# METTL1 promotes Neuroblastoma development through m 7 G tRNA modification and selective oncogenic gene translation

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# Abstract

## Background

Neuroblastoma (NBL) is a common but aggressive malignancy with a poor prognosis in childhood. Despite existing therapy approaches, the 5-year survival rate for patients with advanced NBL remains below 30%, emphasizing urgent necessary for novel therapeutic strategies. Studies have shown that epigenetic disorders play an essential role in the pathogenesis of NBL. However, the function and mechanism of N7-methylguanosine ($m^7G$) methyltransferase in NBL remains unknown.

## Methods

The expression levels of $m^7G$ tRNA methyltransferase METTL1 (Methyltransferase-like 1) were analyzed by querying the GEO database and further confirmed by immunohistochemistry (IHC) assay. The prognostic role of METTL1 were analyzed by the R2 genomics analysis and visualization platform. Function studies were performed to evaluate how METTL1 works in proliferation, apoptosis and migration in cell lines and xenograft mouse models. The role of METTL1 on NBL cells mRNA translation activity was measured using puromycin intake assay and polysome profiling assay. The $m^7G$ modified tRNAs were identified by tRNA reduction and cleavage sequencing (TRAC-seq). Ribosome nascent-chain complex-bound mRNA sequencing (RNC-seq) was utilized to analyzed genes with alterative translation efficiency. Analyzed the codon frequency decoded by $m^7G$ tRNA in genes to clarify the mechanism of $m^7G$ modification in translation regulation.

## Results

This study found METTL1 was significantly upregulated in advanced NBL and was associated with a poor prognosis. Further in vitro and in vivo assays showed METTL1 played a crucial role in promoting NBL progression. Furthermore, $m^7G$ profiling and translation analysis revealed downregulation of METTL1 inhibited puromycin intake efficiency of NBL cells, indicating the crucial METTL1 did count in regulation of NBL cell translation. With all tRNAs with $m^7G$ modification identified in NBL cells, METTL1 knockdown significantly lowered both $m^7G$ modification level and $m^7G$ tRNAs expressions. RNC-seq revealed 339 overlapped genes with impaired translation in NBL cells upon METTL1 knockdown. Further analysis revealed these genes contained higher frequency of codons decoded by $m^7G$-modified tRNAs and were enriched in oncogenic pathways.

## Conclusion

This study revealed the critical role and mechanism of METTL1-mediated tRNA $m^7G$ modification in regulating NBL progression, providing new insights for developing therapeutic approaches for NBL patients.

# 1. Background

Neuroblastoma (NBL) is the most common malignant tumor in infancy and the most common extracranial solid tumor in childhood, accounting for more than 7% of malignant tumors in children under 15 years of age[1]. Despite the availability of multimodal treatment, the prognosis and survival rates are extremely low, which poses many challenges for clinicians in diagnosis and treatment, especially for high-risk patients[1, 2]. Therefore, it is vital to search for potential molecular mechanisms of NBL initiation and progression as well as more effective management strategies.

Epigenetic disorders have been proven to play an essential role in the pathogenesis of NBL and offer a number of potential therapeutic targets[3–8]. In recent years, with the continuous optimization of the sensitivity of detection technologies and high-throughput sequencing technologies, a large number of low-abundance RNA modifications on mRNA, tRNA, and other noncoding RNAs have been shown to be associated with the onset and progression of human diseases[9–12]. a growing number of reports show that aberrant expression of lncRNAs, dysregulated expression and functional disruption of miRNAs play a crucial function in MYCN proto-oncogene (MYCN) expression and malignant progression in high-risk NBL[13–18]. In addition, N6-methyladenosine ($m^6A$) mRNA modification regulators have been reported to influence the clinical prognosis of NBL and may be novel therapeutic targets for NBL[19]. Indeed, tRNAs contain more modifications that contribute to tRNA stability, translation accuracy, and protein synthesis rates[20, 21]. Aberrant expression or mutations of tRNA modification enzymes are increasingly observed in human diseases[22, 23]. However, little is known about the physiological functions of tRNA modifications, especially in cancer.

tRNAs are widely modified in nature, and N7-methylguanosine ($m^7G$) is one of the most prevalent modified nucleosides[24]. This modification is a product of $m^7G$ methyltransferase, which in humans is installed by the METTL1 (methyltransferase-like 1)/WDR4 (WD repeat domain 4) complex[25, 26]. The complex component METTL1 catalyzes methylation of guanine, while its partner WDR4 helps stabilize the methyltransferase complex[25, 27]. Recent reports have revealed a widespread tRNA $m^7G$ methylome in mammals[26, 28–30]. knockout of METTL1 leads to impaired tRNA $m^7G$ modification and abnormal differentiation and growth of embryonic stem cells in mice[26]. Mutations in WDR4 are associated with microcephalic primordial dwarfism and Galloway-Mowat syndrome[31, 32]. This evidence suggests that METTL1/WDR4-mediated tRNA $m^7G$ modifications play a key role in regulating cell fate decisions. Notably, METTL1 was recently reported to impair the sensitivity of colon and cervical cancer cells to chemotherapy[33, 34]. Nevertheless, the oncogenic functions and molecular mechanisms of $m^7G$ tRNA modification in regulating NBL progression remain uncovered.

Here, we found that evaluated METTL1 was associated with poor prognosis in NBL. Functionally, downregulation of METTL1 was attributed to reduced tumorigenesis and progression in vitro and in vivo. Mechanistically, METTL1-mediated tRNA $m^7G$ modification selectively regulates the translation of oncogenic transcripts in a codon frequency-dependent manner. This study revealed the molecular mechanism of tRNA $m^7G$ modification mediated NBL progression, providing a potential strategy for clinical management of NBL.

# 2. Materials And Methods

## 2.1 Patient samples

Clinical data were obtained from 132 children (under 16 years old) with NBL diagnosed pathologically as neuroblastoma at the First Affiliated Hospital of Sun Yat-sen University from January 1, 2014, to December 30, 2019. Clinical data were applied to analyze the relationship between the diagnostic stage and METTL1 expression levels. Paraffin-embedded specimens were used for immunohistochemistry (IHC) analysis of METTL1 expression levels. Ethical approval for the study with human subjects was obtained from the Institutional Review Board of the First Affiliated Hospital of Sun Yat-sen University, and written consent was obtained from each patient.

## 2.2 Experimental Animals

The BALB/c-nu female mice were purchased from GemPharmatech Co., Ltd (Jiangsu, China). All animal care and experimental protocols were approved by the Institutional Ethics Committee for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University. The study complied with all relevant ethical regulations regarding Animal Research: Reporting of in vivo Experiments (ARRIVE) guidelines. Mice were euthanized when their tumor size and overall health status met the institutional euthanasia criteria.

## 2.3 Cell lines and cultures

Human embryonic kidney 293T (HEK 293T) cells were obtained from Prof. Shuibin Lin's laboratory (Guangzhou, China). Human NBL KELLY cells were obtained from Tongpai Biotechnology Co Ltd (Shanghai, China). Human NBL BE2C cells were from Procell Life Science & Technology Ltd (Wuhan, China). 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA), and 1% GlutaMAX (Gibco, USA). KELLY cells were cultured in DMEM (Gibco, USA) supplemented with 10% FBS (Gibco, Australia) and 1% penicillin-streptomycin (Gibco, USA), and 1% GlutaMAX (Gibco, USA). SK-N-BE(2)C (BE2C) cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, Gibco, USA) supplemented with 10% FBS (Gibco, Australia), 1% penicillin-streptomycin (Gibco, USA), 1× GlutaMAX (Gibco, USA), and 1% MEM non-essential amino acid solution (Gibco, USA). Cells were cultured in a 5% CO2 cell culture incubator (Thermo Scientific, USA) at 37°C.

## 2.4 Knockdown of METTL1 in NBL cells

Lentiviral vectors expressing pLKO.1 shRNA as a negative control and two shRNA constructs targeting METTL1 were supplied by KeyGEN BioTECH (Nanjing, China). For lentivirus production, the lentiviral shRNA constructs were co-transfected into HEK 293T cells using Lipofectamine 2000 (Invitrogen, USA) with a packaging plasmid (pCMV-ΔR8.9) and an envelope plasmid (pCMV-VSVG). 48 h later, the viruses were collected and infected with Polybrene (Solarbio, China) (4 μg/ml for KELLY cells and 8 μg/ml for BE2C cells). Stably infected cells were screened with puromycin (Solarbio, China) (2 μg/ml for KEELY

cells and 4 μg/ml for BE2C cells) for 24 hours. Small interfering RNAs (siRNAs) targeting the 3'-UTR of METTL1 were used to knockdown METTL1 with Lipofectamine 2000 (Invitrogen, USA). siRNA sequences are listed in Table S1.

## 2.5 Cell proliferation and migration assays

For the cell proliferation assay, 1000 cells were grown in each well of a 96-well plate with 100 μL of fresh medium. Cell viabilities were measured every 24 h for five days using Cell Counting Kit-8 (Dojindo, Japan). For the migration assay, $7.5\times10^4$ cells in 200 μL of serum-free medium were added to the upper chamber of the transwell insert (Corning Falcon, USA) and placed in receiving wells containing 700 μL of cell culture medium supplemented with 10% fetal bovine serum. Migrated cells were stained with 0.5% crystal violet and counted after 24 hours.

## 2.6 Cell apoptosis assays

According to the manufacturer's instructions, the cell apoptosis assay was performed with Annexin V-FITC Apoptosis Detection Kit (KeyGEN BioTECH, China). The percentage of positive cells was detected by CytoFLEX (Beckman Coulter, USA).

## 2.7 Subcutaneous implantation in a mouse model

Four- to six-week-old BALB/c-nu female mice were randomly divided into shNC and shMETTL1-1 groups (n = 5). NBL cells were resuspended by mixing equal amounts of phosphate-buffered saline (PBS, Gibco, USA) and Matrigel (Corning, China). $7\times 10^6$ NBL cells in 100 μl of the PBS-Matrigel mixture were injected into the back of the mice. The length (a) and width (b) of the tumors were measured every two days with calipers, and the tumor volume (V) was calculated using the formula $V = ab^2/2$. Fifteen days after injection, the mice were humanely killed, and the harvested tumors were used for further analysis.

## 2.8 Immunohistochemical (IHC) staining

IHC was performed with an IHC kit (Agilent, USA) and primary antibodies (anti-METTL1, Proteintech, 1:2000 dilution; anti-Ki67, Proteintech, 1:8000 dilution) to detect the described protein expression. To assessing the level of METTL1 expression, histochemistry score (H-score) is generating by the following formula: H Score = summation (1 + i)×pi, where i is the intensity score and pi is the percent of the cells with that intensity. The intensity score was categorized as 0 (absent), 1 (weak), 2 (moderate) and 3 (strong). The percent of the cells was scored as 0, 1, 2, and 3 for < 5%, 5–25%, 25–50%, 50–75%, and > 75%, respectively. Tissues with H-score ≧ 3 were considered as high METTL1 expression group, and tissues with H-score < 3 were classified as low METTL1 expression group.

## 2.9 RNA isolation and quantitative analysis

According to the manufacturer's instructions, total RNAs were isolated with AG RNAex Pro RNA Reagent (AG, China). For reverse transcription-polymerase chain reaction (RT-PCR), cDNA was synthesized in a 20uL reaction system using HiScript III RT SuperMix for qPCR Kit (Vazyme, China). cDNA samples were then diluted at 1:20 and used for qPCR. Real-time quantitative PCR assays (qPCR) were performed on a

StepOnePlus™ real-time PCR system (Thermo Scientific, USA) with TB Green™ Premix Ex Taq™ II (Takara, Japan). Each sample was repeated three times. Results were calculated by the $2^{-\Delta\Delta Ct}$ method using β-ACTIN as an internal control. The primer sequences used in this study are listed in Table S1.

## 2.10 Northern blot, Northwestern blot, and Western blot

As previously reported, Northern blot and western blot assays were performed[26, 35]. Briefly, for Northern blot, 2 μg of total RNA was separated by electrophoresis on a 15% TBE-UREA gel, and then the RNAs were transferred to a positively charged nylon membrane. Afterward, the nylon membranes were cross-linked with ultraviolet (UV) light. The indicated tRNAs and U6 snRNA were blotted with Digoxigenin-labeled probes. After transfer and cross-linking, nylon membranes were blotted with primary antibody (anti- $m^7G$, MBL International, USA) for Northwestern blotting at 4°C overnight. Anti-digoxigenin or anti- $m^7G$ antibody signals were detected according to the previously described Western blot protocol[26, 35]. Probe sequences are listed in Table S1.

## 2.11 Polysome profiling

Polysome profiling was performed as previously described[36]. Briefly, NBL cells were incubated with 100 μg/ml cycloheximide for 15 min at 37°C. After immediate cold PBS wash, the cells were lysed with multimeric cell extraction buffer (50 mM MOPS, 15 mM MgCl2, 150 mM NaCl, 100 μg/ml cycloheximide, 0.5% Triton X-100, 1 mg/ml heparin, 200 U/ml RNase inhibitor, 2 mM Phenylmethylsulfonyl Fluoride (PMSF), and 1 mM benzamidine) for 10 minutes on ice and centrifuged at 13,000g for 10 min at 4°C. The OD values of the samples were measured and adjusted to be equal. Then 1 ml of cytoplasmic extract was layered onto 11 ml of a 10%-50% sucrose gradient, followed by centrifugation at 36,000 rpm for 3 h at 4°C. Separated samples were fractionated at 0.75 ml/min by a BR-188 density gradient fractionation system (Brandel, USA) and monitored at an absorbance of 254 nm.

## 2.12 Puromycin intake assay

NBL cells were transfected with METTL1 siRNA and negative control oligos (siNC). After 48 hours, cells were incubated with puromycin (final concentration of 1μM) for 30 min at 37°C. After incubation, the cells were lysed to extract proteins, and the levels of puromycin were detected by Western blot with an anti-puromycin antibody (MABE343, Millipore). The siRNA sequences are listed in Table S1.

## 2.13 tRNA $m^7G$ reduction and cleavage sequencing (TRAC-seq)

TRAC-seq was performed as previously described[37]. Small RNAs were isolated from total RNAs using the Quick-RNA™ Microprep Kit (Zymo Research, USA) according to the manufacturer's instructions, followed by recombinant wild-type and D135S AlkB protein treatment. Half of the AlkB-treated RNAs were used as input for the construction of the library. Next, the remaining AlkB-treated RNAs were treated with 0.2 M NaBH4 for 30 min on ice in the dark. The RNAs were then dark-treated with aniline acetate solution (H2O: glacial acetic acid: aniline, 7:3:1) for 2 h at room temperature in the dark to induce the site-specific

cleavage. After the cleavage, the RNAs were purified using the Oligo Clean & Concentrator™ kit (Zymo Research, USA). Finally, the RNA samples were applied for cDNA library construction using NEBNext Multiplex Small RNA Library Prep Set for Illumina (New England BioLabs, USA) and used for high-throughput sequencing on Illumina Nextseq 500. The TRAC-seq data were analyzed as previously described[37]. Briefly, for tRNA $m^7G$ analysis, joint and low-quality sequence data were filtered with Trim-Galore. The filtered data were mapped to human mature tRNA sequences using Bowtie, and the read depth at each site and the number of reads starting from that position were calculated using Bedtools. The cleavage rate at the site was defined as the ratio between the number of reads starting and the read depth at the site. The cleavage score for the site was then calculated as:

$$\text{Cleavage score (i)} = \frac{log2\left(\text{Cleavagerate}_{\text{treat}}\right)}{\log2\left(\text{Cleavageratio}_{\text{non}-\text{treat}}\right)}$$

Sites with a cleavage score > 3 and a cleavage rate > 0.1 were considered as candidate $m^7G$ sites. To analyze tRNA expression, we extracted sequences containing tRNA genes and 100 bp upstream and downstream of tRNA genes as precursor tRNA genes. The predicted introns were deleted for the mature tRNA sequences, and "CCA" was added to the 3' end. During the mapping process with Bowtie2, tRNA reads were calculated and normalized for further analysis.

# 2.14 Ribosome nascent-chain complex-bound mRNA sequencing (RNC-seq)

RNC-seq was performed as previously described[38]. Briefly, cells were pretreated with 100 μg/ml cycloheximide and incubated for 15 min at 37°C. After washing twice with pre-cooled PBS, 1 ml of cell lysate was incubated with 1 ml of ribosomal buffer (RB buffer) containing 1% TritonX-100 [20 mM HEPES-KOH (pH 7.4), 15 mM $MgCl_2$, 200 mM KCl, 100 μg/ml cycloheximide and 2 mM dithiothreitol] for 30 min on ice. The cell lysate was then centrifuged at 16,200g for 10 min at 4°C. 10% of the extract was used as input control. The remaining extract was layered into 11.5 ml sucrose buffer (30% sucrose in RB buffer), and the RNC pellet was collected by centrifugation at 32,000 rpm for 5 h at 4°C. Next, RNA was isolated from the input and RNC samples for sequencing. The isolated RNA was subjected to cDNA library construction and sequencing using the BGISEQ-500 platform (BGI-Shenzhen, China). Gene expression levels were normalized to FPKM. Translational efficiencies were calculated as: TE = (FPKM in polyribosome-seq) / (FPKM in input RNA-seq).

# 2.15 Gene ontology and pathway analysis

Gene ontology and pathway analysis of TE-down mRNAs identified in RNC-seq data were performed using ToppGene Webtool (https://toppgene.cchmc.org/enrichment.jsp). Benjamini-Hochberg adjusted *P* values < 0.05 for ontology terms, and pathways were classified as significantly enriched.

# 2.16 Statistics Analysis

Quantitative data are shown as mean ± SEM. P values in all cases are represented as: ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05. For statistical analysis, Student's t-test, one-way ANOVA, or Mann-Whitney U test were used unless otherwise stated. Event-free survival was analyzed using the log-rank test. Statistical analyses were performed using GraphPad Prism version 8 or SPSS version 25.

# 3. Results

## 3.1 Elevated METTL1 is associated with poor prognosis in NBL patients

To explore the clinical association between METTL1 and NBL, we first analyzed the RNA-seq

and corresponding clinical information of NBL from the GEO database (GSE62564). We found that high mRNA levels of METTL1 mainly occurred in high-risk NBL samples based on the Children's Oncology Group (COG), advanced NBL samples according to the International Neuroblastoma Staging System (INSS), and NBL samples with amplified MYCN (Fig. 1A). In addition, METTL1 mRNA levels were significantly elevated in the advanced INSS staging group and the high-risk group (Fig. 1B-1C). Further Kaplan-Meier analysis showed that 5-year event-free survival was significantly lower in NBL patients with high METTL1 expression than in NBL patients with low METTL1 expression (Fig. 1D). Moreover, the group with high levels of METTL1 had a worse prognosis in non-high group (Fig. 1E) and in high group (Fig. 1F) respectively, suggesting a potential regulatory role in the progression and progression of NBL.

To further validate the potential link between METTL1 and NBL, METTL1 protein levels were examined by immunohistochemistry (IHC) in a cohort of 132 NBL patients (n = 132) (Fig. 2A). IHC was assessed by H-score. The results consistently showed that METTL1 protein levels were significantly increased in advanced NBL (Fig. 2B-2C), which further demonstrated that high METTL1 expression was closely associated with the high INSS stage of NBL. Our findings suggested that METTL1 could play an essential role in the progression of NBL.

## 3.2 METTL1 knockdown inhibited NBL progression in vitro

To explore the role of METTL1 in NBL, two short hairpin RNAs were used to knockdown the expression of METTL1 (shM1-1, shM1-2). Kelly and BE2C were two NBL cell lines with high METTL1 expression. Cells transfected with short hairpin RNA targeting green fluorescent protein (shGFP) were used as the negative control. Western blot assays were performed to confirm the inhibitory effect of METTL1 in Kelly and Be2C cells (Fig. 3A). To further study the biological effects of METTL1 knockdown in NBL cells, we first performed a CCK-8 assay to test the proliferation of NBL cells. The results showed that downregulation of METTL1 significantly inhibited cell proliferation (Fig. 3B). In addition, flow cytometry assays were performed, and the data showed that knockdown of METTL1 resulted in a significant increase in apoptosis of NBL cells (Fig. 3C-3D). Furthermore, the migration ability of NBL cells was significantly

decreased after METTL1 knockdown compared to the control group (Fig. 3E-3F). These results uncovered the critical role of METTL1 in the progression of NBL in vitro.

## 3.3 Inhibition of METTL1 reduced the tumorigenicity of NBL cells in vivo

We then used a xenograft mouse model to verify whether aberrant METTL1 expression affects NBL formation and growth in vivo. The tumorigenicity of mice injected with METTL1 knockdown BE2C cells was suppressed compared to controls, as reflected by a significant decrease in tumor size and weight (Fig. 4A-4E). IHC staining of subcutaneous tumor tissues showed significantly reduced protein levels of METTL1 and Ki67, indicating that METTL1 knockdown significantly inhibited NBL proliferation in vivo (Fig. 4F-4G).

## 3.4 METTL1 regulated tRNA $m^7G$ modification, tRNA expression, and overall mRNA translation in NBL cells

We explored the oncogenic function of METTL1 in regulating NBL progression in vivo and in vitro experiments. To explore the molecular mechanisms underlying METTL1-mediated regulation of NBL progression, northwestern blot and northern blot were first performed, and we found a dramatic decrease in the level of $m^7G$ modification in METTL1 knockdown kelly cells (Fig. 5A). For better understanding, we determined the tRNA $m^7G$ modification profile using previously established tRNA $m^7G$ site reduction and cleavage sequencing (TRAC-seq) (Lin et al., 2019), by which we identified a total of 21 $m^7G$ -modified tRNAs and the corresponding motif sequence "DURGY" (Fig. 5B-5C). The $m^7G$ signal in METTL1 knockdown Kelly cells was significantly lower than in control cells (Fig. 5D). In addition, METTL1 knockdown resulted in a significant decrease in the expression level of $m^7G$ -modified tRNA in Kelly cells (Fig. 5D-5E), indicating that METTL1-mediated tRNA $m^7G$ modification played a key role in regulating tRNA expression.

The tRNA is one of the key components in the mRNA translation process, so we performed a polyribosome analysis to assess the global translation level of METTL1 knockdown and control NBL cells. METTL1 knockdown inhibited translational activity compared to the control group, which was reflected in reducing polyribosomal peaks (Fig. 5F). In addition, the decrease in mRNA translation levels in METTL1 knockdown NBL cells was also confirmed by the puromycin intake assay, as reflected in the reduction of incorporated protein (Fig. 5G). Altogether, our data demonstrated that METTL1-mediated tRNA $m^7G$ modification plays a vital role in controlling tRNA expression and mRNA translation in NBL cells.

## 3.5 Knockdown of METTL1 selectively inhibited the translation of oncogenic mRNAs

To further study the translational mechanism of METTL1-mediated tRNA $m^7G$ modifications in NBL progression, we sequenced actively translated mRNAs using ribosome nascent-chain complex sequencing (RNC-seq). mRNAs bound to ribosome nascent-chain complex (RNC-mRNAs) were isolated from total mRNAs (input-mRNAs) by centrifugation. The translation efficiency (TE) of mRNA was calculated by dividing the FPKM (fragments per kilobase per million) of input-mRNA by the FPKM of RNC-mRNA. mRNAs with down-regulated TE (TE-down) or upregulated TE (TE-up) were identified by RNC-seq (Fig. 6A). To investigate the association between down-regulated $m^7G$ -modified tRNA expression and TE-down, we calculated the frequency of $m^7G$ -modified tRNA-decoded codons on all mRNAs. Interestingly, the frequency of the codons decoded by $m^7G$ -modified tRNAs on TE-down mRNAs was significantly higher than that of other mRNAs, suggesting that METTL1-mediated tRNA $m^7G$ modification selectively regulates mRNA translation in the form of $m^7G$ -related codon dependent manner (Fig. 6B).

We took the intersection of TE-down mRNAs in Kelly and Be2C cells and found 339 candidates (Fig. 6C). Gene ontology analysis of candidate mRNAs showed that TE-down mRNAs in METTL1 knockdown cells were significantly enriched in oncogenic pathways, including the genomes of c-MYC transcriptional activation and validated targets of the cell cycle (Fig. 6D). Metadherin (MTDH) and Programmed Cell Death 10 (PDCD10), two common oncogenes with significantly reduced TE in METTL1 knockdown NBL cells, were selected as candidate genes. The translation level or protein level of the candidate genes were verified by RNC-qPCR and Western blot, and the results confirmed that downregulation of METTL1 in NBL cells significantly suppressed the expression of MTDH and PDCD10 at the transcription and translation level (Fig. 6E-6F). Consistent with our RNC-seq data, our RNC-qPCR and Western blot data indicated that METTL1-mediated tRNA $m^7G$ modification regulated oncogenic expression by interfering with mRNA translation. Overall, our findings uncovered a selective regulatory function of METTL1 in oncogenic mRNA translation and revealed a molecular mechanism for tRNA $m^7G$ modification mediated NBL progression.

# 4. Discussion

Epigenetic plasticity plays a vital role in regulating the phenotype of tumor cells[39, 40]. Notably, aberrant expression of the $m^7G$ -modified methyltransferase METTL1 was associated with a range of cancers[19, 33, 41–43]. The potential role of METTL1 in catalyzing $m^7G$ modifications is different in different cancers. On the one hand, overexpression of METTL1 acts as a tumor suppressor through miRNA $m^7G$ modification in colon and lung cancers[33, 43]. On the other hand, METTL1 exerts oncogenic activity via suppression of PTEN signaling in hepatocellular carcinoma and via the AKT/mTORC1 pathway in lung adenocarcinoma[19, 42]. In this study, we demonstrated for the first time the oncogenic role of METTL1-mediated tRNA $m^7G$ modification in NBL. Higher METTLE expression is associated with a higher risk of COG and advanced INSS stage in NBL patients and confers a survival disadvantage to NBL patients. Furthermore, in vitro and in vivo experiments confirmed that knockdown of METTL1 expression inhibited tumorigenic capacity, cell activity, and migration ability of NBL and promoted apoptosis of cells. Therefore, METTL1 knockdown can inhibit the progression of NBL.

In our study, METTL1-mediated tRNA $m^7G$ modification was shown to selectively regulate the translation of oncogenic transcripts in a codon-dependent manner, indicating METTL1-mediated NBL progression. mRNA translation is closely coordinated by mRNA, tRNA, and ribosomes and is a key link in transmitting genetic information from DNA to proteins. Previous studies have illustrated that dysregulation of mRNA translation encoding oncogenes leads to aberrant expression of oncogenes[44–47]. Therefore, targeting mRNA translation is a vital strategy to regulate gene expression.

A growing body of evidence reveals the oncogenic functions of MTDH and PDCD10 in regulating cancer progression. For example, MTDH has been reported to affect the function of PI3K/AKT pathway genes directly or indirectly and is associated with tumor cell survival, metastasis, and drug resistance[48–55]. In addition, a study showed that knockdown of MTDH in human NBL significantly inhibited cell survival and significantly improved sensitivity to cisplatin[56]. PDCD10 promotes cell migration and tumor metastasis through epithelial-mesenchymal transition and the Wnt signaling pathway and is involved in apoptosis and cell cycle regulation[57, 58]. These studies provided direct evidence for targeting MTDH and PDCD10 for better cancer management. In the present study, we found that METTL1 and its downstream MTDH and PDCD10 have oncogenic functions in the progression and occurrence of NBL, suggesting that METTL1 or its downstream MTDH and PDCD10 may be potential targets for NBL therapy. Unfortunately, we did not perform downstream gene rescue experiments, and further validation is needed.

# 5. Conclusion

In conclusion, enhanced expression of METTL1 in patients predicts a poor prognosis. Furthermore, METTL1 knockdown reduces $m^7G$ tRNA modification and selectively reduces mRNA translation of oncogenes in NBL in a codon frequency-dependent manner, which could explain why METTL1 knockdown inhibits tumorigenesis and progression in NBL cells in vitro and in vivo. The results of this study enrich the network of epigenetics in the regulation of neuroblastoma progression, and METTL1 is expected to provide new biomarkers and novel therapeutic targets for the diagnosis of NBL, significantly advanced NBL.

# Abbreviations

All abbreviations metioned in this study are listed as follow:

**NBL**

Neuroblastoma

**$m^7G$**

N7-methylguanosine

**METTL1**

Methyltransferase-like 1

**IHC**

Immunohistochemistry assay

**TRAC-seq**

tRNA reduction and cleavage sequencing

**RNC-seq**

Ribosome nascent-chain complex-bound mRNA sequencing

**$m^6A$**

N6-methyladenosine

**WDR4**

WD repeat domain 4

**HEK 293T**

Human embryonic kidney 293T

**BE2C**

Neuroblastoma cell line: SK-N-BE(2)C

**H-score**

Histochemistry score

**shRNA**

Short hairpin RNA

**shGFP**

shRNA targeting green fluorescent protein

**shM1**

shRNA targeting METTL1

**siRNA**

Small interfering RNA

**siNC**

siRNA targeting negative control oligos

**siM1**

siRNA targeting METTL1

**PMSF**

Phenylmethylsulfonyl Fluoride

**RB buffer**

Ribosomal buffer

**TE**

Translational efficiencies

**COG**

Children's Oncology Group

**INSS**

International Neuroblastoma Staging System

**MYCN**

MYCN proto-oncogene

**RNC-mRNAs**

mRNAs bound to ribosome nascent-chain complex

**Input-mRNAs**

Total mRNAs

**FPKM**

Fragments per kilobase per million

**TE-down**

Down-regulated Translational efficiencies

**TE-up**

Upregulated Translational efficiencies

**MTDH**

Metadherin

**PDCD10**

Programmed Cell Death 10

# Declarations

### Ethics approval and consent to participate

Ethical approval was given by the medical ethics committee of IEC for Clinical Research and Animal Trails of the First Affiliated Hospital of Sun Yat-sen University.

### Consent for publication

Not applicable

### Availability of data and material

The raw data are available on reasonable request from corresponding author.

### Competing interests

All authors declare that they have no conflict of interests.


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### Authors' contributions

L.B.H., S.B.L. and J.S.L. designed, supervised the study, and revised the manuscript. Y.H. acquired, analyzed and interpreted data; C.Y.Y., J.Y.M., P.J.W., M.H.Y., H.H., T.F.Y., S.X., X.Y.C., D.C.H., Z.Q.L and Y.L.T

helped with some experiments and data interpretation; Y.H., C.Y.Y. and P.J.W. wrote the manuscript with inputs from all author.

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# References

1. Maris, J.M., M.D. Hogarty, R. Bagatell, et al., Neuroblastoma. Lancet, 2007. **369**(9579): p. 2106–20.
2. Shohet, J. and J. Foster, Neuroblastoma. BMJ, 2017. **357**: p. j1863.
3. Du, H.M. and X.K. Zhu, *Genomic imprinting of neuroblastoma*. Chinese Journal of Pediatric Surgery, 2019. **40**(4): p. 374–377.
4. Decock, A., M. Ongenaert, J. Hoebeeck, et al., *Genome-wide promoter methylation analysis in neuroblastoma identifies prognostic methylation biomarkers*. Genome Biol, 2012. **13**(10): p. R95.
5. Djos, A., T. Martinsson, P. Kogner, et al., *The RASSF gene family members RASSF5, RASSF6 and RASSF7 show frequent DNA methylation in neuroblastoma*. Mol Cancer, 2012. **11**: p. 40.
6. Yang, Q., C.M. Kiernan, Y. Tian, et al., *Methylation of CASP8, DCR2, and HIN-1 in neuroblastoma is associated with poor outcome*. Clin Cancer Res, 2007. **13**(11): p. 3191–7.
7. Oehme, I., H.E. Deubzer, D. Wegener, et al., *Histone deacetylase 8 in neuroblastoma tumorigenesis*. Clin Cancer Res, 2009. **15**(1): p. 91–9.
8. Keshelava, N., E. Davicioni, Z. Wan, et al., *Histone deacetylase 1 gene expression and sensitization of multidrug-resistant neuroblastoma cell lines to cytotoxic agents by depsipeptide*. J Natl Cancer Inst, 2007. **99**(14): p. 1107–19.
9. Delaunay, S., F. Rapino, L. Tharun, et al., *Elp3 links tRNA modification to IRES-dependent translation of LEF1 to sustain metastasis in breast cancer*. J Exp Med, 2016. **213**(11): p. 2503–2523.
10. Begley, U., M.S. Sosa, A. Avivar-Valderas, et al., *A human tRNA methyltransferase 9-like protein prevents tumour growth by regulating LIN9 and HIF1-alpha*. EMBO Mol Med, 2013. **5**(3): p. 366–83.
11. Martinez, F.J., J.H. Lee, J.E. Lee, et al., *Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome*. J Med Genet, 2012. **49**(6): p. 380–5.
12. Lemmens, R., M.J. Moore, A. Al-Chalabi, et al., *RNA metabolism and the pathogenesis of motor neuron diseases*. Trends Neurosci, 2010. **33**(5): p. 249–58.
13. Parodi, F., R. Carosio, M. Ragusa, et al., *Epigenetic dysregulation in neuroblastoma: A tale of miRNAs and DNA methylation*. Biochim Biophys Acta, 2016. **1859**(12): p. 1502–1514.
14. Russell, M.R., A. Penikis, D.A. Oldridge, et al., *CASC15-S Is a Tumor Suppressor lncRNA at the 6p22 Neuroblastoma Susceptibility Locus.* Cancer Res, 2015. **75**(15): p. 3155-66.
15. Zhao, Z., X. Ma, D. Sung, et al., *microRNA-449a functions as a tumor suppressor in neuroblastoma through inducing cell differentiation and cell cycle arrest*. RNA Biol, 2015. **12**(5): p. 538–54.

16. Pandey, G.K., S. Mitra, S. Subhash, et al., *The risk-associated long noncoding RNA NBAT-1 controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation*. Cancer Cell, 2014. **26**(5): p. 722–37.
17. Foley, N.H., I. Bray, K.M. Watters, et al., *MicroRNAs 10a and 10b are potent inducers of neuroblastoma cell differentiation through targeting of nuclear receptor corepressor 2*. Cell Death Differ, 2011. **18**(7): p. 1089–98.
18. Beveridge, N.J., P.A. Tooney, A.P. Carroll, et al., *Down-regulation of miR-17 family expression in response to retinoic acid induced neuronal differentiation*. Cell Signal, 2009. **21**(12): p. 1837–45.
19. Wang, C., W. Wang, X. Han, et al., *Methyltransferase-like 1 regulates lung adenocarcinoma A549 cell proliferation and autophagy via the AKT/mTORC1 signaling pathway*. Oncol Lett, 2021. **21**(4): p. 330.
20. Schimmel, P., *The emerging complexity of the tRNA world: mammalian tRNAs beyond protein synthesis*. Nat Rev Mol Cell Biol, 2018. **19**(1): p. 45–58.
21. Kirchner, S. and Z. Ignatova, *Emerging roles of tRNA in adaptive translation, signalling dynamics and disease*. Nat Rev Genet, 2015. **16**(2): p. 98–112.
22. Pan, T., *Modifications and functional genomics of human transfer RNA*. Cell Res, 2018. **28**(4): p. 395–404.
23. Okamoto, M., S. Hirata, S. Sato, et al., *Frequent increased gene copy number and high protein expression of tRNA (cytosine-5-)-methyltransferase (NSUN2) in human cancers*. DNA Cell Biol, 2012. **31**(5): p. 660–71.
24. D'Silva, S., S.J. Haider, and E.M. Phizicky, *A domain of the actin binding protein Abp140 is the yeast methyltransferase responsible for 3-methylcytidine modification in the tRNA anti-codon loop*. RNA, 2011. **17**(6): p. 1100–10.
25. Alexandrov, A., E.J. Grayhack, and E.M. Phizicky, *tRNA m7G methyltransferase Trm8p/Trm82p: evidence linking activity to a growth phenotype and implicating Trm82p in maintaining levels of active Trm8p.* RNA, 2005. **11**(5): p. 821–30.
26. Lin, S., Q. Liu, V.S. Lelyveld, et al., *Mettl1/Wdr4-Mediated m(7)G tRNA Methylome Is Required for Normal mRNA Translation and Embryonic Stem Cell Self-Renewal and Differentiation*. Mol Cell, 2018. **71**(2): p. 244–255 e5.
27. Alexandrov, A., M.R. Martzen, and E.M. Phizicky, *Two proteins that form a complex are required for 7-methylguanosine modification of yeast tRNA*. RNA, 2002. **8**(10): p. 1253–66.
28. Malbec, L., T. Zhang, Y.S. Chen, et al., *Dynamic methylome of internal mRNA N(7)-methylguanosine and its regulatory role in translation*. Cell Res, 2019. **29**(11): p. 927–941.
29. Enroth, C., L.D. Poulsen, S. Iversen, et al., *Detection of internal N7-methylguanosine (m7G) RNA modifications by mutational profiling sequencing*. Nucleic Acids Res, 2019. **47**(20): p. e126.
30. Zhang, L.S., C. Liu, H. Ma, et al., *Transcriptome-wide Mapping of Internal N(7)-Methylguanosine Methylome in Mammalian mRNA*. Mol Cell, 2019. **74**(6): p. 1304–1316 e8.

31. Shaheen, R., G.M. Abdel-Salam, M.P. Guy, et al., *Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism*. Genome Biol, 2015. **16**: p. 210.
32. Braun, D.A., S. Shril, A. Sinha, et al., *Mutations in WDR4 as a new cause of Galloway-Mowat syndrome*. Am J Med Genet A, 2018. **176**(11): p. 2460–2465.
33. Liu, Y., C. Yang, Y. Zhao, et al., *Overexpressed methyltransferase-like 1 (METTL1) increased chemosensitivity of colon cancer cells to cisplatin by regulating miR-149-3p*/S100A4/*p53 axis*. Aging (Albany NY), 2019. **11**(24): p. 12328–12344.
34. Okamoto, M., M. Fujiwara, M. Hori, et al., *tRNA modifying enzymes, NSUN2 and METTL1, determine sensitivity to 5-fluorouracil in HeLa cells*. PLoS Genet, 2014. **10**(9): p. e1004639.
35. Lin, S. and R.I. Gregory, *Identification of small molecule inhibitors of Zcchc11 TUTase activity*. RNA Biol, 2015. **12**(8): p. 792–800.
36. Luo, E.C., J.L. Nathanson, F.E. Tan, et al., *Large-scale tethered function assays identify factors that regulate mRNA stability and translation*. Nat Struct Mol Biol, 2020. **27**(10): p. 989–1000.
37. Lin, S., Q. Liu, Y.Z. Jiang, et al., *Nucleotide resolution profiling of m(7)G tRNA modification by TRAC-Seq*. Nat Protoc, 2019. **14**(11): p. 3220–3242.
38. Zhang, M., K. Zhao, X. Xu, et al., *A peptide encoded by circular form of LINC-PINT suppresses oncogenic transcriptional elongation in glioblastoma*. Nat Commun, 2018. **9**(1): p. 4475.
39. Robichaud, N., N. Sonenberg, D. Ruggero, et al., *Translational Control in Cancer*. Cold Spring Harb Perspect Biol, 2019. **11**(7).
40. Flavahan, W.A., E. Gaskell, and B.E. Bernstein, *Epigenetic plasticity and the hallmarks of cancer*. Science, 2017. **357**(6348).
41. Liu, Y., Y. Zhang, Q. Chi, et al., *Methyltransferase-like 1 (METTL1) served as a tumor suppressor in colon cancer by activating 7-methyguanosine (m7G) regulated let-7e miRNA/HMGA2 axis*. Life Sci, 2020. **249**: p. 117480.
42. Tian, Q.H., M.F. Zhang, J.S. Zeng, et al., *METTL1 overexpression is correlated with poor prognosis and promotes hepatocellular carcinoma via PTEN*. J Mol Med (Berl), 2019. **97**(11): p. 1535–1545.
43. Pandolfini, L., I. Barbieri, A.J. Bannister, et al., *METTL1 Promotes let-7 MicroRNA Processing via m7G Methylation*. Mol Cell, 2019. **74**(6): p. 1278–1290 e9.
44. Choe, J., S. Lin, W. Zhang, et al., *mRNA circularization by METTL3-eIF3h enhances translation and promotes oncogenesis*. Nature, 2018. **561**(7724): p. 556–560.
45. Lin, S., J. Choe, P. Du, et al., *The m(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells*. Mol Cell, 2016. **62**(3): p. 335–345.
46. Yang, F., H. Jin, B. Que, et al., *Dynamic m(6)A mRNA methylation reveals the role of METTL3-m(6)A-CDCP1 signaling axis in chemical carcinogenesis*. Oncogene, 2019. **38**(24): p. 4755–4772.
47. Zheng, G., J.A. Dahl, Y. Niu, et al., *ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility*. Mol Cell, 2013. **49**(1): p. 18–29.

48. Du, Y., B. Jiang, S. Song, et al., *Metadherin regulates actin cytoskeletal remodeling and enhances human gastric cancer metastasis via epithelial-mesenchymal transition*. Int J Oncol, 2017. **51**(1): p. 63–74.
49. Liu, Y., X. Kong, X. Li, et al., *Knockdown of metadherin inhibits angiogenesis in breast cancer*. Int J Oncol, 2015. **46**(6): p. 2459–66.
50. Du, C., X. Yi, W. Liu, et al., *MTDH mediates trastuzumab resistance in HER2 positive breast cancer by decreasing PTEN expression through an NFkappaB-dependent pathway*. BMC Cancer, 2014. **14**: p. 869.
51. Hu, G., Y. Wei, and Y. Kang, *The multifaceted role of MTDH/AEG-1 in cancer progression*. Clin Cancer Res, 2009. **15**(18): p. 5615–20.
52. Yu, C., K. Chen, H. Zheng, et al., *Overexpression of astrocyte elevated gene-1 (AEG-1) is associated with esophageal squamous cell carcinoma (ESCC) progression and pathogenesis*. Carcinogenesis, 2009. **30**(5): p. 894–901.
53. Yoo, B.K., L. Emdad, Z.Z. Su, et al., *Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression*. J Clin Invest, 2009. **119**(3): p. 465–77.
54. Hu, G., R.A. Chong, Q. Yang, et al., *MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer*. Cancer Cell, 2009. **15**(1): p. 9–20.
55. Kikuno, N., H. Shiina, S. Urakami, et al., *Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity*. Oncogene, 2007. **26**(55): p. 7647–55.
56. Liu, H., X. Song, C. Liu, et al., *Knockdown of astrocyte elevated gene-1 inhibits proliferation and enhancing chemo-sensitivity to cisplatin or doxorubicin in neuroblastoma cells*. J Exp Clin Cancer Res, 2009. **28**: p. 19.
57. Fu, X., W. Zhang, Y. Su, et al., *MicroRNA-103 suppresses tumor cell proliferation by targeting PDCD10 in prostate cancer*. Prostate, 2016. **76**(6): p. 543–51.
58. Fan, L., H. Lei, S. Zhang, et al., *Non-canonical signaling pathway of SNAI2 induces EMT in ovarian cancer cells by suppressing miR-222-3p transcription and upregulating PDCD10.* Theranostics, 2020. **10**(13): p. 5895–5913.

# Figures

### Figure 1

METTL1 is elevated in advanced NBLs and is associated with poor prognosis in NBL patients in the GEO database (GSE62564). (A) The R2 genomics analysis and visualization platform showed a heatmap of correlations between METTL1 mRNA levels, COG risk, INSS stage, and MYCN status in NBL samples from the GEO database. (B) The GEO database showed the mRNA level of METTL1 in NBL samples at different stages (except stage4S, n=53). (C) GEO database showed METTL1 mRNA levels in high-risk

NBL samples and non-high-risk NBL samples. (D) R2 genomics analysis and visualization platform showed high METTL1 expression was associated with low event-free survival of NBL patients in the GEO database(n= 498). (E-F) R2 genomics analysis and visualization platform showed high METTL1 expression was associated with low event-free survival of NBL patients in the non-high(n=322) and high(n=276) risk groups from the GEO database respectively. Data are presented as mean ± SEM. ****p < 0.0001 (Mann-Whitney U test; log-rank test).

## Figure 2

METTL1 is elevated in advanced NBLs and is associated with poor prognosis in patients with clinical NBL. (A) Representative images of METTL1 IHC staining with different staining intensities in clinical NBL tumors. (B) Quantification of METTL1 IHC scores in different INSS stages of clinical NBL tumors. (C) Proportion of METTL1expression cases at different stages of clinical NBL tumors. ****p < 0.0001 (One-way ANOVA; Student's t-test).

## Figure 3

METTL1 knockdown inhibits NBL progression in vitro. (A) Western blot confirmed stable knockdown of METTL1 in KELLY and BE2C cells. (B) The CCK-8 assay (n = 3 per group) determined the proliferation of METTL1 knockdown and control NBL cells. (C-D) Flow cytometry assay (C) and quantitative analysis (D) of apoptosis rates in METTL1 knockdown and control NBL cells. (E-F) Invasion assay (E) and quantitative analysis (F) of METTL1 knockdown and control NBL cells. Data are presented as mean ± SEM. ***p < 0.001, ****p < 0.0001 (One-way ANOVA; Student's t-test).

## Figure 4

Inhibition of METTL1 reduces the tumorigenicity of NBL cells in vivo. (A-B) Tumor profiles of mice implanted with METTL1 knockdown and control NBL cells. n=5. (C-D) Quantification of tumor weight (C) and size (D) at endpoints. (E)) Growth curves of tumor volumes in the METTL1 knockdown and control groups. (F) Representative images of METTL1 and Ki67 IHC staining. (G) Quantitative analysis of IHC scores for METTL1 and Ki67. Data are presented as mean ± SEM. *p < 0.05, ****p < 0.0001.(One-way ANOVA; Student's t-test).

## Figure 5

METTL1 regulates tRNA $m^7G$ modification, tRNA expression and global mRNA translation in NBL cells. (A) Northwestern blot confirmed decreased levels of $m^7G$ modification in METTL1 knockdown NBL cells （kelly shM1-1）. U6 snRNA was used as a loading control. (B) A total of 21 $m^7G$ -modified tRNAs were identified by TRAC-seq. (C) Motif sequences at the tRNA $m^7G$ site. (D) Quantification of $m^7G$-modified tRNAs in METTL1 knockdown and control cells (kelly shM1-1 and kelly shNC). (E) Knockdown of METTL1 in NBL cells resulted in reduced expression of $m^7G$ -modified tRNAs. (F) Polysome profiling of METTL1 knockdown and control NBL cells. (G) Puromycin intake assay of METTL1-inhibited and control kelly cells. Data are expressed as mean ± SEM. ***p < 0.001, ****p < 0.0001 (Mann-Whitney U test).

**Figure 6**

Knockdown of METTL1 selectively inhibits translation of oncogenic mRNAs. (A) TE Scatter plot in METTL1 knockdown and control NBL cells. (B) Frequency of $m^7G$ tRNA decoding codons for increased TEs (up), decreased TEs (down), and unaltered TEs (non). (C) Venn diagram of genes with decreased TEs in KELLY and BE2C cells. (D) Pathway enrichment of TE-down genes. (E) RNC-qPCR confirmed translational downregulation of MTDH and PDCD10 after METTL1 knockdown. (F) Western blot confirmed the decreased protein levels of MTDH and PDCD10 in METTL1 knockdown NBL cells. Data are presented as mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001 (Student’s t-test; Mann-Whitney U test).

# Supplementary Files

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