

METTL1-mediated m7G modification of CD47 promotes lung adenocarcinoma escape from macrophage phagocytosis

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Abstract

Introduction: Emerging evidence suggests that reduced phagocytosis by macrophages is a significant event contributing to the immune escape of cancer cells. METTL1 has been implicated in the malignant progression of various cancers, but whether it affects macrophage phagocytosis of lung adenocarcinoma (LUAD) remains unclear.

Material and methods: METTL1 expression was analyzed using The Cancer Genome Atlas (TCGA) database and LUAD cell models. The impact of METTL1 expression on macrophage phagocytosis of LUAD was analyzed using CFSE staining and flow cytometry based on METTL1 knockdown and overexpression cell models. Prediction via the RM2Target database identified the gene CD47, which might be regulated by METTL1 through m7G modification. Meanwhile, RIP-qPCR, qPCR, and MeRIP-qPCR were used to detect the effects of METTL1-regulated m7G modification on CD47 mRNA levels, and rescue experiments were designed to explore its regulatory effect on macrophage phagocytic ability.

Results: METTL1 was highly expressed in LUAD tissues and cells. Higher METTL1 expression was associated with poor patient prognosis. Knockdown of METTL1 enhanced macrophage phagocytosis by approximately 109%, whereas its overexpression reduced phagocytosis by about 54%. Mechanistic research showed that METTL1, through its methyltransferase activity, mediates m7G modification of CD47 mRNA, thereby enhancing its stability and increasing its expression, promoting LUAD escape from macrophage phagocytosis.

Conclusions: Taken together, our study demonstrates that METTL1-driven m7G modification of CD47 promotes LUAD escape from macrophage phagocytosis, suggesting that targeting the METTL1-CD47 axis may represent a potential strategy for enhancing LUAD immunotherapy.

Key words: METTL1, CD47, m7G modification, macrophages, LUAD.

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Introduction

Lung adenocarcinoma (LUAD) is a common subtype of lung cancer. Progress has been made in diagnosis and treatment, but the prognosis of LUAD patients remains generally poor, with a 5-year survival rate still below 30% [1, 2]. In recent years, cancer immunotherapy has become an important research direction for inhibiting tumor growth, recurrence, and metastasis due to its ability to relieve immune suppression in the tumor microenvironment (TME) or activate the body's immune system to eliminate cancer cells [3-5]. However, clinical data show significant differences in the response of LUAD patients to existing immunotherapy regimens [6]. Moreover, tumor cells can evade immune surveillance by inducing immune cell dysfunction, which limits the efficacy of immunotherapy [7, 8], posing a major challenge to LUAD treatment.

Macrophages are one of the important phagocytic cells in the body, but in the TME, their function and polarization state are affected, forming tumor-associated macrophages (TAMs) [9]. TAMs constitute one of the most abundant immune cell populations in the TME, with two polarization phenotypes, M1 and M2 [10]. The M1 polarization phenotype is characterized by high expression of molecules such as CD80, CD86, MHCII, iNOS, and CD68, and M1 macrophages can phagocytose cancer cells and recruit T cells, thereby exerting anti-tumor effects [11]. In contrast, the M2 polarization phenotype is generally considered to be an immunosuppressive phenotype, closely related to tumor immune escape [12]. In LUAD, enhancing the phagocytic ability of macrophages can restore innate immune surveillance function and inhibit tumor cell escape [7]. Therefore, exploring factors or potential molecular mechanisms that regulate macro-

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phage phagocytosis is of great significance for improving the effectiveness of LUAD immunotherapy.

METTL1 is an RNA methyltransferase encoded by a gene located on human chromosome 12. It primarily regulates the functions of RNA molecules, including those derived from multiple proto-oncogenes and tumor suppressor genes by forming a complex with WDR4, thereby participating in biological processes such as cell proliferation, apoptosis, and metabolic reprogramming [13, 14]. In cancers such as LUAD, hepatocellular carcinoma, and nasopharyngeal carcinoma, METTL1 is often highly expressed and associated with tumor malignancy, indicating that METTL1 may be a promising anti-cancer target [15-17]. Notably, recent studies showed that METTL1 also plays an important role in tumor immune regulation. The abundance of CD8⁺ T cells, CD4⁺ T cells, and macrophages varies depending on the level of METTL1 expression [18, 19]. Additionally, evidence suggests that downregulating METTL1 expression can enhance the ability of macrophages to phagocytose cancer cells [20]. However, it remains unclear whether METTL1 can help cancer cells evade immune surveillance by regulating macrophage phagocytosis in LUAD.

CD47 is a highly N-glycosylated 47-52 kDa integral membrane glycoprotein that is highly expressed in various tumors [21]. CD47 is now recognized as a tumor phagocytosis checkpoint because it sends a “do not eat me” signal to macrophages through binding to SIRPα on the surface of macrophages. This interaction allows cancer cells to evade phagocytosis and disrupt the antigen presentation of macrophages, thereby potentially limiting the initiation of adaptive immune responses [22, 23]. In LUAD, it has been confirmed that inhibiting CD47 expression can enhance the phagocytic ability of macrophages and restore innate immune surveillance against cancer cells [7]. Therefore, dysregulation of CD47 expression may be an important mechanism underlying tumor immune evasion.

In this study, bioinformatics analysis and LUAD cell models confirmed the upregulation of METTL1 expression in LUAD. Its high expression was significantly negatively associated with macrophage infiltration. Moreover, its overexpression reduced the phagocytic ability of macrophages. Mechanistically, we found that METTL1 mediated m7G modification of CD47 mRNA, thereby increasing its stability and expression and promoting the escape of LUAD cells from macrophage-mediated phagocytosis. This discovery not only highlights the role of METTL1 in regulating macrophage phagocytosis but also provides a basis for further research into the METTL1-CD47 axis as a potential therapeutic target in LUAD.

Material and methods

Bioinformatics analysis

We downloaded LUAD mRNA expression data (Normal: 59, Tumor: 539) from The Cancer Genome Atlas

(TCGA) database. The Limma package in R software was used to analyze the expression of METTL1 in LUAD patients, and the Survival package in R software was used to analyze the relationship between METTL1 expression and overall survival. Subsequently, based on METTL1 expression levels, LUAD samples from TCGA were divided into high-expression and low-expression groups. Then, the IOBR package was used for CIBERSORT immune infiltration to calculate the enrichment scores of immune cells in these samples. Combined with the Tumor Immune Estimation Resource database, the correlation between METTL1 and macrophage infiltration was further evaluated. Finally, the RM2Target database was used to predict CD47, a gene that might be modified by METTL1 through m7G.

Cell culture and plasmid transfection

Human normal bronchial epithelial cells (BEAS-2B), human LUAD cells (A549 and H1299), and human monocytic leukemia cells (THP-1) were all purchased from ATCC (USA). Human LUAD cells (PC9) were purchased from the Cell Bank of the Chinese Academy of Sciences (China). All cells were cultured in DMEM medium (Cytoc, China) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) under standard conditions.

Small interfering RNA (si-METTL1), pcDNA3.1 overexpression plasmids (oe-METTL1, oe-METTL1 WT, oe-METTL1 MUT, and oe-CD47) and corresponding negative controls were purchased from Gene Pharma (China). Cell transfection was performed using the Lipofectamine 3000 kit (Invitrogen, USA) according to the instructions, and transfection efficiency was detected 24 hours after transfection.

qPCR

Total RNA was extracted from cells using TRIzol reagent (Absin, China) and reverse transcribed into cDNA using the PrimeScript RT reagent Kit (TAKARA, Japan). Subsequently, qPCR was performed on the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, USA) using TB Green Premix Ex Taq (TAKARA, Japan) as the fluorescent DNA-binding dye. Relative expression was calculated by the $2^{-\Delta\Delta C_t}$ method using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference control. The primer sequences were as follows (5'-3'): METTL1-F: GGCAACGTGCTCACTCAAA; METTL1-R: CACAGCCTATGTCTGCAAACT; CD47-F: AGAAGGTGAAACGATCATCGAGC; CD47-R: CTCATCCATACCACCGGATCT; GAPDH-F: ACAACTTTGGTATCGTGGAAGG; GAPDH-R: GC-CATCACGCCACAGTTTC.

Macrophage polarization

THP-1 cells were differentiated into M0 macrophages using 100 ng/ml PMA (Selleck, USA) and incubated over-

night. Subsequently, the cells were washed twice with medium, and M0 macrophages were treated with 20 ng/ml IFN- γ (MCE, USA) and 10 ng/ml LPS (Selleck, USA) for 48 hours to induce polarization into M1 macrophages.

Microscopy-based phagocytosis assay

M1 macrophages were seeded into a 24-well plate containing cell climbing slides. After 24 hours of incubation, the complete medium was replaced with serum-free medium to starve the macrophages for 2 hours. Subsequently, LUAD cells were digested into a cell suspension using trypsin (Beyotime, China) and stained with the live-cell fluorescent dye 5(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Abcam, UK) for 30 min. Then, the stained cell suspension was added to the starved macrophages and co-cultured for 2 hours. Subsequently, the cells were washed with phosphate-buffered saline (PBS). Macrophages phagocytosing cancer cells were observed under a fluorescence microscope, and the number was counted.

In vitro phagocytosis assay

Following the method described in the Microscopy-based phagocytosis assay section, after co-culturing LUAD cells and macrophages for 2 hours, the suspended cells were washed with PBS, and the adherent cells were digested into a cell suspension using trypsin. Cells were stained with PerCP-conjugated anti-CD80 (Thermo Fisher, MA1-10289, USA) in the dark for 30 min. Then, the proportion of fluorescently labeled cells in macrophages was detected by flow cytometry to calculate the phagocytosis rate [24].

RIP-qPCR

LUAD cells were lysed using radioimmunoprecipitation assay (RIPA) lysis buffer (Millipore, USA) containing protease inhibitors to release intracellular RNA-protein complexes. Anti-METTL1 (Abcam, ab271063, UK) was added to the lysate and incubated overnight at 4°C to allow the anti-body to specifically bind to the METTL1 protein, forming antibody-METTL1 protein-RNA complexes, with non-specific anti-IgG (Abcam, ab133470, UK) used as a negative control. Protein A/G magnetic beads (MCE, USA) were then added to the immunocomplexes and incubated at 4°C for 1-2 hours to allow the antibody complexes to bind to the magnetic beads. Proteinase K (Promega, USA) was added to the antibody-magnetic bead complex to remove cross-linked proteins and release RNA. Finally, qPCR was performed to determine the enrichment of CD47.

MeRIP-qPCR

Cell samples were collected, and RNA was extracted into microcentrifuge tubes. An ultrasonic disruptor was used to fragment the total RNA into 100-150 bp fragments, and an aliquot of the fragmented RNA was retained as the

input sample. Next, the remaining samples at 4°C with anti-m7G (MBL Life Science, RN016M, Japan) or anti-IgG (negative control) were incubated for 2 hours to enrich methylated RNA fragments. Subsequently, the samples were eluted using an elution buffer to release the enriched RNA fragments. Finally, qPCR was performed to assess m7G enrichment on CD47 mRNA.

Western blot

RIPA lysis buffer was used to extract total protein. Then, the protein extract was separated on SDS-PAGE, and the separated proteins were transferred to a PVDF membrane (Millipore, USA). After blocking with 5% skim milk for 1 hour, the membrane with the primary antibody anti-CD47 (Abcam, ab300435, UK) or anti-GAPDH (Abcam, ab8245, UK) was incubated at 4°C overnight. The membrane with HRP-conjugated secondary antibody (Abcam, UK) was incubated at room temperature for 1 hour, followed by protein labeling using an enhanced chemiluminescence detection kit (Vazyme, China).

Flow cytometry

Cell samples were collected, and the cell concentration was adjusted to approximately 1×10^6 /ml. An appropriate amount of cell suspension was transferred to a flow cytometry tube, and PE-conjugated anti-CD47 (Elabscience, E-AB-F1016D, China) was added and incubated in the dark at 4°C for 30 min. After incubation, the cells were washed twice with PBS to remove unbound antibodies. Finally, the cells were resuspended in PBS and analyzed by flow cytometry. Cell surface CD47 expression was evaluated based on the fluorescence intensity.

Data analysis

All statistical analyses were performed using Graph-Pad Prism 8.0 software. The data were expressed as mean $\pm$ standard deviation. A two-tailed Student's *t*-test was used to compare the results between two different groups, and one-way analysis of variance was used for multiple comparisons. Each experiment was repeated at least three times. Differences were considered significant at $p < 0.05$.

Results

METTL1 is upregulated in LUAD and associated with poor prognosis

First, LUAD mRNA expression data were downloaded from the TCGA database. Through analysis, the gene METTL1, which was significantly highly expressed in tumor tissues of LUAD patients, was identified (Fig. 1A). Subsequently, METTL1 expression was further detected at the cellular level. As shown in Figure 1B, compared to the immortalized human bronchial epithelial cell line

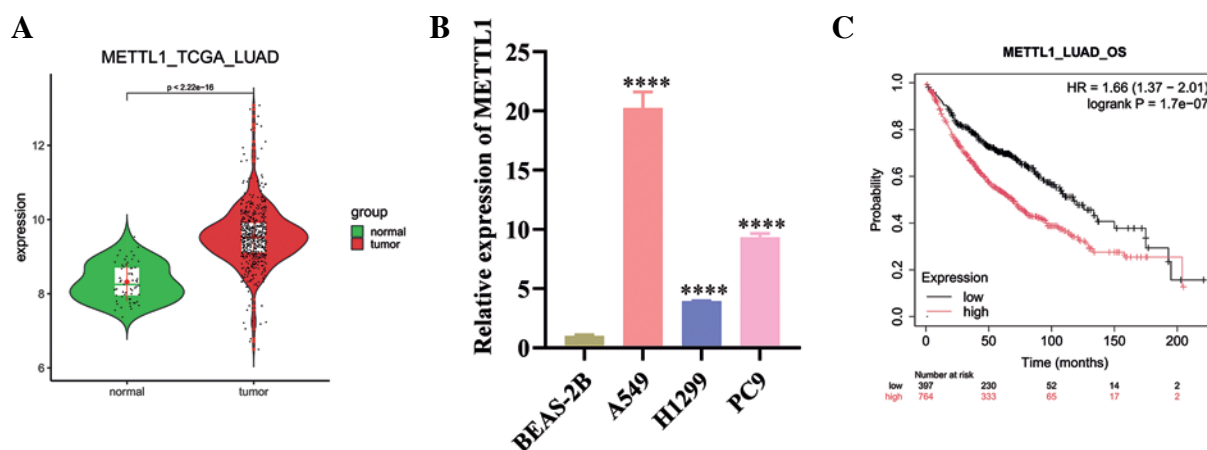


Fig. 1. METTL1 is upregulated in lung adenocarcinoma (LUAD) and associated with poor prognosis. **A)** Analysis of METTL1 expression differences between LUAD tumor tissues and adjacent normal tissues in LUAD patients using the TCGA database. **B)** qPCR detection of METTL1 expression in the immortalized human bronchial epithelial cell line BEAS-2B and the LUAD cell lines A549, H1299, and PC9. **C)** Kaplan-Meier survival analysis curve of METTL1 expression levels and overall survival in LUAD patients. **** $p < 0.0001$

BEAS-2B, METTL1 expression was upregulated in the LUAD cell lines A549, H1299, and PC9. Additionally, Kaplan-Meier survival analysis results showed that high expression of METTL1 was significantly associated with shorter overall survival in LUAD patients (Fig. 1C). The above results indicate that METTL1 is highly expressed in LUAD and is associated with poor prognosis.

METTL1 inhibits macrophage phagocytosis of LUAD cells

METTL1 has been reported to be involved in immune regulation in malignancies such as hepatocellular carcinoma [25], but whether it can regulate immunity in LUAD remains unknown. We divided LUAD samples into high and low expression groups based on METTL1 expression levels. Immune infiltration analysis showed that METTL1 expression was associated with the enrichment scores of CD4⁺ T cells and macrophages (Fig. 2A). Correlation analysis revealed that METTL1 expression was significantly negatively associated with macrophage infiltration (Fig. 2B). Next, macrophage phagocytosis assays were used to detect changes in the ability of M1 macrophages to phagocytose tumor cells after METTL1 knockdown or overexpression in LUAD cells (Fig. 2C, D). After METTL1 knockdown, the macrophage phagocytosis index significantly increased, indicating that METTL1 knockdown can promote macrophage phagocytosis of LUAD cells (Fig. 2E, G). Conversely, after METTL1 overexpression, the macrophage phagocytosis index decreased, indicating that increased METTL1 expression can inhibit macrophage phagocytosis of LUAD cells (Fig. 2F, H). These findings suggest that METTL1 expression is negatively

associated with macrophage infiltration and suppresses macrophage phagocytosis of LUAD cells.

METTL1 regulates CD47 mRNA through m7G modification

The METTL1 gene contains multiple exons and introns, and the protein it encodes contains a methyltransferase domain that can catalyze m7G modification of various RNA molecules, including mRNAs [26]. Therefore, we sought to investigate METTL1-mediated m7G modification in LUAD. First, using the RM2Target database, we predicted that METTL1 might mediate m7G modification of CD47 mRNA (Fig. 3A). To determine whether METTL1 regulates CD47 mRNA through m7G modification, we first performed RIP-qPCR experiments and found that, compared with non-specific IgG, METTL1-specific antibody enriched CD47, indicating that METTL1 can bind to CD47 (Fig. 3B). Subsequently, A549 cells with METTL1 knockdown or overexpression of wild-type (WT) or mutant (MUT) METTL1 were generated. qPCR results showed that METTL1 knockdown reduced METTL1 expression, whereas transfection with METTL1 WT or MUT plasmids significantly increased METTL1 expression in A549 cells (Fig. 3C). Next, we examined the effect of METTL1 on CD47 mRNA expression. METTL1 knockdown reduced CD47 expression, whereas transfection of WT METTL1 plasmid overexpression significantly increased CD47 expression; in contrast, overexpression of MUT METTL1 did not significantly change CD47 mRNA levels (Fig. 3D). In addition, MeRIP-qPCR showed that m7G enrichment on CD47 mRNA changed in parallel with CD47 mRNA expression (Fig. 3E). These results suggest that the meth-

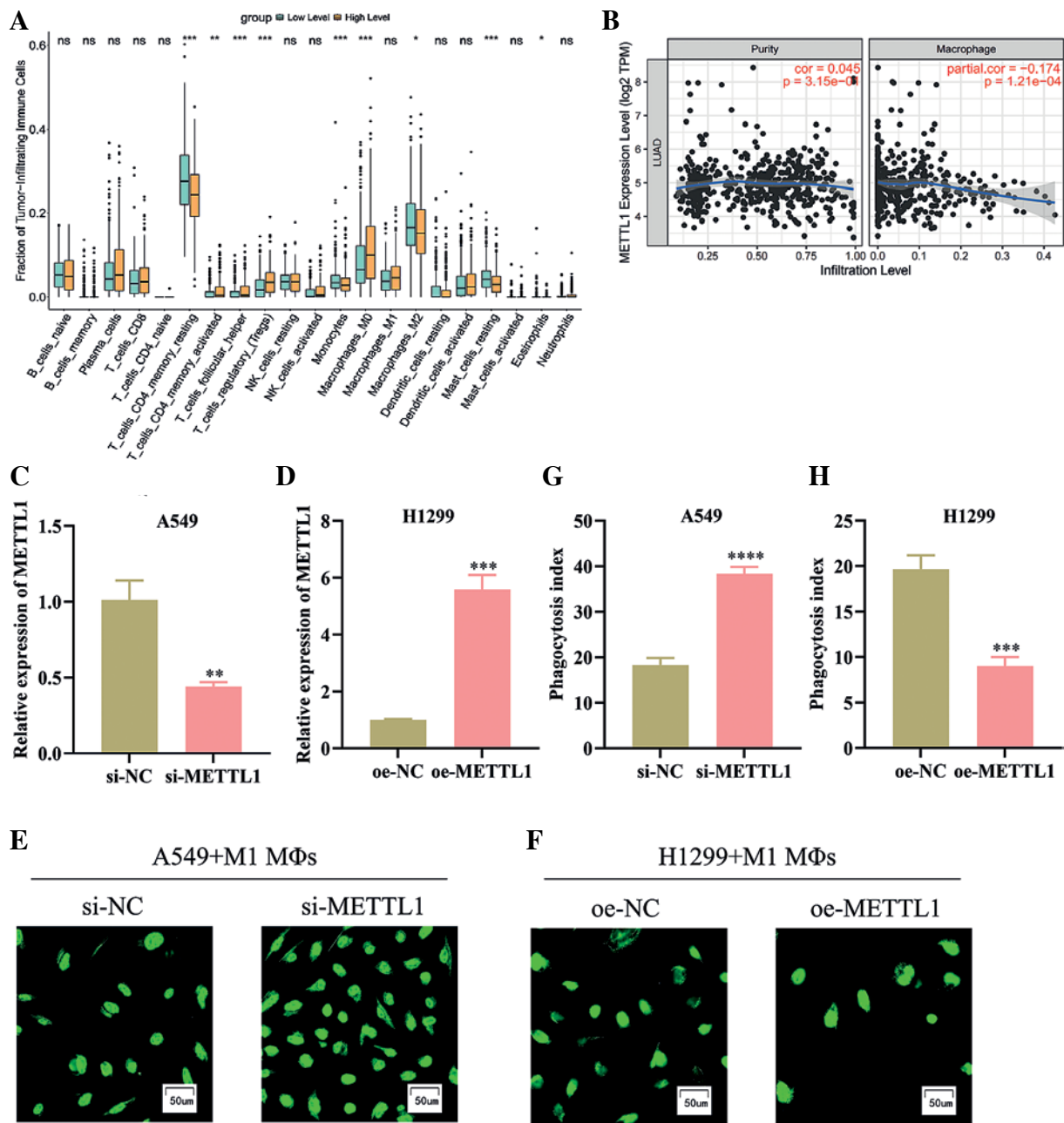
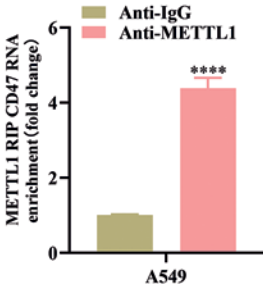


Fig. 2. METTL1 inhibits macrophage phagocytosis of lung adenocarcinoma (LUAD) cells. **A**) Relationship between METTL1 copy number and the infiltration levels of different immune cell types. **B**) Correlation analysis of METTL1 expression and macrophage infiltration. **C, D**) Based on A549 cells with high METTL1 expression and H1299 cells with low METTL1 expression, cell groups with knockdown or overexpression of METTL1 were constructed, and transfection efficiency was detected by qPCR. **E-H**) The above different groups of LUAD cells were co-cultured with M1 macrophages, and the phagocytic ability of macrophages to tumor cells was analyzed by macrophage phagocytosis assay. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns – not significant

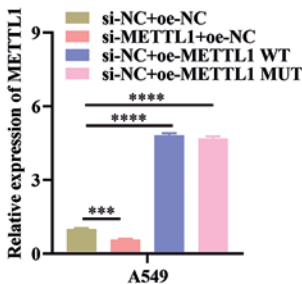
A

RM2Target ID	WERs Name	WERs Type	Target Gene	Modification	Organism
RM2Target_143417	METTL1	Writer	CD47	m7G	Homo sapiens

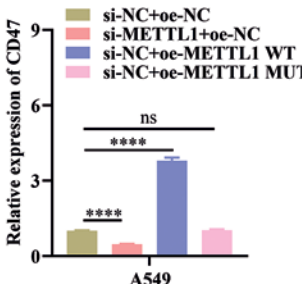
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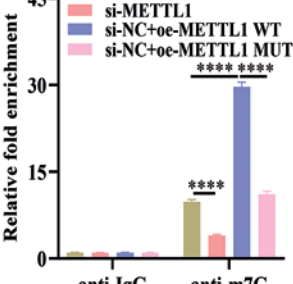


Fig. 3. METTL1 regulates CD47 mRNA through m7G modification. **A)** The RM2Target database predicted that METTL1 may modify genes through m7G. **B)** RIP-qPCR analysis of the association between METTL1 and CD47. **C, D)** qPCR analysis of METTL1 and CD47 mRNA expression levels. **E)** MeRIP-qPCR analysis of m7G enrichment on CD47 mRNA in different treatment groups. *** $p < 0.001$, **** $p < 0.0001$; ns – not significant

yltransferase activity of METTL1 promotes m7G modification of CD47 mRNA and increases its expression.

METTL1 promotes LUAD escape from macrophage phagocytosis by upregulating CD47

Next, we constructed A549 cell groups with METTL1 knockdown and CD47 overexpression. qPCR results showed that METTL1 knockdown reduced CD47 expression, while CD47 overexpression reversed the reduction in CD47 expression caused by METTL1 knockdown (Fig. 4A). Western blot results were consistent with those of qPCR (Fig. 4B). Considering that CD47 is a cell surface transmembrane protein [27], flow cytometry was used to detect the relative mean fluorescence intensity of CD47 on the A549 cell membrane. The results showed that METTL1 knockdown reduced the CD47 fluorescence signal on the cell surface, whereas CD47 overexpression reversed this effect (Fig. 4C). To clarify whether the regulation of CD47 by METTL1 affects the escape of LUAD cells from macrophage phagocytosis, we conducted a macrophage phagocytosis assay. The results showed that CD47 overexpression significantly attenuated the increase in macrophage phagocytosis induced by METTL1 knockdown (Fig. 4D). Additionally, we labeled macrophages with CD80 and further verified the effect of METTL1-mediated CD47 upregulation on macrophage phagocytosis of LUAD cells using flow cytometry. The results showed that after METTL1 knockdown, the macrophage phagocytosis rate significantly increased, whereas CD47 overexpression reduced the increase in phagocytosis induced by METTL1 (Fig. 4E). Taken together, these findings suggest that METTL1 pro-

motes LUAD escape from macrophage phagocytosis by upregulating CD47.

Discussion

Immunotherapy, as an emerging anti-cancer strategy, has shown considerable therapeutic potential [6, 28]. However, a deeper understanding of the mechanisms underlying tumor immune evasion is needed to improve its efficacy and overcome the complex strategies employed by cancer cells to evade immune surveillance. This study provides evidence that the METTL1-CD47 axis can promote LUAD escape from macrophage phagocytosis, suggesting that targeting this axis may represent a potential strategy for improving LUAD immunotherapy.

METTL1, as a potential marker of tumor progression, has been reported to be aberrantly expressed in various cancers, including osteosarcoma, LUAD, breast cancer, and nasopharyngeal carcinoma, and to be involved in tumorigenesis, metastasis, and chemotherapy resistance [17, 29-31]. With the growing body of evidence, recent studies have gradually revealed that the expression level of METTL1 is associated with the degree of immune cell infiltration in various cancers. METTL1 upregulation can contribute to an immunosuppressive microenvironment by inducing myeloid-derived suppressor cells, whereas METTL1 deficiency may restore anti-tumor immunity [25]. METTL1 also plays a role in regulating the immune phenotype of macrophages, and its elevated expression is associated with increased M2 macrophage infiltration, whereas METTL1 inhibition can promote M1-like polarization and enhance macrophage phagocytic ability [20]. However, research on

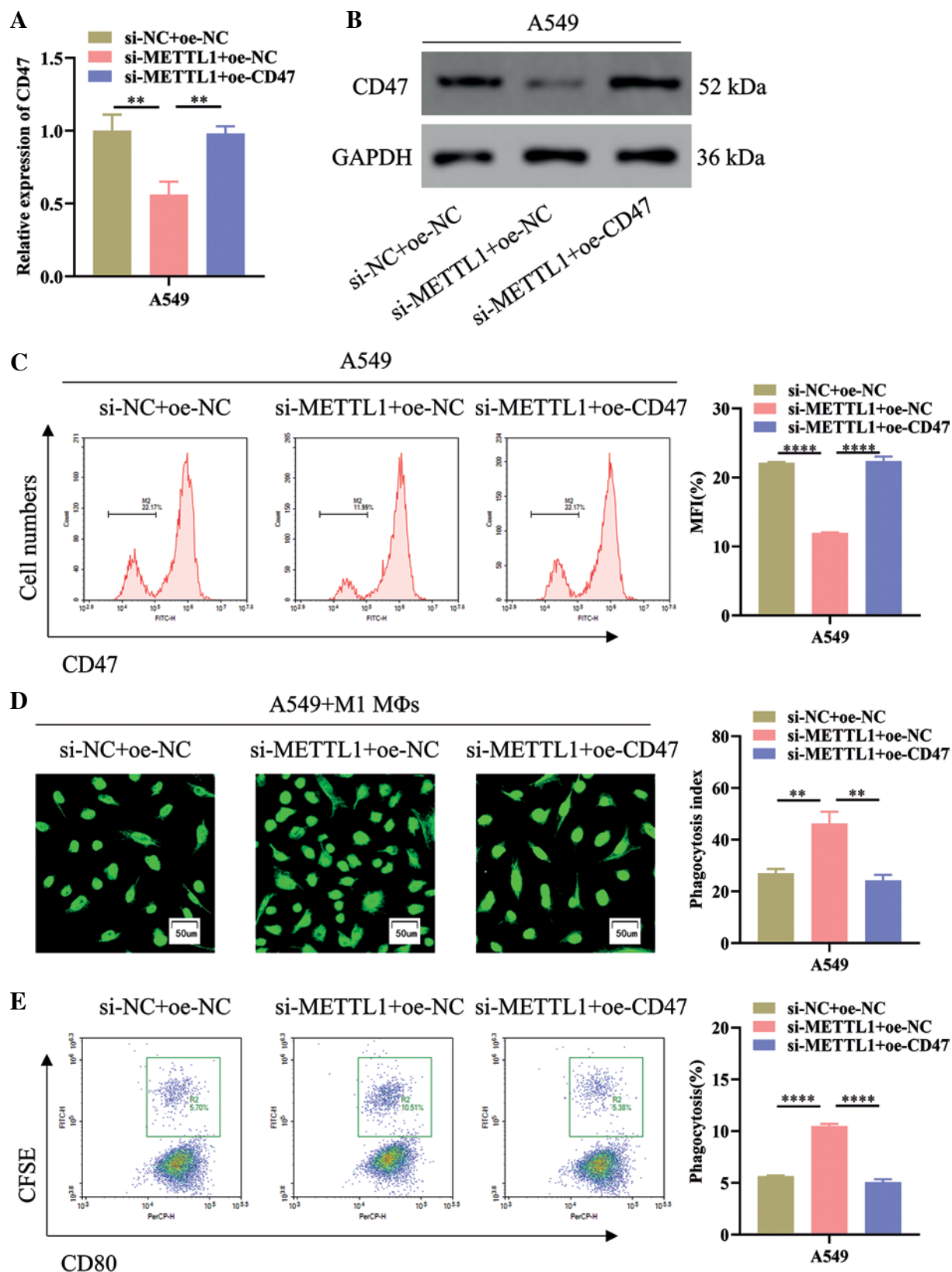


Fig. 4. METTL1 promotes lung adenocarcinoma (LUAD) escape from macrophage phagocytosis by upregulating CD47. **A, B**) qPCR and western blot detection of CD47 expression. **C**) Flow cytometric analysis of CD47 fluorescence signal on the membrane of A549 cells. **D**) Co-culture of the above different groups of LUAD cells with M1 macrophages, and analysis of the changes in macrophage phagocytosis of tumor cells by macrophage phagocytosis assay. **E**) Flow cytometric analysis of macrophage phagocytosis of the indicated LUAD cell groups in the co-culture system. $**p < 0.01$, $****p < 0.0001$

the regulation of macrophage phagocytosis by METTL1 remains limited. Due to the complexity of tumor cells, the effects of METTL1 may differ among cancer types and experimental models. Therefore, in this study, based on the LUAD model, we explored the role of METTL1 in regulating tumor escape from immune surveillance and found that METTL1 upregulation inhibits macrophage phagocytosis of LUAD cells. This finding supports a role for METTL1 in regulating macrophage-mediated antitumor immunity in LUAD and suggests that targeting METTL1 may have potential for modulating the functional state of TAMs.

In this study, we found that METTL1 may influence tumor immunity by promoting m7G modification of CD47 mRNA. Previous studies have mainly focused on the regulation of CD47 at the transcriptional and post-translational levels. For example, oncogenes such as MYC and IFN- γ can induce CD47 transcription [32, 33], and HIF-1 can directly upregulate its expression by binding to the CD47 promoter [34]. Recent studies have shown that oncogenes are also involved in the post-translational regulation of CD47. For instance, in glioblastoma, EGFR activation can induce CD47 phosphorylation, preventing its polyubiquitination and degradation [35]. These findings suggest that CD47 expression is subject to regulation at multiple levels. Based on these mechanistic insights, therapeutic strategies targeting the CD47/SIRP α axis have evolved from monotherapy to multimodal combination approaches. Blocking CD47 in lung cancer has been shown to induce ferroptosis via the Nrf2/FPN axis [36]. Furthermore, when combined with CTLA-4 inhibitors, this blockade can enhance anti-tumor immunity in non-small cell lung cancer by remodeling the tumor vasculature and immune microenvironment [37]. Furthermore, for specific molecular subtypes such as ALK-positive tumors, ALK inhibitors can synergize with CD47 blockade to enhance macrophage phagocytosis and overcome targeted therapy resistance [38]. Inhibiting the deubiquitinase USP2 promotes CD47 degradation and may improve the response to PD-1 therapy [39]. Additionally, bispecific chimeric peptides simultaneously targeting the CD47/SIRP α and PD-1/PD-L1 pathways can activate macrophages and CD8⁺ T cells [40]. These advances underscore the potential of CD47 as a therapeutic target for combination immunotherapy and highlight the complexity of its regulation. Notably, m7G modification is a common post-transcriptional RNA modification. It affects tumor proliferation, apoptosis, and invasion by regulating mRNA stability, translation efficiency, and subcellular localization, and is closely related to the tumor immune microenvironment [41, 42]. Given that METTL1 catalyzes m7G modification of various RNAs, including mRNA, our findings suggest that METTL1 can enhance the stability of CD47 mRNA through m7G modification, thereby promoting LUAD cells to evade macrophage phagocytosis. This finding identifies a potential post-transcriptional regulatory mechanism for CD47 expression and

provides a basis for further investigation of the METTL1–CD47 axis as a therapeutic target in LUAD.

In summary, our results indicate that METTL1 promotes m7G modification of CD47 mRNA, thereby increasing its stability and cell-surface expression in LUAD cells, which inhibits macrophage phagocytosis and may contribute to LUAD immune evasion. However, this study has some limitations. Given that this study relies heavily on *in vitro* data without confirmation in Rag2-/- γ c-/- murine models [43] or clinical specimens, the broader applicability and clinical translational significance of these conclusions warrant further investigation through future studies. In addition, although we found that METTL1 can regulate CD47 expression through m7G modification, the specific sites of METTL1-mediated CD47 mRNA modification and the detailed molecular mechanisms have not been fully elucidated. Therefore, future research should further validate these findings and characterize the specific modification sites and mechanisms, which may provide a basis for developing new therapeutic strategies for LUAD.

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Disclosures

Approval of the Bioethics Committee was not required. The authors declare no conflict of interest.

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