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Original Article

METTL16 promotes PD-L1 expression and mediates immunosuppression in B-cell lymphomas via m^{6}A methylation

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Abstract: Objectives: Immune Checkpoint Inhibitors (ICIs) have changed cancer treatment. Altered expression of methyltransferase-like 16 (METTL16), one of the initial m^{6}A "writers", is associated with malignant progression. METTL16 is a methyltransferase that may influence the treatment response of B-cell lymphoma (BCL). Methods: Overexpression and knockdown strategies were used to regulate METTL16 expression in BCL cells, and then qPCR and western blot were employed to measure the levels of METTL16 and programmed death ligand 1 (PD-L1). A BCL-T cell co-culture system was employed to study immune evasion. T cell activation and infiltration into tumor and spleen tissues were assessed by flow cytometry. METTL16 function in regulating the m^{6}A modification of PD-L1 mRNA was explored by MeRIP and RNA stability assays. Using a xenograft mouse model, we determined that METTL16 promotes BCL tumor growth. Results: Silencing METTL16 in BCL cells inhibited their proliferation and reduced neoplastic expansion in vivo. METTL16 silencing enhanced T cell activation and cytokine release. METTL16 promoted the association of Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) and PD-L1 mRNA by regulating m^{6}A modification. Reduction of METTL16 in BCL cells led to a lower proportion of PD-1-positive T cells in the co-culture system. In addition, an increase in METTL16 elevated the resistance of BCL cancer to inhibition by anti-PD-L1 therapy. Conclusion: Our findings indicate that METTL16 promotes BCL progression and immune evasion through epigenetic regulation of the PD-L1/PD-1 pathway.

Keywords: B-cell lymphoma, METTL16, PD-L1, N6-methyladenosine, Immune evasion

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma and relatively aggressive [1]. DLBCL is the most common subtype of non-Hodgkin's lymphoma, accounting for about 30%-35% of all cases worldwide [2]. More than half of patients can be cured with standard R-CHOP chemotherapy. However, about one-third of patients experience disease recurrence or refractory diseases, and 90% of these cases have had poor clinical outcomes [3, 4]. Therefore, it is critical to elucidate DLBCL pathogenesis and develop novel therapeutic strategies.

Next-generation immunotherapies have shown promising efficacy in hematological malignancies, including lymphomas [5, 6]. B7 Homolog 1 (B7-H1) is also known as programmed death ligand 1 (PD-L1), a B7 family member that can interact with and modulate the immune response of tumor-infiltrating T lymphocytes through the PD-1 receptor [7]. PD-L1 is expressed by neoplastic cells and reduces the proliferation and function of cytotoxic CD8$^+$ T cells by binding to PD-1 [8]. Recent studies have shown that an increase in PD-L1 leads to impaired T-cell function, thereby enabling tumors to evade immune recognition [9, 10]. Monoclonal antibodies targeting PD-L1 enhance the killing power of CD8$^+$ T cells by preventing apoptosis induced by PD-1 [11]. Therefore, tumor microenvironment markers may help predict response to anti-PD-L1 drugs in B-cell lymphoma.

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Epigenetic modifications refer to heritable changes in gene expression without alterations to the DNA sequence [12]. Dysregulation of epigenetic factors is a hallmark of various human diseases, particularly malignant tumors [13]. RNA molecules are subject to various post-transcriptional chemical modifications [14, 15]. N6-methyladenosine (m^6A) is the most widespread type of mRNA modification in eukaryotes [16]. The m^6A mark is a reversible biochemical modification, and its amount is strictly regulated by the equilibrium function of methyltransferases (writers) and demethylases (erasers) [17]. m^6A modifications have been shown to regulate RNA translation, stability, and alternative splicing. Therefore, aberrant m^6A modification has been implicated in human diseases, including cancer [18, 19]. Dysregulation of m^6A "writers" and enzymes, including methyltransferase-like 3 (METTL3), METTL14, and METTL16, has been reported in lung cancer, hepatocellular carcinoma and glioblastoma [20-23]. In addition, the m^6A "reader" Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) has also been identified to promote the development of acute myeloid leukaemia and lung cancer [24, 25]. METTL16 has not been studied in the context of lymphoma and immune evasion yet. In this study, we investigated the role of METTL16 on BCL cell proliferation and immune evasion both in vitro and vivo. We have also investigated how METTL16 regulates the immune evasion of BCL through epigenetics.

Materials and methods

Cell lines and transfection

DLBCL cell lines (Farage and DB) were obtained from the American Type Culture Collection (ATCC). These cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (BI, Israel), supplemented with 10% Fetal Bovine Serum (FBS) (BI, Israel) and 1% penicillin and streptomycin (Sigma, USA). Culture was performed at 37°C in a 5% CO_2 atmosphere and high-humidity conditions. siRNAs targeting METTL16 and IGF2BP2, as well as METTL16 overexpression plasmids and a negative control (NC), were obtained from GenePharma (Shanghai, China). Cells were transfected with the above-mentioned specific siRNAs or vectors using Lipofectamine 2000 (Thermo, USA) according to the manufacturer's instructions.

T-cell co-culture model

Peripheral blood was obtained from healthy donors. Ficoll-Paque density gradient centrifugation was employed to separate peripheral blood mononuclear cells (PBMCs). T cells were activated using Dynabeads Human T-Activator CD3/CD28 (Gibco, USA) for 48 hours according to the manufacturer's instructions. T cell and B cell lines (BCL cells) were combined, cultured in a 24-well plate at a 10:1 ratio for co-culture experiments, and then maintained for 72 hours. Cells were stained with antibodies against CD4, CD8, IFN-γ, TNF-α, PD-1 and PD-L1, and analyzed by flow cytometry.

Cell viability and proliferation detection

Cell viability was assessed using a CCK-8 kit (Solarbio, China) according to the manufacturer's instructions. BCL cells were seeded at a concentration of 5,000 cells/well in 96-well plates and incubated at 37°C for indicated durations. CCK-8 reagent was added, and the plate was incubated for another two hours. Absorbance was measured at 450 nm using a microplate reader.

Cellular apoptosis

Apoptosis was measured using an Annexin V/PI apoptosis detection kit (Beyotime, China) after the above procedures. Cells were harvested and stained with Annexin V-FITC/PE and Propidium Iodide (PI). After incubation in the dark for 30 minutes, the number of apoptotic cells was determined by flow cytometry (BD Biosciences, USA).

Quantitative PCR (qPCR) experiment

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) in accordance with manufacturer's protocol. Then, 1 mg total RNA served as the substrate for reverse transcription in a high-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA). The generated cDNA was used as the template for quantitative PCR amplification with SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA). Relative mRNA expression was determined by the 2-ΔΔCt

method, and β-actin served as the internal reference.

Western blotting assay

Cells were lysed in chilled RIPA buffer and supplemented with protease (Thermo; USA) and phosphatase inhibitors (Beyotime; China). BCA Protein Assay Kit (Thermo, USA) was used to determine protein amounts, and an equal volume of protein was loaded in an SDS-PAGE gel. Electrophoresis was used to separate the proteins, and then a nitrocellulose membrane was prepared. A 5% skim-milk suspension served as the blocking agent. Then, using the primary antibody for METTL16 (1:1000, 17,676, CST), IFG2BP2 (1:1000, 11601-1-AP, Proteintech), and PD-L1 (1:1000, ab252420, ab213480, Abcam), β-actin (1:1000, A1978, Sigma-Aldrich, USA), incubate with the HRP-linked secondary antibody (1:5000, 7074, CST). Protein bands were visualized using an enhanced chemiluminescence (ECL) kit and a Tanon imaging system (China).

RNA immunoprecipitation (RIP)

The interaction between IGF2BP2 and PD-L1 mRNA was examined by EZ-Magna RIP kit (Millipore, USA). Samples were incubated overnight at 4°C, followed by incubation with anti-IGF2BP2 antibody-conjugated magnetic beads, and then centrifuged. After the above overnight incubation, the antibody-bound beads were combined with isolated total RNA at 4°C for another 6 hours. Purify and purify the obtained RNA; then, by means of qPCR, quantify the abundance of the specific PD-L1 mRNA.

MeRIP-qPCR assay

Total cellular RNA was collected before addition of an anti-m^{6}A antibody (Thermo, USA) for 6 hours at 4°C. Dynabeads (Invitrogen, USA) were added to 2% BSA (Thermo, USA), incubated at 4°C for 3 hours, and then treated with the RNA-antibody complex overnight at 4°C. Finally, after adding an SDS buffer, extract RNA and then use qPCR to determine the PD-L1 expression level.

RNA stability measurement

BCL cells were transfected with siMETTL16 or siIGF2BP2, and then 50 μM of actinomycin D (Sigma, USA) was added. Collect total RNA at different times and then convert it into cDNA. Relative PD-L1 mRNA expression was normalized to the 0-hour control, and qPCR was used for further analysis.

Elisa

Immune mediators were obtained by centrifugation of the incubation fluid to remove cells, and then the resulting supernatant was collected. The levels of IFN-γ, TNF-α and IL-2/GzmB were determined by an individual ELISA kit (Thermo, USA) following the instructions provided by the company.

Syngeneic tumor model and in vivo treatment

All biological studies conducted in this paper received approval from the Ethics Committee of The Fourth Hospital of Hebei Medical University (Approval No. 2023KS142) and followed the ARRIVE guidelines. Four- to six-week-old wild-type BALB/c mice of different ages were obtained from Vital River Laboratory (Beijing, China). Murine B-cell lymphoma cell line A20 was transfected with control, siMETTL16 or METTL16 overexpression vectors. A total of 5×10^6 engineered A20 cells (resuspended in 100 μL PBS) were subcutaneously transplanted into the right flank of BALB/c mice. Arbitrarily divide the subjects into two groups at the time when palpable tumors appeared for in vivo immune checkpoint blockade treatment. The cohort of anti-PD-L1 therapy received intraperitoneal administrations of anti-mouse PD-L1 mAb (10 mg/kg, clone 10F.9G2, Bio X Cell) or an IgG isotype reference every 3 days for a total of four sessions. The size of the tumor is a length and width; its mass can be calculated by the formula $V = (\text{length} \times \text{width}^2)/2$. The study will be about to end, so we will euthanize the subjects by inhalation of carbon dioxide (CO_2). All the animals in the experiment were moved to the living-beasts area for regular maintenance.

Analysis of immune cells in tumor and spleen tissues

Tumor samples were obtained by surgery, and then, before mechanical dissociation, were washed with PBS using a gentle MACS dissociator (Miltenyi Biotec, Germany). Filter the cell suspension through a 100 μm cell strainer,

then centrifuge to obtain the pellet, and finally resuspend in PBS. Then, these individual cells were stained with specific antibodies to identify different subsets of immune cells. For T-lymphocyte profiling, cells were co-stained with FITC-labelled anti-CD3 (555274, clone 17A2, BD Biosciences), PE-labelled anti-CD8 (553032, clone 53-6.7, BD Biosciences), PE-labelled anti-CD4 (553730, clone GK1.5, BD Biosciences), APC-anti-PD-1 (329908, clone EH12.2H7, BioLegend), APC-anti-IFN-γ (502512, clone 4S.B3, BioLegend), and PE-anti-TNF-α (502909, clone MAb11, BioLegend) antibodies at a 1:100 ratio. To isolate T helper cells, PE-Cy7-conjugated CD4 (25-0042-82, clone RM4-5, Invitrogen) and APC-conjugated CD25 (17-0251-82, clone PC61.5, Invitrogen) antibodies were used at a 1:100 dilution for 30 minutes at 4°C, followed by permeabilization and labeling with PE-conjugated Foxp3 (12-5773-80, clone FJK-16S, Invitrogen) at a 1:100 concentration. Spleen tissues were also homogenized and sieved, then centrifuged. Add ACK lysis buffer to remove red blood cells, then prepare the isolated cells for staining as shown below. All of the above specimens were analyzed by FACS Calibur flow cytometry.

Statistics analysis

Results are shown as the mean ± standard deviation (SD). A two-tailed Student's t-test was conducted on the cohorts to see if there was a statistically significant difference at $P < 0.05$.

Results

METTL16 regulates PD-L1 expression in DLBCL cells

First, we determined whether METTL16 alters the level of PD-L1 using METTL16 siRNA and overexpression plasmids. Both METTL16 siRNA and OE-METTL16 vectors effectively reduced or increased the abundance of METTL16 RNA and protein levels in the Farage and DB cell models (**Figure 1A-D**). Then, we found that suppression of METTL16 resulted in a significant reduction in both RNA and protein levels of PD-L1 (**Figure 1E** and **1F**), and overexpression of METTL16 increased PD-L1 expression substantially (**Figure 1G** and **1H**). We selected siMETTL16-1 for the above studies.

METTL16 modifies T-cell activity in the presence of DLBCL cells

We established a DLBCL-T cell coculture system to assess the tumor cell behavior and T cell function. Flow cytometry showed that METTL16 modulates PD-L1 expression on DLBCL cells upon T cell contact (**Figure 2A**). Moreover, METTL16 knockdown inhibited proliferation and induced apoptotic of Farage and DB cells co-cultured with T cells (**Figure 2B** and **2C**). At the same time, the proportion of $CD8^+$ T cells was significantly higher in coculture with DLBCL cells that had been depleted of METTL16 (**Figure 2D**). In addition, co-culture with METTL16-depleted DLBCL cells enhanced T cell secretion of IL-2, IFN-γ, TNF-α, and granzyme B (**Figure 2E** and **2F**). Notably, silencing METTL16 in DLBCL cells also reduced the expression of PD-1 in co-cultured T cells (**Figure 2G**).

METTL16 regulates the growth of in vivo DLBCL by modifying the immune system

Then, a xenograft tumor model was built to examine the in vivo effect of METTL16. Knockdown of METTL16 reduced the level of PD-L1 in tumors (**Figure 3A**) and inhibited in vivo tumor growth (**Figure 3B-D**). In addition, silencing METTL16 increased the fraction of $CD3^+CD8^+$ T cells in the tumor and spleen (**Figure 3E**). IFN-γ-expressing T cells and TNF-α-expressing T cells were also increased after siMETTL16 treatment (**Figure 3F**). Notably, the proportion of $CD25^+Foxp3^+$ T regulatory cells was significantly reduced in tumors, spleens, and peripheral blood (**Figure 3G**). These findings suggest that METTL16 knockdown enhances antitumor immunity in vivo.

METTL16 epigenetically regulates the m^6A modification of PD-L1

Next, we investigated the molecular mechanisms by which METTL16 regulates PD-L1 expression. METTL16 is a methyltransferase for m^6A modification. Silencing METTL16 significantly reduced m^6A abundance on PD-L1 mRNA, while overexpression of METTL16 increased it (**Figure 4A**). MeRIP experiments also showed similar results (**Figure 4B** and **4C**). As IGF2BP2 functions as an m^6A reader pro-

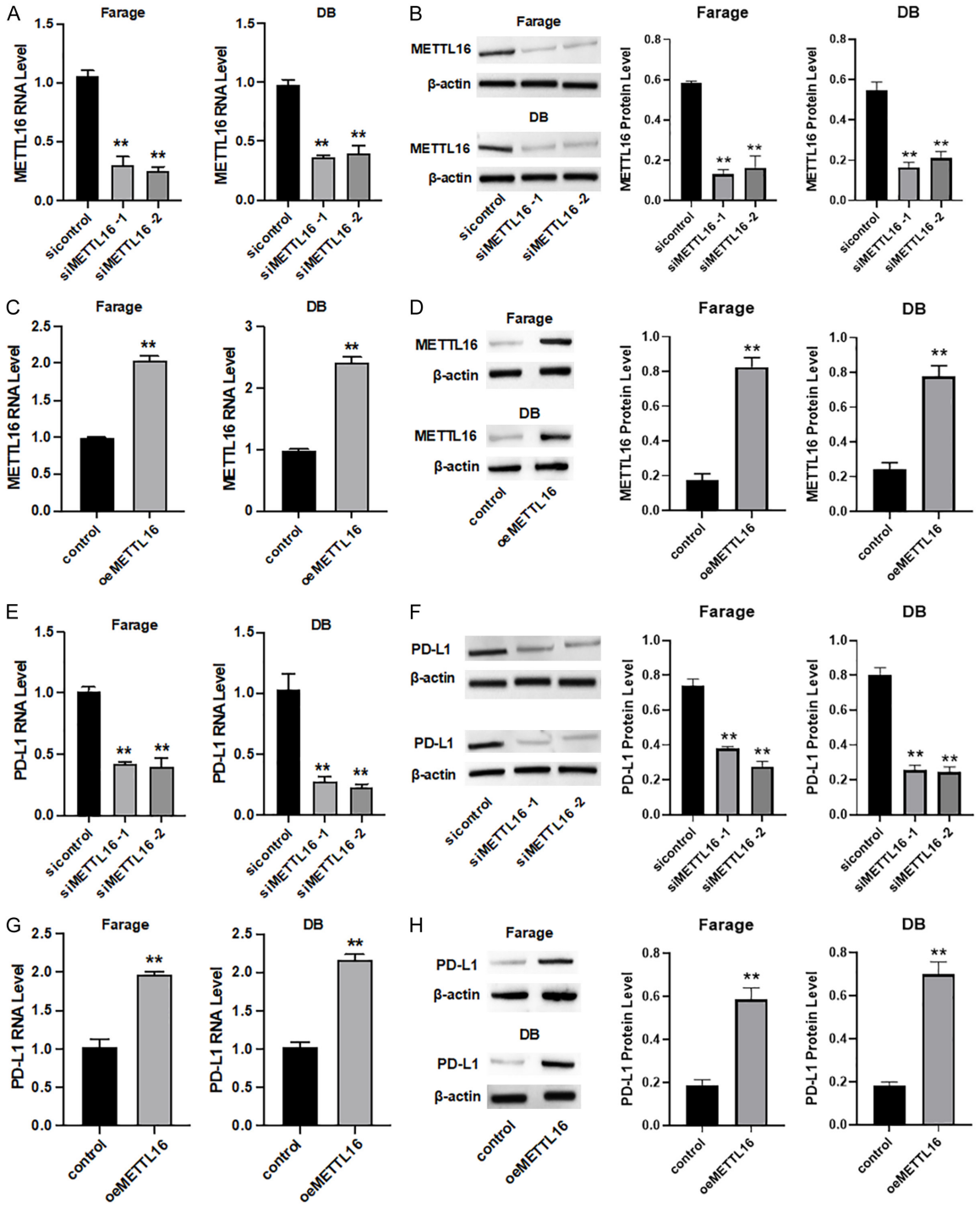
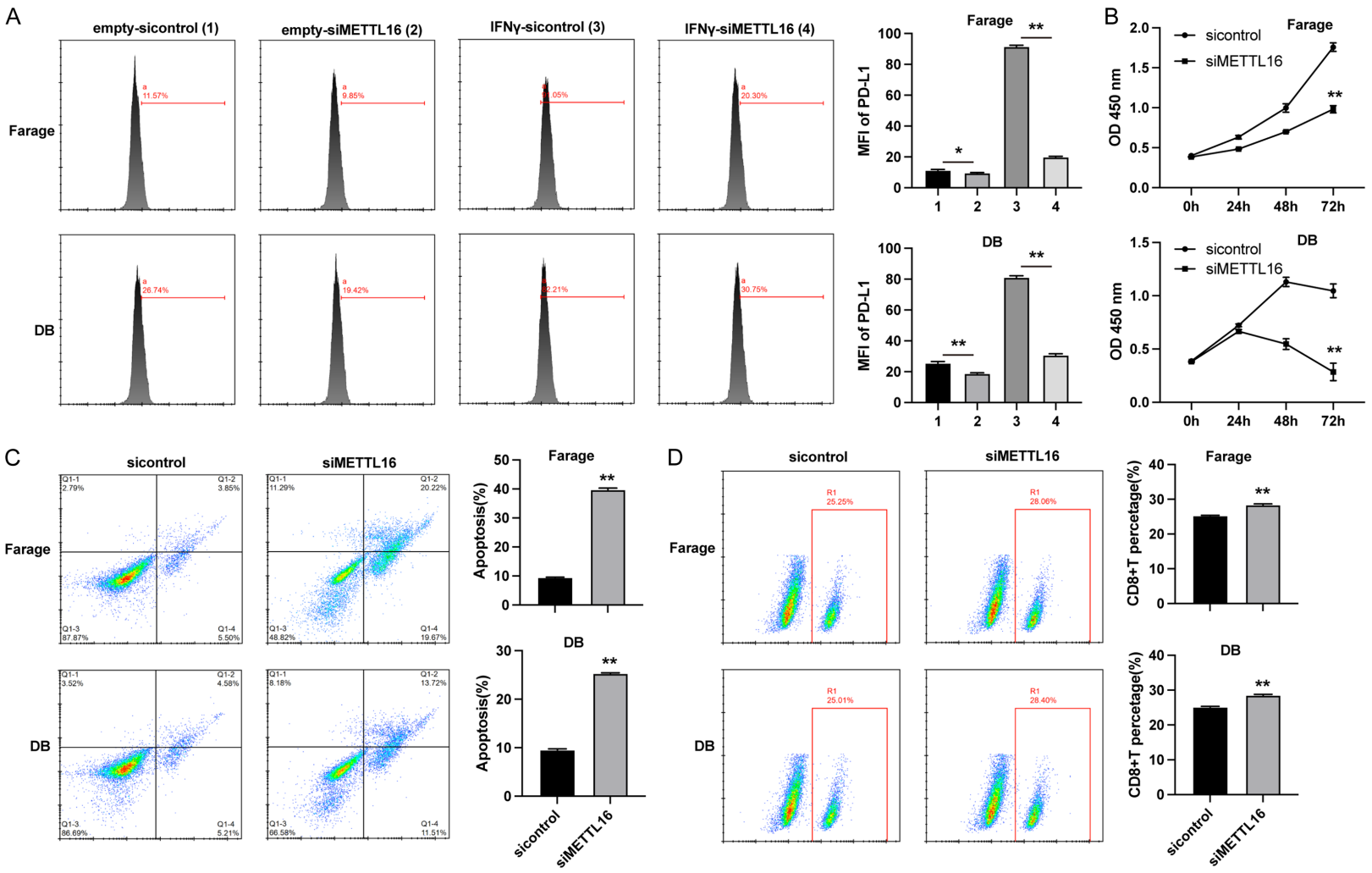


Figure 1. Methyltransferase-like 16 (METTL16) regulates programmed death ligand 1 (PD-L1) in Diffuse large B-cell lymphoma (DLBCL) cell lines. (A, B) qPCR and western blot showing the effects of si-METTL16 transfection on RNA (A) and protein (B) levels of METTL16 in Farage and DB cells. (C, D) qPCR and western blot showing the effects of OE-METTL16 transfection on RNA (C) and protein (D) levels of METTL16 in Farage and DB cells. (E, F) qPCR and western blot showing the effects of si-METTL16 transfection on RNA (E) and protein (F) levels of PD-L1 in Farage and DB cells. (G, H) qPCR and western blot showing the effects of OE-METTL16 transfection on RNA (G) and protein (H) levels of PD-L1 in Farage and DB cells. $^{**}P < 0.01$.

tein, we further examined its role in PD-L1 regulation. IGF2BP2 knockdown significantly reduced PD-L1 protein expression (**Figure 4D** and **4E**). RIP-qPCR analysis showed direct bind-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Q1-1 2.79%
Q1-2 3.85%
Q1-3 87.87%
Q1-4 5.50%
Q1-1 11.29%
Q1-2 20.22%
Q1-3 48.82%
Q1-4 19.67%
Q1-1 3.52%
Q1-2 4.58%
Q1-3 86.69%
Q1-4 5.21%
Q1-1 8.18%
Q1-2 13.72%
Q1-3 66.58%
Q1-4 11.51%
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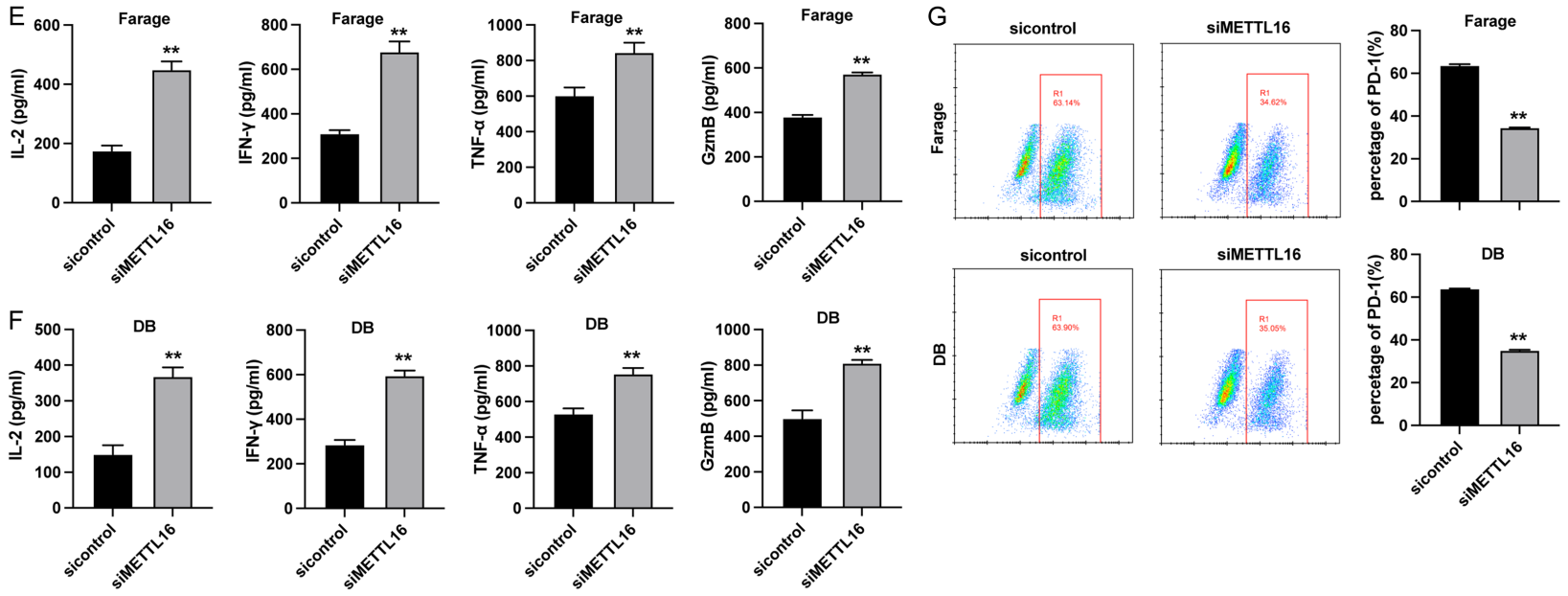

Figure 2. Methyltransferase-like 16 (METTL16) regulates T-cell activity in Diffuse large B-cell lymphoma (DLBCL) cells. Farage and DB cells were transfected with si-METTL16 or OE-METTL16 plasmids, and subsequently co-cultured with T cells. (A) The expression of programmed death ligand 1 (PD-L1) on DLBCL cells was detected by flow cytometry. (B, C) Cell viability (B) and apoptosis (C) of DLBCL cells were measured using CCK-8 and flow cytometry. (D) $CD8^+$ T cells were quantified by flow cytometry. (E, F) Cytokine secretion levels was measured by ELISA. (G) PD-1^+ T cells populations were determined by flow cytometry. $^{**}P < 0.01$.

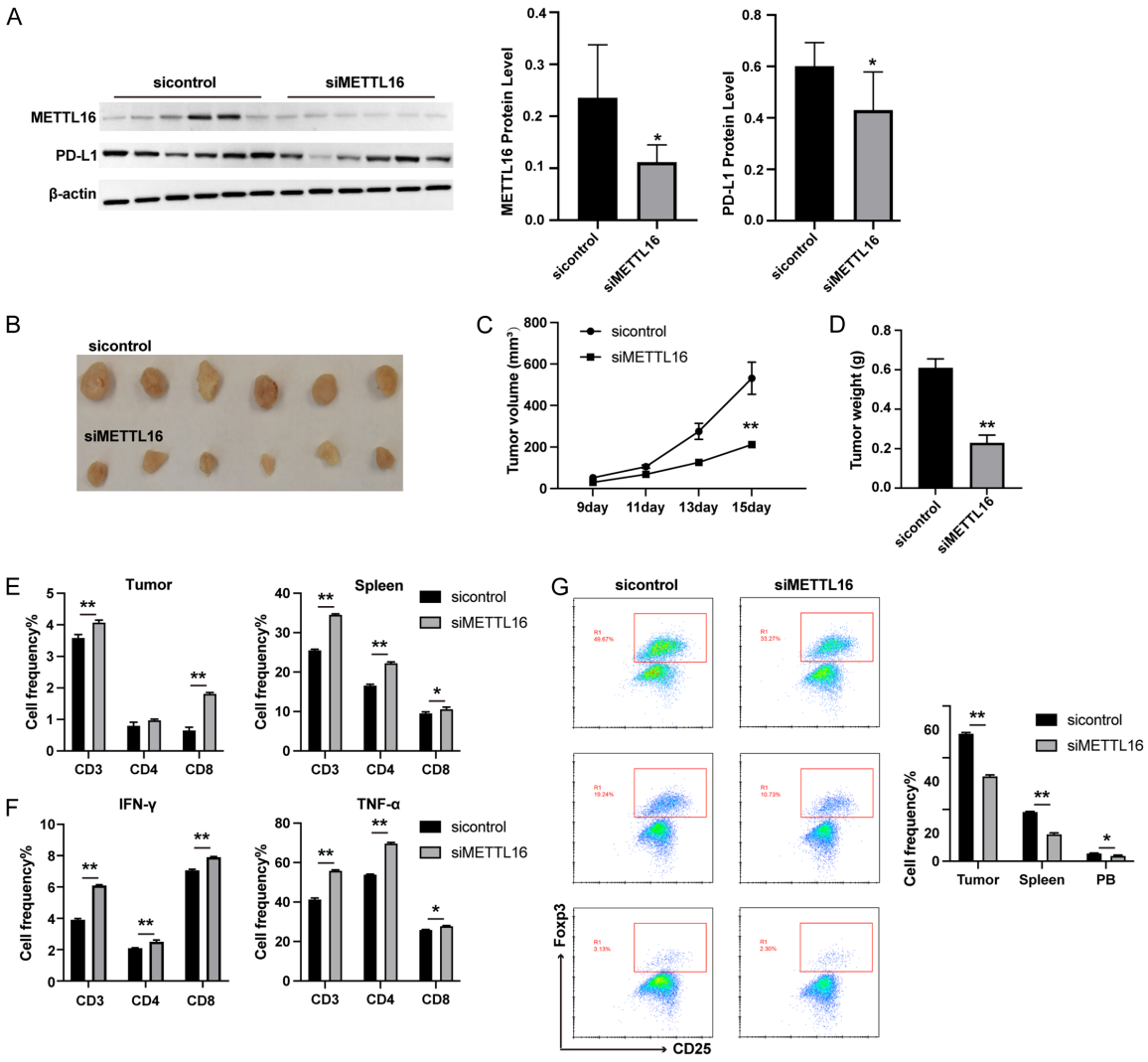

Figure 3. Methyltransferase-like 16 (METTL16) regulates the in vivo progression of Diffuse large B-cell lymphoma (DLBCL) by modulating the immune response. An orthotopic tumor model was established, followed by treatment with si-METTL16. A. Protein expression levels of METTL16 and programmed death ligand 1 (PD-L1) were analyzed by Western blot. B-D. Tumor size, growth rate, and final tumor weight were evaluated. E. T-cell proportions in tumor tissues and spleen were analyzed by flow cytometry. F. Interferon-gamma (IFN-γ)- and tumor necrosis factor-α (TNF-α)-producing T cells within tumor tissues were quantified by flow cytometry. G. Treg cell population in tumor tissues was assessed by flow cytometry. $^{*}P < 0.05$, $^{**}P < 0.01$.

ing between IGF2BP2 and PD-L1 RNA (**Figure 4F**), and METTL16 depletion reduced this binding in Farage and DB cells (**Figure 4G** and **4H**). Furthermore, mRNA stability assays showed that simultaneous silencing METTL16 and IGF2BP2 significantly reduced PD-L1 mRNA levels after actinomycin D treatment compared with the siControl group (**Figure 4I**).

METTL16 modulating immune evasion in DLBCL via regulation of the PD-L1/PD-1 axis

Next, we validated the in vivo anti-tumor and immunostimulatory effects of the METTL16-PD-L1 axis. METTL16 overexpression promoted tumor growth in vivo, which was effectively inhibited by anti-PD-L1 antibody treatment. Notably, when METTL16 was overexpressed at the same time as anti-PD-L1 antibody treatment, the anti-tumor effect was completely abolished, and the resulting tumors were significantly larger than those in the anti-PD-L1 single-treatment group (**Figure 5A** and **5B**). Additionally, only the anti-PD-L1 treatment increased the infiltration of $CD3^{+}$, $CD4^{+}$ and $CD8^{+}$ T cells within the tumor (**Figure 5C**), and simultaneously augmented the secretion of IFN-gamma and TNF-alpha (**Figure 5D** and **5E**).

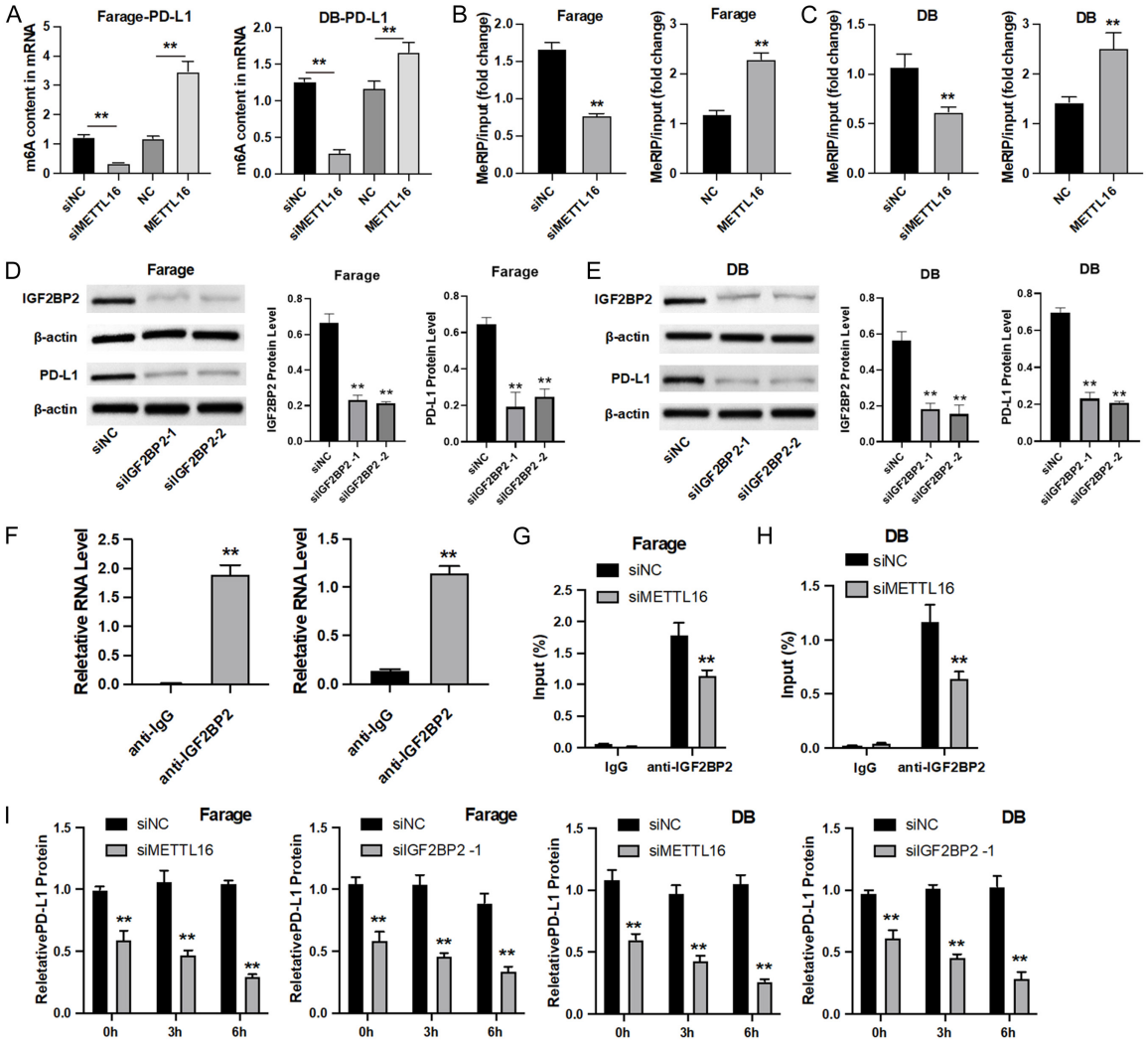

Figure 4. Methyltransferase-like 16 (METTL16) regulates programmed death ligand 1 (PD-L1) expression via m^{6}A epigenetic modification in Diffuse large B-cell lymphoma (DLBCL) cells. (A-C) Farage and DB cells were transfected with si-METTL16 or OE-METTL16 plasmids, and m^{6}A enrichment of PD-L1 mRNA was quantified by crosslinking-immunoprecipitation -qPCR (A) and Methylated RNA Immunoprecipitation (MeRIP) sequencing (B, C). (D, E) Farage and DB cells were transfected with Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) siRNA, and the protein levels of IGF2BP2 and PD-L1 were examined by Western blotting. (F-H) The interaction of IGF2BP2 on PD-L1 mRNA was measured by RNA immunoprecipitation-qPCR. (I) Farage and DB cells were co-transfected with si-METTL16 and si-IGF2BP2, followed by actinomycin D treatment, and PD-L1 mRNA levels were determined by qPCR. $^{**}P < 0.01$.

However, METTL16 has been forced to be expressed and thus creates a highly immunosuppressive microenvironment that effectively counteracts the anti-PD-L1-mediated immunosuppressive effects of immunotherapy (**Figure 5D** and **5E**).

Discussion

N6-Methyladenosine (m^{6}A) is the most abundant mRNA modification in eukaryotes and regulates post-transcriptional gene expression [26]. m^{6}A modifications influence cell proliferation, metastasis, and immune evasion [27]. This m^{6}A modification is inherently reversible, and it is in a state of equilibrium between "writers" that add it, "erasers" that remove it, and "readers" that recognize it [28]. In cancer, m^{6}A regulators may function as oncogenes or tumor suppressors depending on the cellular context and target mRNAs. METTL16 is a "writer" of m^{6}A and exhibits potential functional heterogeneity across different cancer types. METTL16 has been shown to be overexpressed in glio-

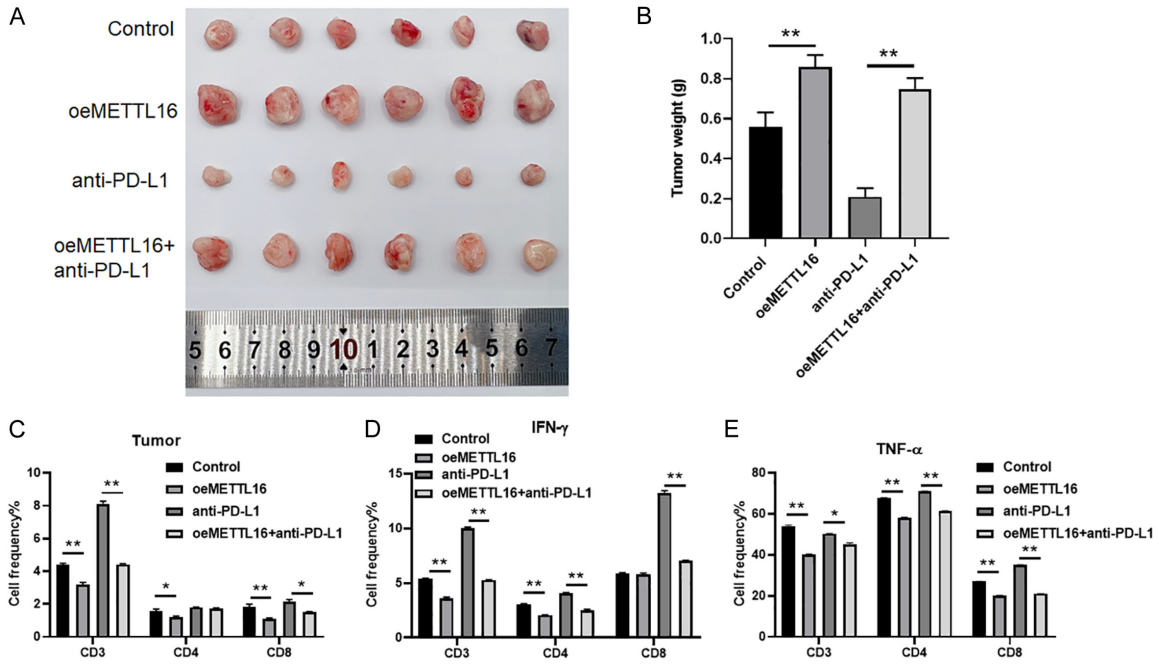

Figure 5. Methyltransferase-like 16 (METTL16) regulates immune evasion in Diffuse large B-cell lymphoma (DLBCL) through modification of the programmed death ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) pathway. A xenograft tumor system was established, followed by treatment with OE-METTL16 vectors and anti-PD-L1 antibodies. A, B. Tumor size and tumor weight were measured in each group. C. The proportion of T cells within tumor tissues was analyzed by flow cytometry. D, E. Interferon-gamma (IFN-γ)- and tumor necrosis factor-α (TNF-α)-producing T-cell populations in tumor tissues were quantified by flow cytometry. $^{*}P < 0.05$, $^{**}P < 0.01$.

mas [29] and is associated with poor prognosis in melanoma [30]. METTL16 promotes gastric cancer growth by upregulating cyclin D1 expression [31]. Notably, METTL16 acts as a tumor suppressor in pancreatic adenocarcinoma and correlates with immune regulatory profiles and immune cell infiltration [32, 33]. In bladder cancer, METTL16 reduces cell proliferation and cisplatin resistance via the HIF-2α-PMEPA1-autophagy axis [34]. These observations suggest that METTL16 predominantly acts as an oncogene across most solid tumors, but its downstream effectors and interacting pathways vary depending on the cellular context. We found that METTL16 regulated PD-L1 expression in BCL. METTL16 modulation affected BCL cell growth in vitro and in vivo, and also altered T cell activity and immune evasion. Mechanistically, METTL16 promoted m^{6}A modification and stability of PD-L1 RNA by recruiting the "reader" IGF2BP2, and thus increased the expression of PD-L1 in BCL cells to support T cell activation. Although our findings demonstrated that IGF2BP2 enhances PD-L1 mRNA stability, IGF2BP proteins are also heavily implicated in promoting target mRNA translation. It is highly plausible that IGF2BP2 concurrently facilitates the translational efficiency of PD-L1, which requires further validation using polysome profiling in future studies.

METTL16 and IGF2BP2 are necessary regulators of m^{6}A RNA methylation in post-transcriptional modifications that affect RNA function, including splicing, stability and translation [35-37]. METTL16 is known to work with IGF2BP2 to alter the half-life of SENP3 mRNA via m^{6}A-specific mechanisms. IGF2BP2, on the other hand, can reduce the proteasomal degradation of Lactotransferrin (LTF) by blocking its de-SUMOylation and thus promote ferroptosis and the progression of HCC [36]. Research by Jiang and his team has found that IGF2BP2 is strongly associated with a poor prognosis for pancreatic adenocarcinoma (PDAC), and both IGF2BP2 and METTL16 have shown considerable connections with advanced-stage pancreatic adenocarcinoma (PAAD). In addition, these m^{6}A mediators have been linked to the expression level of immune modulators, including

immune suppressors, immune activators and MHC proteins, as well as the density of immune cell infiltration in PAAD [37].

Our study demonstrate that METTL16 upregulates PD-L1 in BCL cells by enhancing m^{6}A modification and prolonging PD-L1 mRNA stability. As a tumor-derived ligand, PD-L1 binds to the PD-1 receptor on T cells [38]. This engagement suppresses T cell activation and prevents tumor cell destruction, thereby enabling immune evasion [39, 40]. In our BCL-T cell coculture system, METTL16 silencing enhanced cytotoxic T cells activation and cytokine release. However, due to the lack of an independent clinical cohort of patients with BCL, this study was unable to directly assess the expression of METTL16 and PD-L1 proteins or their prognostic value. Future studies with tissue microarrays and clinical follow-up are warranted to validate the METTL16/PD-L1 axis at the protein level and its potential as a prognostic biomarker.

Conclusion

In summary, our findings demonstrate that METTL16 regulates PD-L1 and promotes B-cell lymphoma proliferation. Mechanistically, METTL16 regulates m^{6}A modification of PD-L1 transcripts via IGF2BP2 recruitment, thereby promoting immunosuppression and BCL progression both in vitro and in vivo.

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Disclosure of conflict of interest

None.

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