



Methyltransferase-like 3 upregulation is involved in the chemoresistance of non-small cell lung cancer

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Background: Treatments for non-small cell lung cancer (NSCLC) have improved tremendously, but therapeutic resistance is a common and major clinical challenge in treatment. Methyltransferase-like 3 (METTL3) is a ribonucleic acid (RNA) methyltransferase that has crucial functions in the development and progression of cancers, including drug resistance, by regulating N⁶-methyladenosine (m⁶A) modification. However, the role of METTL3 in the progression and drug resistance of NSCLC is poorly understood.

Methods: The expression levels of METTL3 and AKT serine/threonine kinase 1 (AKT1) in NSCLC tissues were detected using quantitative real-time PCR (RT-qPCR), Western blots, and immunohistochemical assays. The m⁶A levels of AKT1 messenger RNA (mRNA) in NSCLC tissues were detected using m⁶A methylated RNA immunoprecipitation–quantitative polymerase chain reaction.

Results: The expression levels of METTL3 and the AKT1 protein were significantly increased in NSCLC tissues, and m⁶A expression levels of AKT1 mRNA were dramatically upregulated in NSCLC tissues. Additionally, METTL3, AKT1 protein, and m⁶A levels of AKT1 mRNA were overexpressed in chemoresistant NSCLC samples, and high expression levels of METTL3 and AKT1 were correlated with poor patient survival, especially in chemoresistant NSCLC patients. Further, AKT1 protein expression and m⁶A levels of AKT1 mRNA were positively correlated with METTL3 expression, and AKT1 protein expression was positively correlated with m⁶A levels of AKT1 mRNA. Moreover, METTL3 and AKT1 protein expression levels were significantly associated with cisplatin susceptibility, tumor, node, metastasis stage, and lymph node metastasis.

Conclusions: Taken together, our results indicate that METTL3 contributes to the progression and chemoresistance of NSCLC by promoting AKT1 protein expression through regulating AKT1 mRNA m⁶A levels, and may provide an efficient therapeutic intervention target for overcoming chemoresistance in NSCLC.

Keywords: Non-small cell lung cancer (NSCLC); methyltransferase-like 3 (METTL3); AKT serine/threonine kinase 1 (AKT1); chemoresistance; biomarker

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Introduction

Lung cancer is a malignant cancer with the highest mortality rate in the world (1). Non-small cell lung cancer (NSCLC) is one of the biggest causes of cancer mortality worldwide (2), and accounts for >80% of all lung cancer cases (3). Chemotherapy is a traditional therapy for NSCLC (4), and cisplatin (DDP) is a clinical drug used for chemotherapy and has become the standard treatment for NSCLC patients due to its good performance (5). Unfortunately, long-term cisplatin treatment leads to drug resistance, which in turn usually leads to NSCLC treatment failure (6). Inducing DDP susceptibility is a sound strategy for improving the treatment of NSCLC patients.

Epigenetics refers to the differential expression of genes based on changes in non-gene sequences, including chromatin conformation, deoxyribonucleic acid (DNA) methylation, and (ribonucleic acid) RNA methylation changes (7-11). In the last few decades, epigenetics has been found to be closely related to tumorigenesis and development (12,13). DNA methylation has been found involved in cancer progression and chemoresistance (14,15), and played as potential target for cancer treatment (16). The LINE-1 methylation level in hepatocellular carcinomas (HCC) tissues was significantly lower than in matched normal liver tissues and reduces the disease-free survival rates of HCC patients, the results suggested that LINE-1 methylation level might play as a biomarker for identifying patients who will experience an unfavorable clinical outcome (17). Zhang *et al.* found that the methylation-induced downregulation of *HOXA11* might be a diagnostic and prognostic marker and contribute to cisplatin resistance in patients with lung adenocarcinoma (18). Recently, more and more studies have focused on RNA methylation, which was discovered to be a post-transcription epigenetic modification (19,20). N⁶-methyladenosine (m⁶A) is an important, and the most abundant, type of RNA methylation modification, and regulates RNA translation, stability, splicing, and nucleation (21,22). Recently, studies have shown that m⁶A methylation is bound up with the occurrence, progression, and drug resistance of human cancers (23-25). In recently, studies have revealed that the tumor microenvironment (TME) is associated with m⁶A. It has been reported that m⁶A enhances the anti-cancer response of tumor-infiltrating CD8⁺ T cells, improves the therapeutic effect of anti-PDL1 receptor blockers (26), and Liu *et al.* demonstrated that m⁶A modification plays an important role in tumorigenesis and TME infiltration characterization of low-grade gliomas (27).

Also, Wang *et al.* found that an m⁶A score based on IGF2BP2, IGF2BP3, KIAA1429, METTL3, EIF3H and LRPPRC expression were proposed as an indicator of tumor microenvironment status and were instrumental in predicting the prognosis of pancreatic cancer patients (28). Furthermore, the tumor microenvironment characterization revealed that the identified m⁶A patterns were highly consistent with immune-inflamed, immune-excluded and immune-desert, and patients with lower m⁶A scores showed a better immune responses and durable clinical benefits in three independent immunotherapy cohorts (29). m⁶A methylation is catalyzed by component “writer” methyltransferase complexes [methyltransferase-like 3 (METTL3), methyltransferase-like 14, and WT1 associated protein (WTAP)] and is removed by “eraser” demethylases [FTO alpha-ketoglutarate dependent dioxygenase (FTO) and ALKB homolog 5 (ALKBH5)] (30).

METTL3 has been identified as the main methyltransferase complex and plays a critical role in m⁶A methylation (31). The dysregulation of METTL3 regulates the total m⁶A methylation level, which directly affects the decay and translation of messenger RNA (mRNA) and microRNA biogenesis, which contribute to human diseases (32-35). METTL3 was found to be upregulated in gastric cancer, and the high expression of METTL3 is predictive of a poor prognosis (36). Peng *et al.* found that the upregulation of METTL3 results in abnormal m⁶A modification and is positively correlated with tumor metastasis in colorectal cancer (37). Additionally, METTL3 serves as an oncogenic gene in bladder cancer by interacting with DGCR8 microprocessor complex subunit (DGCR8) and positively regulating the pri-miR221/222 process (38). In NSCLC, METTL3 promotes the Warburg effect of NSCLC by promoting ABHD11-AS1 expression via installing the m⁶A modification and enhancing ABHD11-AS1 transcript stability (39). The m⁶A reader YT521-B homology domain containing 2 (YTHDC2) is frequently downregulated in lung adenocarcinoma and low expression of YTHDC2 was associated with poor clinical outcome (40). The m⁶A mRNA methylation initiated by METTL3 promotes YAP mRNA translation via recruiting YTHDF1/3 and eIF3b to the translation initiation complex and increases YAP mRNA stability through regulating the MALAT1-miR-1914-3p-YAP axis. The increased YAP expression and activity induce NSCLC drug resistance and metastasis (41). The results of previous studies showed different roles of m⁶A mRNA methylation in progression of NSCLC. However, few studies have reported on the functions of

RNA methylation in lung cancer drug resistance, and very little is known about the important role of METTL3 in the chemoresistance of NSCLC.

In this study, we found that METTL3 was upregulated in NSCLC tissues, and its expression levels in chemoresistant NSCLC patients were significantly higher than those of chemosensitive NSCLC patients. Further, higher expression levels of METTL3 were also highly associated with shorter overall survival (OS). We also showed that AKT1 protein expression was negatively correlated with METTL3 expression. Our findings show the important role of the METTL3-AKT1 axis in the progression and chemoresistance of NSCLC, and may provide a therapeutic intervention target for NSCLC treatment.

We present the following article in accordance with the MDAR checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6608/rc>).

Methods

Patients and tissue samples

The data of 180 NSCLC patients, who were treated with DDP therapy at the Sun Yat-sen University Cancer Center from March 2015 to September 2016, were collected. During the DDP treatment, these patients were evaluated once every 2 weeks for drug effectiveness. Recurrent NSCLC was defined (solid tumors, RECIST 1.1) as a 5 mm or 20% increase in the total lesion diameter from the baseline (42,43). The NSCLC tissues and adjacent normal tissues (>3 cm from tumor tissues) were collected during the surgical resection, and a pathological puncture biopsy was performed. Ninety NSCLC tissues were identified as sensitive NSCLC tissues (primary), and another 90 were identified as resistant NSCLC tissues (recurrent). Written informed consent was obtained from all the patients before the study. This study was approved by the Ethics Committee of Sun Yat-sen University Cancer Center (No. GZR2020-297) and performed in accordance with the Declaration of Helsinki (as revised in 2013).

RT-qPCR

Total RNA from tissues were isolated using TRIzol reagent (TransGen Biotech, Beijing, China) and be quantitated using NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific™). The reverse transcription of 2 µg of total RNA into first-strand complementary DNA

(cDNA) was performed using the PrimeScript™ RT Master Mix (Takara Biotechnology, Beijing, China). RT-qPCR assay was performed using the SYBR Green qPCR Master Mix (MedChemExpress) in the Applied Biosystems 7500 Real-Time PCR system (ABI, USA) under the following reaction conditions: a denaturation step for 5 min at 94 °C, 38 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C. The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control, and the data were analyzed using the $2^{-\Delta\Delta C_t}$ method. The primers for qPCR were as follows: forward primer for METTL3: 5'-AGAGTGTCGGAGGTGATT-3' and reverse primer for METTL3: 5'-TAGTACGGGTATGTTGAGC-3'; forward primer for AKT1: 5'-TCTTTGCCGGTATCGTGT-3' and reverse primer for AKT1: 5'-TGTCATCTTGGTCAGGTGGT-3'; and forward primer for GAPDH: 5'-CTCCTCCTGTTTCGACAGTCAGC-3' and reverse primer for GAPDH: 5'-CCCAATACGACCAAATCCGTT-3'.

m⁶A methylated RIP-qPCR

The methylated RNA immunoprecipitation–quantitative polymerase chain reaction (RIP-qPCR) analyses were performed as previously described (44). Briefly, the extracted RNA was fragmented by RNA fragmentation reagents (Thermo Scientific™) and 2 µg of RNA was mixed with protein A beads (Thermo Fisher Scientific, Waltham, MA) and an anti-m⁶A antibody (Abcam, ab151230) in an immunoprecipitation buffer for m⁶A-immunoprecipitation. Later, the beads were eluted with elution buffer containing 20 mg/mL of proteinase K (10401ES60, Yeasen, Shanghai, China) twice. After elution and recovery, RNA enrichment was analyzed by qPCR.

Western blot

Total protein from tissues were isolated using the cell-lysis buffer for the Western blot containing 1 mM of phenylmethanesulfonyl fluoride (Catalog number: 36978, Thermo Scientific™), and was quantified using a Bicinchoninic Acid Assay (BCA) Protein Assay Kit (P0012S, Beyotime Biotechnology, Shanghai, China). Western blot was performed as previously described (45). Primary antibodies, including METTL3 antibody (ab195352, dilution 1:1,000), AKT1 antibody (ab233755, dilution 1:1,000), and GAPDH antibody (ab9485, Dilution 1:1,000), were purchased from Abcam. Second antibodies, including

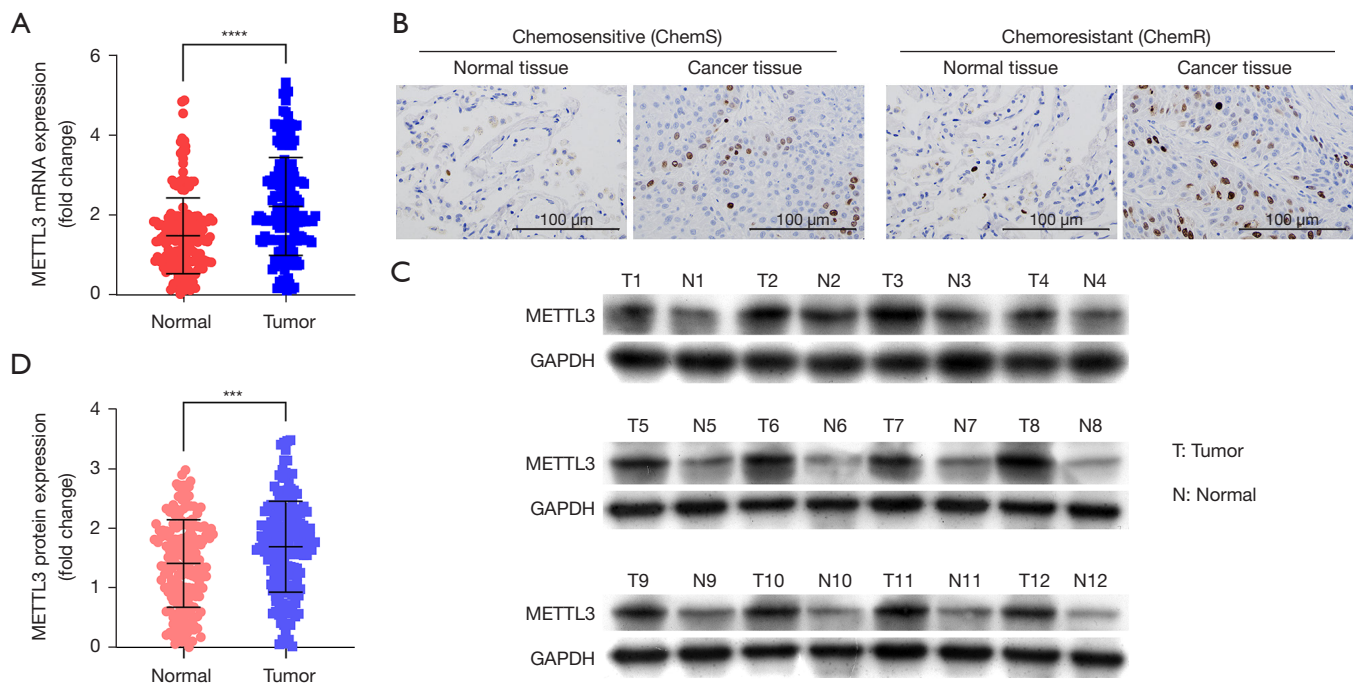


Figure 1 METTL3 is upregulated in NSCLC tissues. (A) The RNA levels of METTL3 were analyzed by qPCR assay; (B) IHC staining images of METTL3 from NSCLC tissues and adjacent normal tissues; (C,D) Western blot analysis of METTL3 protein expression. *** $P < 0.001$, **** $P < 0.0001$. METTL3, methyltransferase-like 3; NSCLC, non-small cell lung cancer.

Horseradish Peroxidase (HRP) goat anti-mouse (or anti-rabbit) IgG (BA1051 and BA1054), were purchased from Boster Biological Technology (Wuhan, China).

Immunohistochemical assay

Immunohistochemical (IHC) assays were performed to analyze the expression of METTL3 and AKT1 as previously described (45). The tissues were cut into 5- μm thick sections and deparaffinized with xylene. METTL3 (1:500 dilution; ab195352) and AKT1 (1:500 dilution; ab233755) antibodies were used in the study.

Statistical analysis

The data are showed as mean \pm standard deviation, and were statistically analyzed using SPSS (standard version 20.0; SPSS, Chicago, IL) software. The χ^2 -test was used to analyze the correlations between METTL3 or AKT1 expression and the clinicopathologic features of NSCLC patients. The correlation between METTL3 expression levels and AKT1 expression levels or m⁶A levels of AKT1 mRNA in NSCLC tissues was determined by Pearson

correlation coefficients. A univariate survival analysis was conducted using the Kaplan-Meier method, and the log-rank test was used to evaluate differences between the survival curves. The independent sample T test was used for statistical analyses between 2 groups. A P value > 0.05 was considered statistically significant.

Results

METTL3 expression is correlated with chemotherapy resistance and poor prognosis in NSCLC patients

To investigate whether METTL3 expression was related to the progression of NSCLC and chemosensitivity in patients, we evaluated the expression levels of METTL3 in 180 pairs of NSCLC tissues. The qPCR results showed that the expression levels of METTL3 mRNA were dramatically increased in cancer tissues ($n=180$; $P < 0.0001$; see *Figure 1A*). Additionally, the IHC and Western blot results revealed that METTL3 protein expression levels in cancer tissues were increased ($P < 0.001$; see *Figure 1B-1D*). Further, the expression of METTL3 in chemoresistant NSCLC tissues and chemosensitive tissues were quantified,

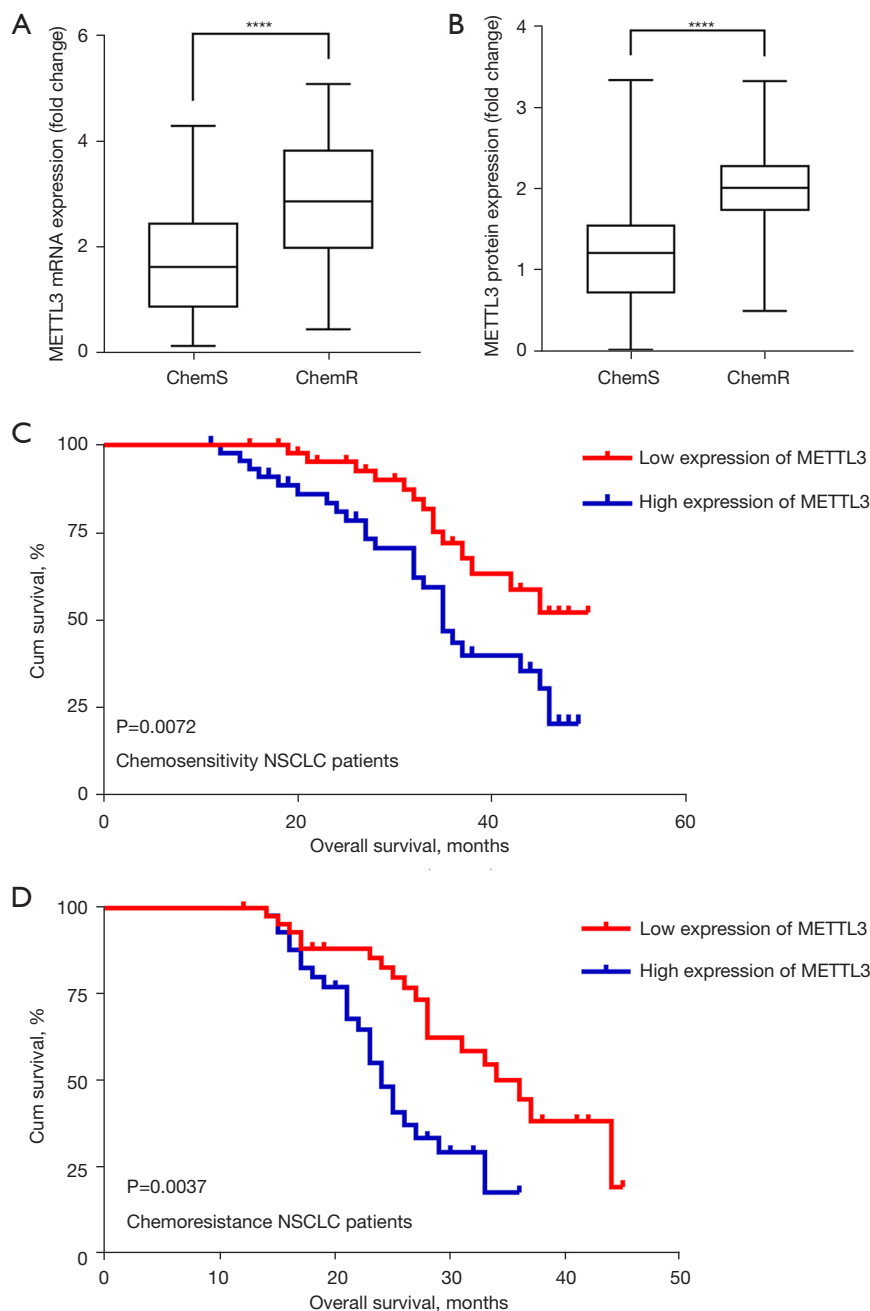


Figure 2 METTL3 is correlated with poor OS in NSCLC patients. (A,B) Quantification of METTL3 mRNA and protein expression in chemoresistant and chemosensitive NSCLC tissues; (C,D) the relationship between METTL3 expression and OS of NSCLC patients was analyzed using Kaplan-Meier survival curves. **** $P < 0.0001$. METTL3, methyltransferase-like 3; OS, overall survival; NSCLC, non-small cell lung cancer.

and the mRNA and protein expression levels of METTL3 were higher in the chemoresistant NSCLC tissues ($n=90$) than the chemosensitive NSCLC tissues ($n=90$; $P < 0.0001$; see *Figure 2A,2B*).

Additionally, the association between METTL3 protein expression and the clinicopathological features of NSCLC patients was also explored. To assess the statistical significance, NSCLC patients were divided into 2 groups

Table 1 Correlation between the protein expression of METTL3 and clinicopathological features in 180 cases of NSCLC

Factors	METTL3 protein expression (n=180)		P value
	Low expression (n=90)	High expression (n=90)	
Age			0.5509
<60 years	46	42	
≥60 years	44	48	
Gender			0.7656
Male	46	44	
Female	44	46	
TNM stage			<0.0001
I-II	64	25	
III-IV	26	65	
DDP			<0.0001
Sensitive	78	12	
Resistant	12	78	
Lymph node metastasis			<0.0001
Yes	18	70	
No	72	20	
Smoking status			0.8815
Smoker	46	45	
Non-smoker	44	45	

METTL3, methyltransferase-like 3; NSCLC, non-small cell lung cancer; DDP, cisplatin.

(high and low groups) according to the median (cut-off =1.729) of METTL3 protein expression. Our results showed that METTL3 protein expression was significantly associated with a number of clinicopathological features, including DDP susceptibility ($P<0.0001$), tumor, node, metastasis (TNM) stage ($P<0.0001$), and lymph node metastasis ($P<0.0001$; see *Table 1*). Additionally, the Kaplan-Meier analysis showed that the high protein expression of METTL3 was correlated with shorter OS, especially in chemoresistant NSCLC patients (see *Figure 2C,2D*). Thus, METTL3 is a potential biomarker that can predict chemotherapy resistance and the outcomes of NSCLC patients.

AKT1 expression is correlated with chemotherapy resistance and a poor prognosis in NSCLC patients

A previous study indicated that m⁶A mRNA methylation regulates AKT1 activity, and promotes the tumorigenicity

of endometrial cancer (46). However, it is not yet known whether AKT1 is involved in the progression of NSCLC and chemoresistance. We evaluated the expression levels of AKT1 in 180 pairs of NSCLC tissues. The qPCR results showed that there was no statistically significant difference between NSCLC tissues and tumor-adjacent normal tissues in terms of AKT1 mRNA expression (see *Figure 3A*). Notably, the IHC and Western blot results revealed increased AKT1 protein expression in NSCLC tissues ($P<0.0001$; see *Figure 3B-3D*). Further, the expression of AKT1 in chemoresistant NSCLC tissues and chemosensitive tissues were quantified, and the protein expression levels of AKT1 were higher in the chemoresistant NSCLC tissues (n=90) than the chemosensitive NSCLC tissues (n=90), but no significant difference in mRNA levels was found (see *Figure 4A,4B*).

Additionally, the association between AKT1 protein expression and the clinicopathological features of NSCLC patients was also explored. To assess the statistical

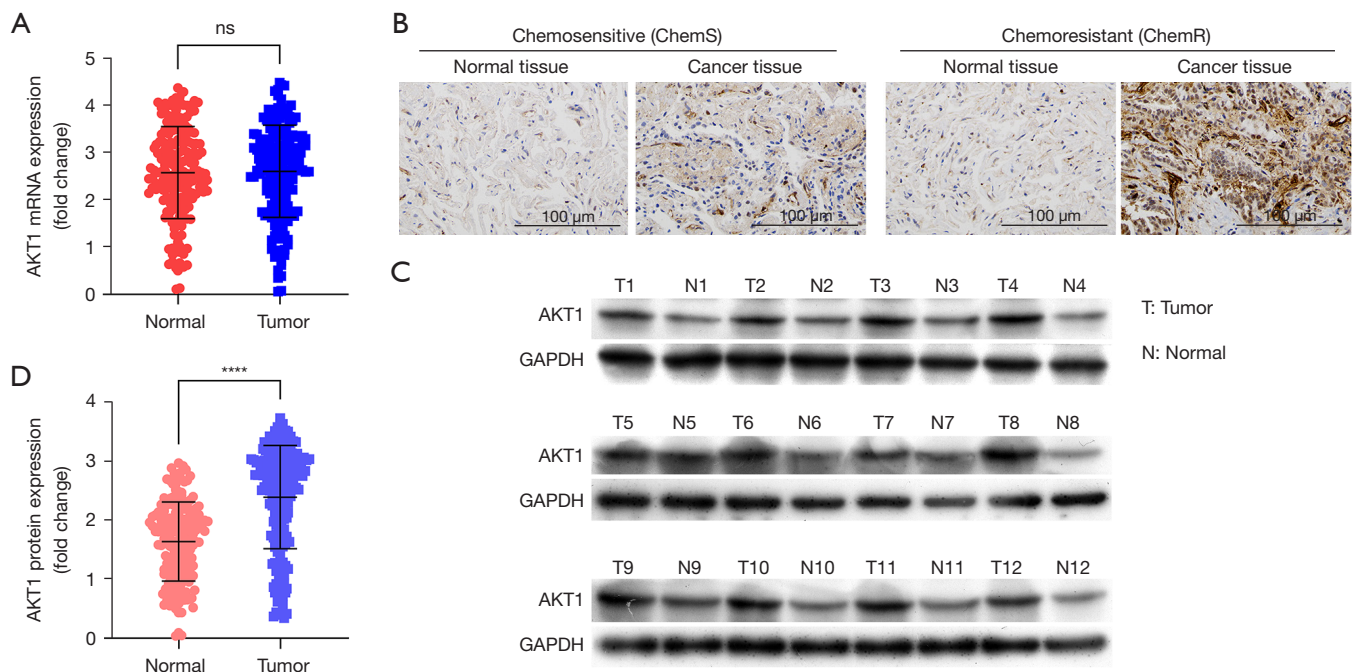


Figure 3 AKT1 is upregulated in NSCLC tissues. (A) The RNA level of AKT1 was analyzed by qPCR assays; (B) IHC staining images of AKT1 from NSCLC tissues and adjacent normal tissues; (C,D) Western blot analysis of AKT1 protein expression. **** $P < 0.0001$. AKT1, AKT serine/threonine kinase 1; NSCLC, non-small cell lung cancer.

significance, the NSCLC patients were divided into high and low AKT1 protein expression groups according to the median (cut-off = 2.642) of AKT1 protein expression. As Table 2 shows, AKT1 protein expression was significantly associated with clinicopathological features, including DDP susceptibility ($P < 0.0001$), TNM stage ($P = 0.0006$), and lymph node metastasis ($P < 0.0001$). Additionally, the high protein expression of AKT1 was significantly correlated with poor survival, especially in chemoresistant NSCLC patients (see Figure 4C,4D).

Correlation between METTL3 protein expression and AKT1 expression in NSCLC

Based on our findings, we examined whether METTL3 regulated AKT1 expression through m^6A . First, we analyzed the correlation between METTL3 and AKT1 expression levels using Pearson correlation coefficients, and found that the mRNA expression of AKT1 was not correlated with METTL3 protein expression ($R = 0.05453$, $P = 0.4672$; see Figure 5A), but the protein expression of AKT1 was positively correlated with METTL3 protein expression ($R = 0.6647$, $P < 0.0001$; see Figure 5B). Further, we evaluated

the m^6A levels of AKT1 in 180 NSCLC tissues using the MeRIP-qPCR method, and found the m^6A levels of AKT1 were significantly upregulated in NSCLC tissues ($P < 0.01$; see Figure 5C). Moreover, the m^6A levels of AKT1 were positively correlated with METTL3 protein expression ($R = 0.5814$, $P < 0.0001$; see Figure 5D), and were significantly positively correlated with the m^6A levels of AKT1 ($R = 0.7774$, $P < 0.0001$; see Figure 5E). All above, our results indicated that METTL3 contributes to the progression and chemoresistance of NSCLC by promoting AKT1 protein expression via the regulation of AKT1 mRNA m^6A levels.

Discussion

In this study, we examined the prognostic effect of METTL3 expression levels in 180 NSCLC patients. METTL3 plays a critical role in m^6A methylation and regulates the total m^6A methylation level, which contributes to human diseases (32-35). This study showed that METTL3 was overexpressed in NSCLC cancer tissues, and its expression levels in chemoresistant NSCLC patients were significantly higher than those in chemosensitive NSCLC patients, and higher METTL3 expression levels

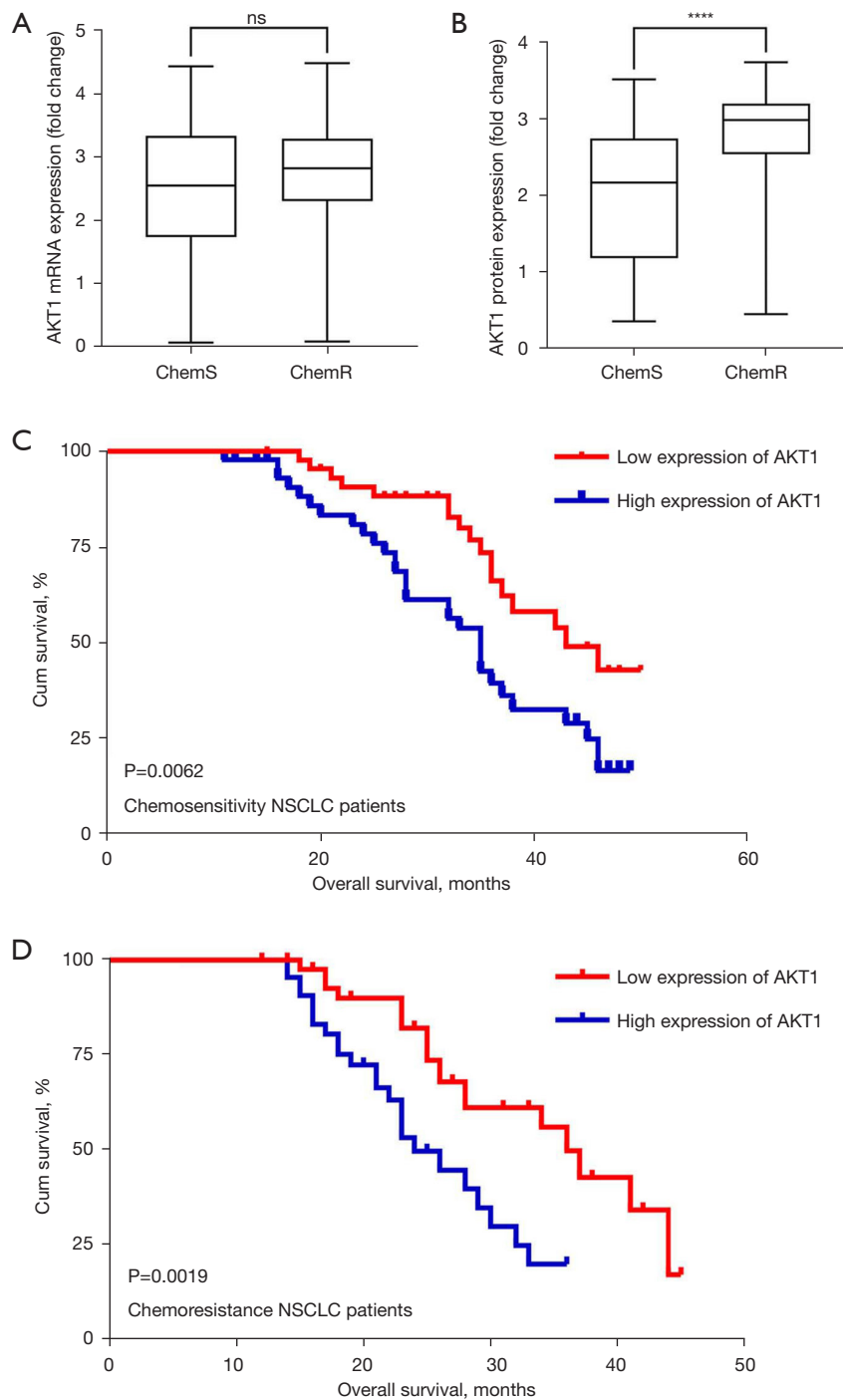


Figure 4 AKT1 protein is correlated with the poor OS of NSCLC patients. (A,B) AKT1 mRNA and protein were quantified in chemoresistant and chemosensitive NSCLC tissues; (C,D) the relationship between AKT1 expression and the OS of NSCLC patients was analyzed using Kaplan-Meier survival curves. **** $P < 0.0001$. AKT1, AKT serine/threonine kinase 1; OS, overall survival; NSCLC, non-small cell lung cancer.

Table 2 Correlation between protein expression of AKT1 and clinicopathological features in 180 cases of NSCLC

Factors	AKT1 protein expression (n=180)		P value
	Low expression (n=90)	High expression (n=90)	
Age			0.1797
<60 years	49	40	
≥60 years	41	50	
Gender			>0.9999
Male	46	46	
Female	44	44	
TNM stage			0.0006
I-II	56	33	
III-IV	34	57	
DDP			<0.0001
Sensitive	64	26	
Resistant	26	64	
Lymph node metastasis			<0.0001
Yes	29	59	
No	61	31	
Smoking status			0.2967
Smoker	49	42	
Non-smoker	41	48	

AKT1, AKT serine/threonine kinase 1; NSCLC, non-small cell lung cancer; DDP, cisplatin.

were significantly correlated with a poor prognosis. The results suggest that METTL3 is a potential biomarker for identifying NSCLC patients who will experience unfavorable clinical outcomes and develop chemoresistance. Additionally, METTL3 expression levels were correlated with AKT1 protein expression.

The relationship between METTL3 expression levels and prognosis has been examined in different types of human cancer (47-50). METTL3 was shown to be downregulated in glioma tissues, and the downregulation of METTL3 was shown to stimulate the malignant development of glioma (51). METTL3 expression was also found to be significantly increased in clear cell renal cell cancer (ccRCC), and higher METTL3 expression predicted shorter OS in ccRCC patients (49). Further, METTL3-mediated autophagy was shown to revise gefitinib resistance in NSCLC cells (52). In this study, we demonstrated that the expression of METTL3 is correlated with a poor prognosis and chemoresistance in NSCLC patients.

METTL3 plays a critical role in regulation of m⁶A methylation, which directly affects the decay and translation of mRNA and microRNA biogenesis. A previous study indicated that m⁶A mRNA methylation regulates AKT1 activity, which promotes the tumorigenicity of endometrial cancer (46). Protein kinase B (AKT) is a pleckstrin homology (PH) domain comprising a serine threonine kinase (53). AKT1 is an important isoform of AKT, which acts as a vital subtype and is closely related to many types of human tumors (54-56). Wang *et al.* found that the overexpression of MNAT1 component of CDK activating kinase (MAT1) mediates the upregulation of AKT1 expression, which promotes the lung metastasis of osteosarcoma (57). However, the chemokine receptor C-X-C motif chemokine receptor 2 (CXCR2) moderates AKT1 results by suppressing breast cancer metastasis and chemoresistance (58). In the progression of bone marrow mesenchymal stem cell (BMSC) adipogenesis, overexpression or knockdown of METTL3 decreased

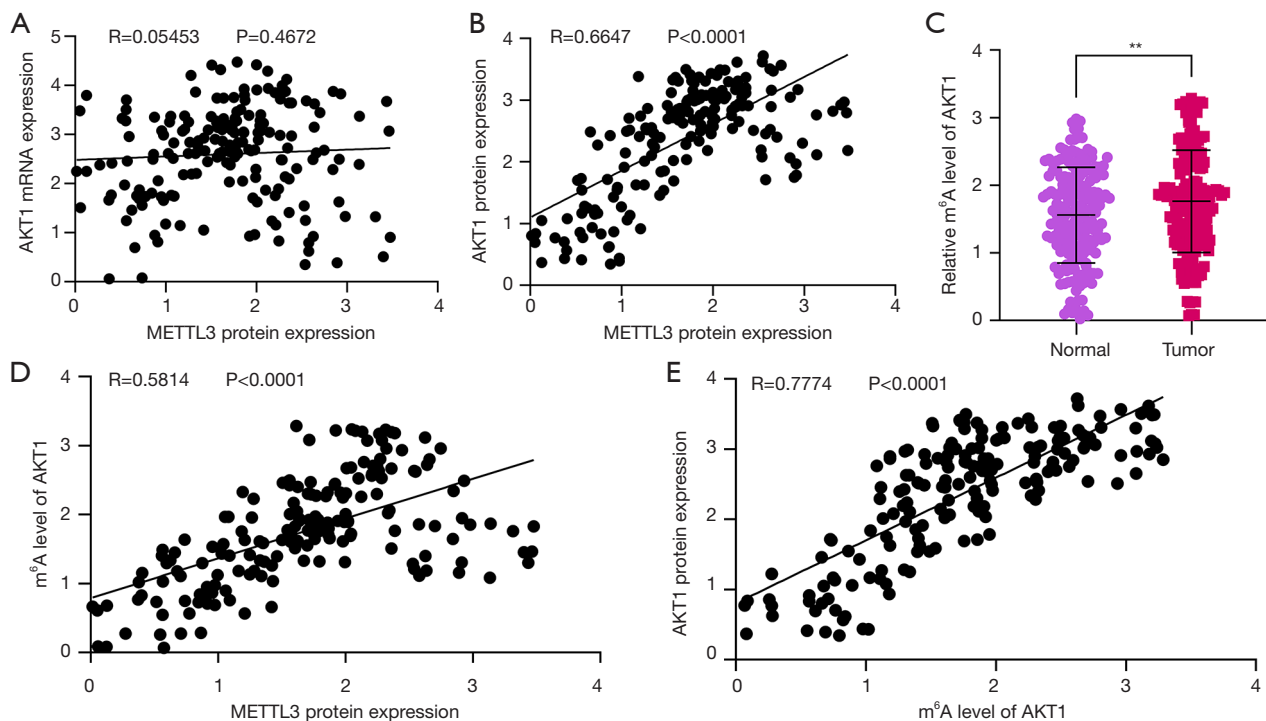


Figure 5 Correlation between METTL3 protein expression and AKT1 expression in NSCLC. (A) The relationship between the METTL3 protein and AKT1 mRNA in NSCLC tissues was analyzed; (B) the relationship between the METTL3 protein and AKT1 protein in NSCLC tissues was analyzed; (C) the m⁶A levels of AKT1 mRNA were analyzed by MeRIP-qPCR assays; (D) the relationship between the METTL3 protein and m⁶A levels of AKT1 mRNA in NSCLC tissues was analyzed; (E) the relationship between the AKT1 protein and m⁶A levels of AKT1 mRNA in NSCLC tissues was analyzed. **P<0.01. METTL3, methyltransferase-like 3; AKT1, AKT serine/threonine kinase 1; NSCLC, non-small cell lung cancer.

or increased AKT1 expression at the mRNA level, respectively. The results showed that METTL3 could negatively regulate AKT protein expression in MSCs by mediating the m⁶A modification of *AKT1-mRNA* (59). In the current study, AKT1 protein (not RNA expression) was increased in NSCLC tissues and positively related with METTL3 expression, and more highly expressed in chemoresistant NSCLC tissues than chemosensitive tissues. To evaluate the potential difference in METTL3 mediates AKT1 expression, we predicted m⁶A sites in *AKT1-mRNA* (NM_005163.2) using SRAMP (<http://www.cuilab.cn/sramp/>). The results revealed 10 m⁶A sites (see Figure S1) of which three high confidence sites in 5'UTR, four sites (one high confidence site, one moderate confidence site and two low confidence sites) in CDS region, and three sites (two moderate confidence sites and one low confidence site) in 3'UTR of *AKT1-mRNA*. Further investigation is needed to determine which of the predicted sites are functional to measure the difference in METTL3 mediates AKT1

expression.

m⁶A is one of the most common RNA modifications. Gene expression can be regulated by the quite prevalent and dynamic regulation of m⁶A modifications (31,60). Recently, studies have demonstrated that m⁶A is a potential biomarker for predicting the progression of cancer (61,62). We found that the m⁶A levels of AKT1 were significantly upregulated in the NSCLC tissues. Additionally, the m⁶A levels of AKT1 were positively correlated with METTL3 protein expression, and the expression of AKT1 protein was positively correlated with the m⁶A levels of AKT1. Our results indicate that METTL3 may promote AKT1 protein expression by regulating AKT1 mRNA m⁶A levels.

Accumulating evidence suggests that m⁶A RNA methylation greatly impacts RNA metabolism and is involved in the pathogenesis of cancers (63). The rapidly evolving research on m⁶A modifications can help reveal the biological mechanisms underlying cancer development and may provide new targets for cancer treatment. Although

fully understand the underlying mechanism of m⁶A modification is still far off, the future research on m⁶A modifications will be focused on regulatory network of m⁶A in a single cancer and screening factors for early diagnosis and prognosis in large number of clinical specimens, and development of potential m⁶A-related targets for cancer treatment.

Conclusions

In summary, our results indicate that METTL3 contributes to the progression and chemoresistance of NSCLC by activating AKT1 protein via the regulation of m⁶A levels of AKT1 mRNA. METTL3 is overexpressed in chemoresistant NSCLC tissues; thus, the METTL3-AKT1 axis may provide an efficient therapeutic intervention target for overcoming chemoresistance in NSCLC.

Highlights

- (I) METTL3 expression is elevated in NSCLC tissues.
- (II) METTL3 expression is higher in chemoresistant than chemosensitive NSCLC tissues.
- (III) METTL3 promotes AKT1 protein expression by regulating AKT1 mRNA m⁶A levels.
- (IV) METTL3 is correlated with NSCLC chemoresistance and OS.

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Footnote

Reporting Checklist: The authors have completed the MDAR checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6608/rc>

Data Sharing Statement: Available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6608/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6608/coif>).

The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Written informed consent was obtained from all the patients before the study. This study was approved by the Ethics Committee of Sun Yat-sen University Cancer Center (No. GZR2020-297) and performed in accordance with the Declaration of Helsinki (as revised in 2013).

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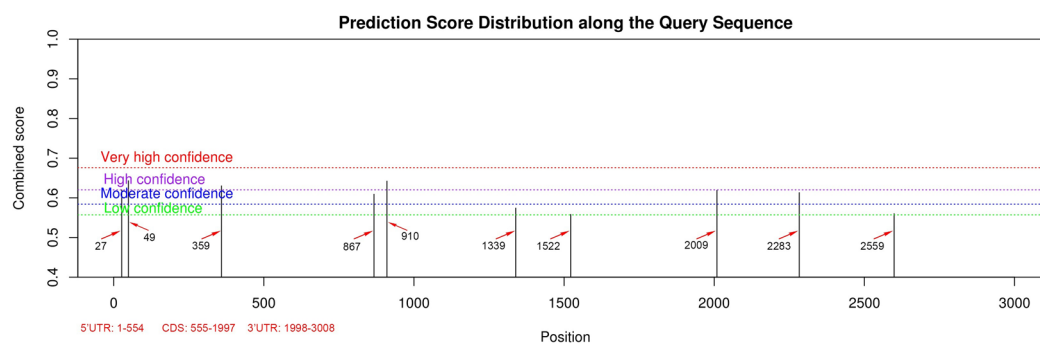


Figure S1 Potential m⁶A sites in full-length AKT gene (NM_005163.2) predicted using SRAMP (<http://www.cuilab.cn/sramp/>). Arrows and numbers: base positions corresponding to the *AKT1-mRNA*.