COAD, using data from TCGA, analysed using the online software UALCAN (http://ualcan. path.uab.edu/index.html). The correlation between the expression of METTL3 and SNHG1 in (E) READ r=0.35 and (F) COAD r=0.44, assessed using Spearman correlation analysis. (G) and (H) METTL3 binding motifs in the exon region of SNHG1, predicted using RMBase (version 2.0; http://rna.sysu.edu. cn/rmbase/). (I) m6A level of SNHG1 in different METTL3-expressing cells, assessed using methylated RNA immunoprecipitation quantitative PCR. The expression of SNHG1 after METTL3 knockdown in (J) HT29 and (K) LOVO cells, measured using RT-qPCR. The level of SNHG1 in METTL3-expressing cells (L) HT29 and (M) LOVO after actinomycin D intervention, quantified using RT-qPCR. *P<0.05; **P<0.01; ***P<0.001. METTL3, methyltransferase-like 3; m6A, N6-methyladenosine; SNHG1, small nucleolar RNA host gene 1; READ, rectal adenocarcinoma; COAD, colon adenocarcinoma; TCGA, The Cancer Genome Atlas; UALCAN, University of ALabama at Birmingham CANcer data analysis portal; RT-qPCR, reverse transcription-quantitative PCR; TPM, transcripts per million; RIP, RNA immunoprecipitation; NC, negative control; sh, short hairpin RNA.

SNHG1 (Table SII; Fig. 3G and H). The m6A level of SNHG1 in cells with different METTL3 expression levels was then assessed and compared with that in the shNC groups, the m6A level of SNHG1 was significantly decreased in the METTL3 knockdown groups in both HT29 and LOVO cells (Fig. 3I). SNHG1 expression was also significantly reduced in METTL3 knockdown groups in both HT29 and LOVO cells, compared with that in shNC groups (Fig. 3J and K). Furthermore, to evaluate the relationship between METTL3-mediated m6A modification and SNHG1 upregulation, 2 μmol/l actinomycin D was added to HT29 and LOVO cells with knocked down METTL3 and RT-qPCR was used to assess the half-life of SNHG1. It was demonstrated that the half-life of SNHG1 was significantly reduced in groups with downregulated METTL3 in HT29 and LOVO cells, compared with that in the shNC groups (Fig. 3L and M). These results indicated that METTL3 affected the stability of SNHG1 in an m6A-dependent manner.

SNHG1 knockdown partially attenuates the facilitative effect of METTL3 on migration and proliferation in HT29 and LOVO cells. SNHG1-related loss-of-function assays were performed to further assess the relationship between SNHG1- and METTL3-mediated promotion of cell migration and proliferation. Initially, HT29 and LOVO cell models with stable

METTL3 overexpression were constructed (Fig. 4A). Specific SNHG1 siRNAs were transfected into METTL3-overexpressing HT29 and LOVO cells and SNHG1 expression was quantified by RT-qPCR (Fig. S1A and B). Overexpression of METTL3 significantly increased the level of SNHG1 expression, compared with vector group, whilst specific SNHG1 siRNAs significantly reduced SNHG1 expression, compared with METTL3-siNC group (Fig. 4B and C). Functional CCK-8 and Transwell assays were then performed to evaluate the role of SNHG1 in METTL3-mediated cell proliferation and cell motility. Overexpression of METTL3 significantly promoted proliferation and migration compared with the empty vector control group; however, this facilitative effect was significantly reversed by knockdown of SNHG1 in HT29 and LOVO cells when compared with the METTL3-siNC group (Fig. 4D and E). These findings indicated that knockdown of SNHG1 partially attenuated the facilitative effect of METTL3 on HT29 and LOVO cell migration and proliferation.

Discussion

RNA modifications are common features in epigenetic regulation in numerous human diseases, including cancer (33). One of the most common RNA modifications is m6A, accounting
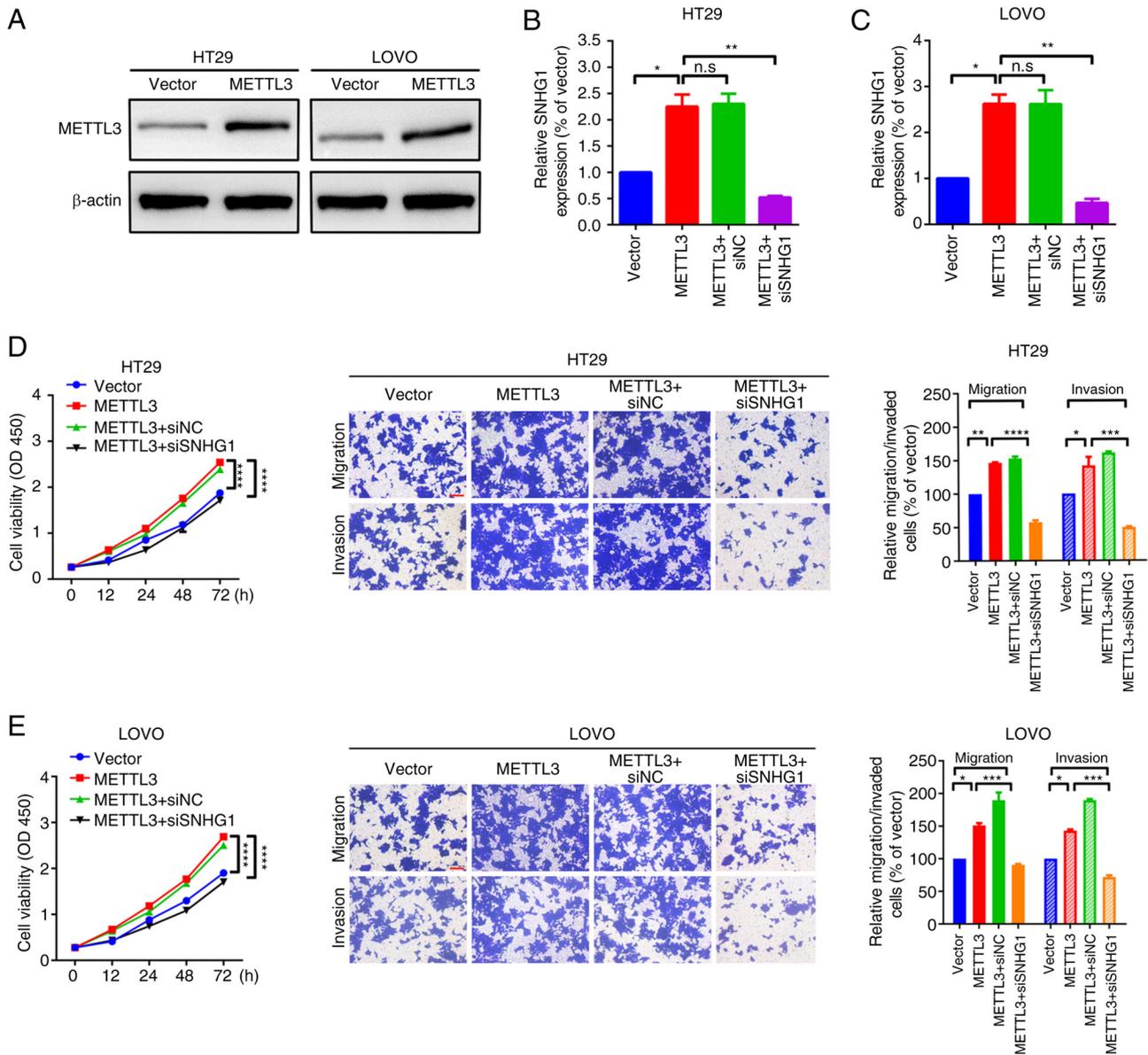
Figure 4. Knockdown of SNHG1 partially attenuated the facilitative effect of METTL3 on migration and proliferation in HT29 and LOVO cells. (A) The expression of METTL3 protein in HT29 and LOVO cells, measured using western blotting. The expression level of SNHG1 in (B) HT29 and (C) LOVO cells, assessed using RT-qPCR. Cell proliferation, invasion and migration of (D) HT29 and (E) LOVO cells, determined using CCK-8 assays (left panel) and Transwell assays (right panel). Scale bar, 200 μm. $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. SNHG1, small nucleolar RNA host gene 1; METTL3, methyltransferase-like 3; CCK-8; Cell Counting Kit-8; NC, negative control; si, small interfering RNA; n.s, non-significance; OD, optical density.

for ~50% of total methylated ribonucleotides and 0.1-0.4% of all adenosine in total cellular RNA (34). m6A regulates cellular processes, including cell self-renewal, differentiation, invasion and apoptosis, and it is also extensively implicated in neoplastic diseases, including CRC, osteosarcoma, lung cancer and ovarian cancer (29,35-37). m6A methyltransferases, demethylases and reader proteins write, remove and recognise m6A, respectively (38).

METTL3, first purified in 1994 from HeLa cell nuclei (39), is the core component of the m6A methyltransferase complex (MTC) and contains 580 amino acids; it functions as the catalytic subunit in the MTC, using adenosylmethionine as the methyl donor (9,40). METTL3 acts as an m6A methyltransferase in numerous cancers, including liver, gastric, lung, pancreatic, bladder, prostate and breast cancers, CRC and acute myeloid leukaemia (10). In line with previously reported findings, analysis of GEO datasets GSE41258 and GSE41328 and experimental research in the present study demonstrated that METTL3 was upregulated and correlated with poor features such as lymph node and metastasis stages, and a significantly shorter overall survival time in patients with CRC. Functional CCK-8 and Transwell assays demonstrated that METTL3 knockdown suppressed the migration and proliferation of HT29 and LOVO cells, suggesting that METTL3 serves as an oncogene in CRC. The downstream targets of METTL3 in CRC include SOX2, chromobox 8, hexokinase 2 and solute carrier family 2 member 1 (13,41,42). Additionally, METTL3 participates in the m6A modification of several noncoding RNAs, including lncRNA RP11-138 J23.1, primary-microRNA-1246 and circular RNA

NOP2/Sun RNA methyltransferase 2 (43-45). In the present study, only the relationship between METTL3 and SNHG1 was evaluated.

SNHG1, located in the 11q12.3 region of the chromosome, contains 11 exons and is a host to eight small nucleolar RNAs from its spliced intron (46). As an oncogenic lncRNA, SNHG1 is aberrantly expressed in numerous malignancies, including colorectal, liver, lung and prostate cancer, and promotes tumourigenesis via diverse signalling pathways (16,31). SNHG1 upregulation promotes CRC progression through multiple mechanisms, such as microRNA sponging, Wnt/β-catenin signal activation and p53 pathway modulation (14,18,32,47). Previous studies have reported that m6A modification affects the stability of several lncRNAs, such as X inactive-specific transcript, growth arrest-specific 5 and metastasis associated lung adenocarcinoma transcript 1 (48-50). In the present study, it was demonstrated that SNHG1 was positively correlated with METTL3. Additionally, it was demonstrated that METTL3 affected the m6A level of SNHG1 and the half-life of SNHG1. Furthermore, it was demonstrated via online bioinformatics analysis that SNHG1 supplied 18 m6A modification sites. Furthermore, knockdown of SNHG1 was demonstrated to have partially attenuated the facilitative effect of METTL3 on migration and proliferation in CRC cells. The findings from the present study together suggest that SNHG1 is a downstream target of METTL3 in CRC.

CRC carcinogenesis is an intricate biological process involving numerous factors and molecules. In the present study, it was demonstrated that METTL3 promotes the proliferation and migration of CRC cells via SNHG1 m6A modification. The findings of the present study therefore suggest that the inhibition of METTL3 may provide a new therapeutic target in the molecular treatment of CRC.

Acknowledgements

Availability of data and materials

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

Authors' contributions

XW conceived and designed the study, and revised the manuscript. YX, YB and GQ performed the experiments, prepared the figures, and designed Tables I, SI and SII. YB and GQ confirm the authenticity of all the raw data. YX, HY and MH developed the methods and performed data analysis. YX and MH acquired and interpreted the data, and wrote and revised the paper. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All experiments were approved by the Animal Ethics and Laboratory Committee of Affiliated Central Hospital of Shenyang Medical Science (approval no. 20220528). All individuals provided written informed consent for the use of their tissues in the present study

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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