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ORIGINAL ARTICLE

METTL3-Driven Maturation of miR-103 Promotes the Progression of Non-Small Cell Lung Cancer

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SUMMARY

Background: **This study aimed to elucidate the molecular mechanism underlying the m6A modification of miR-103 and its role in promoting proliferation and epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) resistance in non-small cell lung cancer (NSCLC).**
Methods: **The expression levels of miR-103 in EGFR-TKI-resistant versus sensitive tissues were analyzed using data from the GEO database, and its potential regulatory pathways were predicted. The effects of miR-103 overexpression or inhibition on NSCLC cell proliferation and drug resistance were evaluated using CCK-8 assays, Transwell migration assays, colony formation assays, and IC50 assays. The influence of miR-103 on the PI3K/AKT/mTOR signaling axis was investigated through Western blotting, rescue experiments, and dual-luciferase reporter assays. Additionally, the N6-methyladenosine (m6A) modification mechanism of miR-103 was confirmed via methylated RNA immunoprecipitation (MeRIP), RNA pull-down, and RNA immunoprecipitation (RIP) assays.**
Results: **Bioinformatics analyses demonstrated that miR-103 is significantly upregulated in EGFR-TKI-resistant tissues ($p < 0.001$) and modulates the PI3K/AKT/mTOR pathway. Compared with parental A549 cells, EGFR-TKI-resistant A549-R cells exhibited markedly elevated miR-103 expression levels ($p < 0.001$). Overexpression of miR-103 significantly promoted cell proliferation, colony formation, and invasion, while reducing sensitivity to osimertinib ($p < 0.001$). In contrast, inhibition of miR-103 yielded the opposite effects ($p < 0.001$). Mechanistically, miR-103 may activate the PI3K/AKT/mTOR pathway by inhibiting PTEN expression, thus promoting NSCLC proliferation and resistance. Moreover, METTL3 enhances the stability and expression of miR-103 via catalyzing its m6A modification. Targeting METTL3 inhibits NSCLC proliferation and drug resistance by downregulating miR-103 and blocking the PI3K/AKT/mTOR axis.**
Conclusions: **METTL3-mediated m6A modification of miR-103 facilitates NSCLC progression and EGFR-TKI resistance through activation of the PI3K/AKT/mTOR signaling pathway. Targeting METTL3 and miR-103 represents a promising therapeutic strategy for NSCLC treatment.**
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KEYWORDS

non-small cell lung cancer, m6A modification, miR-103, PI3K/AKT/mTOR signaling axis, EGFR-TKI

INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide and is among the most frequently diagnosed malignancies [1]. Non-small cell lung cancer (NSCLC) accounts for over 80% of all lung cancer cases [2]. Current treatment strategies for NSCLC primarily involve surgery combined with radiotherapy and chemotherapy, tailored according to tumor stage and disease progression [3]. Despite advances in treatment, the 5-year survival rate for postoperative NSCLC patients remains approximately 20 - 30%, and patients with advanced-stage disease have a median survival of only 18 months following diagnosis [4].

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are one of the primary treatments for advanced NSCLC; however, the development of resistance significantly contributes to disease progression and poor prognosis. Therefore, elucidating the mechanisms underlying EGFR-TKI resistance and identifying potential therapeutic targets are critical.

MicroRNAs (miRNAs) are non-coding, single-stranded small RNAs that play essential roles in tumor development and progression. miR-103 has been reported to be markedly upregulated in several malignancies, including tongue squamous cell carcinoma [5], ovarian [6], and breast cancers [7]. Our bioinformatics analyses revealed that miR-103 is significantly overexpressed in EGFR-TKI-resistant NSCLC tissues and modulates the PI3K/AKT/mTOR signaling pathway. The PI3K/AKT/mTOR axis is a key regulator of tumor cell growth and survival [8] and has been implicated in NSCLC progression and drug resistance [9]. The maturation process of miRNAs directly influences their intracellular levels, and m6A modification has emerged as a critical regulatory mechanism.

To date, the regulatory mechanisms governing miR-103 maturation have not been well elucidated. Growing evidence suggests that m6A modification plays a crucial role in RNA translation and maturation. Methyltransferase-like protein 3 (METTL3), an RNA methyltransferase, directly mediates m6A modification [10] and has a pivotal role in miRNA maturation [11, 12]. In this study, we propose and demonstrate that METTL3-mediated m6A modification promotes the maturation of miR-103, which subsequently facilitates NSCLC progression and resistance to EGFR-TKIs through activation of the PI3K/AKT/mTOR signaling pathway. These findings provide novel insights into NSCLC pathogenesis and suggest potential therapeutic targets for overcoming EGFR-TKI resistance in clinical settings.

MATERIALS AND METHODS

Cell culture

The NSCLC cell line A549 was used in this study. Cells were maintained in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and cultured at 37°C in a humidified incubator with 5% CO_2. To establish the EGFR-TKI-resistant cell line (A549-R), parental A549 cells were gradually exposed to increasing concentrations of osimertinib, starting at 25 nM, with medium refreshed every 4 - 5 days. If no significant cell death occurred, the concentration was further increased. This process was continued until the IC50 value was approximately 20-fold higher than that of parental cells, with a final concentration of 10 μM in A549-R cells.

Bioinformatics analysis

Gene expression data (GSE101586; 8 samples total) from EGFR-TKI-resistant and -sensitive NSCLC tissues were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Differential expression analysis was performed using the Limma package in R to identify significantly upregulated miRNAs ($|\log2FC| > 1$, $p < 0.05$). Volcano and swarm plots were generated using the ggplot2 package to visualize miR-103 expression differences. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using the DAVID database to explore the biological functions and potential signaling pathways of miR-103. Enrichment results were visualized in R using ggplot2, with an adjusted p-value < 0.05 considered statistically significant.

miR-103 overexpression and knockdown

A549-R cells were divided into six groups: miR-103 mimic, miR-103 inhibitor, mimic negative control (NC), inhibitor NC, miR-103 mimic + LY294002, and miR-103 inhibitor + SC79. Cells at approximately 80% confluence were transfected using Lipofectamine 3000 (Invitrogen, USA) with miR-103 mimics, inhibitors, or their corresponding controls (RiboBio, Guangzhou, China) diluted in Opti-MEM. Cells were collected 48 hours post-transfection for subsequent experiments. In the miR-103 mimic + LY294002 group, LY294002 (10 μM) was added 24 hours after transfection; in the miR-103 inhibitor + SC79 group, SC79 (10 μM) was added 24 hours after transfection.

METTL3 overexpression and knockdown

The full-length METTL3 sequence was cloned into the pcDNA3.1 vector (GenePharma, China) to generate the

METTL3 overexpression plasmid. sh-METTL3 was designed to silence METTL3 expression using Gene Pharma Designer 3.0 software to select optimal target sequences. Recombinant psi-LVRU6MP-METTL3-shRNA plasmids and lentiviral particles were constructed by a biotechnology company. A549-R cells were assigned to five groups: OE-METTL3, sh-METTL3, sh-NC, OE-METTL3 + LY294002, and OE-METTL3 + miR-103 inhibitor. Transfection was performed using Lipofectamine 3000 as described in Section "miR-103 overexpression and knockdown". For OE-METTL3 + LY294002, LY294002 (10 μM) was added 24 hours after transfection; for OE-METTL3 + miR-103 inhibitor, SC79 (10 μM) was added 24 hours after transfection.

Luciferase reporter assay

The full-length PTEN sequence potentially binding to miR-103 was amplified by PCR, and a mutant sequence was designed. These fragments were cloned into the PGL3 vector to generate PTEN wild-type (PTEN-WT) and mutant (PTEN-MUT) reporter plasmids. Cells were seeded in 24-well plates at approximately 80% confluence and co-transfected with the reporter plasmids and either miR-103 mimics or mimic NC using Lipofectamine 3000. After 48 hours, cells were harvested and lysed. Firefly and Renilla luciferase activities were sequentially measured using a dual-luciferase reporter assay system. The ratio of firefly to Renilla luciferase activity was calculated to evaluate the direct binding between miR-103 and PTEN.

CCK-8 assay

Cells were seeded in 96-well plates at a density of 3×10^3 cells per well. After attachment, cells were incubated at 37°C with 5% CO_2 for 0, 6, 24, 48, 72, and 96 hours. Subsequently, 100 μL of complete medium and 10 μL of CCK-8 reagent (Sigma-Aldrich, St. Louis, MO, USA) were added to each well and incubated for 2 hours in the dark. Absorbance at 450 nm was measured using a microplate reader. All measurements were performed in triplicate.

Transwell assay

Cells (2×10^3 per well) were seeded into Transwell chambers with serum-free medium, and the lower chamber was filled with medium containing 10% FBS. After 3 days, chambers were removed, and non-migrated cells were wiped off. Migrated cells on the lower membrane surface were fixed and stained with crystal violet for 20 minutes. Images were captured using the Bio-Rad ChemiRS system, and cell counts were analyzed using ImageJ software (version 1.8.0) in five randomly selected fields.

Colony formation assay

Cells were seeded at 300 cells per well in 6-well plates, with three replicates per group. After 14 days, colonies were fixed with PBS, stained with crystal violet for 20 minutes, and imaged using the Bio-Rad ChemiRS system. Colonies containing ≥ 50 cells were counted using ImageJ software (version 1.8.0).

RT-qPCR

Total RNA was extracted using an RNA isolation kit and dissolved in DEPC-treated water. RNA integrity was assessed by agarose gel electrophoresis, and concentrations were measured using a UV spectrophotometer. Reverse transcription was performed using 2 μg of RNA and random primers, followed by incubation at 37°C for 90 minutes and inactivation at 70°C for 10 minutes. qPCR was performed under standard conditions, and relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Western blot

Total protein was extracted, quantified, and separated by 10% SDS-PAGE. Proteins were transferred to PVDF membranes, blocked with 5% skim milk overnight, and incubated with primary antibodies at 4°C for 24 hours. After incubation with HRP-conjugated secondary antibodies at 37°C for 1.5 hours, signals were visualized using an ECL detection system and quantified using ImageJ software. The following primary antibodies were obtained from Cell Signaling Technology: anti-p-PI3K (#17,366), anti-PI3K (#4,292), anti-p-AKT (#4,060), anti-AKT (#4,691), anti-p-mTOR (#5,536), anti-mTOR (#2,983), and anti-PTEN (#9,552).

MeRIP assay

METTL3 mutants (K144A) were generated by site-directed mutagenesis and cloned into the pCDNA3.1 vector. Cells were transfected with either wild-type or mutant METTL3 plasmids using Lipofectamine 3000. The SRAMP database was used to predict m6A modification sites on miR-103. Total RNA was extracted, enriched using the PolyATtract® mRNA Isolation System, fragmented, and immunoprecipitated with the Magna MeRIP m6A kit [13]. miR-103 m6A modification levels were quantified by RT-qPCR.

RNA pull-down assay

Biotin-labeled miR-103 and its antisense RNA were synthesized and incubated with cell lysates using the BersinBio™ RNA pull-down kit (Thermo Fisher Scientific, USA). The enriched RNA-protein complexes were analyzed by Western blotting, with random probes serving as negative controls.

RIP assay

RIP assays were conducted using the EZ-Magna RIP kit (Millipore, USA) with an anti-METTL3 antibody (Abcam). After immunoprecipitation, RNA was purified and analyzed by RT-qPCR. Enrichment was calculated relative to the IgG control group.

RNA stability assay

A549-R cells transfected with sh-METTL3 or sh-NC were treated with 5 μg/mL actinomycin D and collected

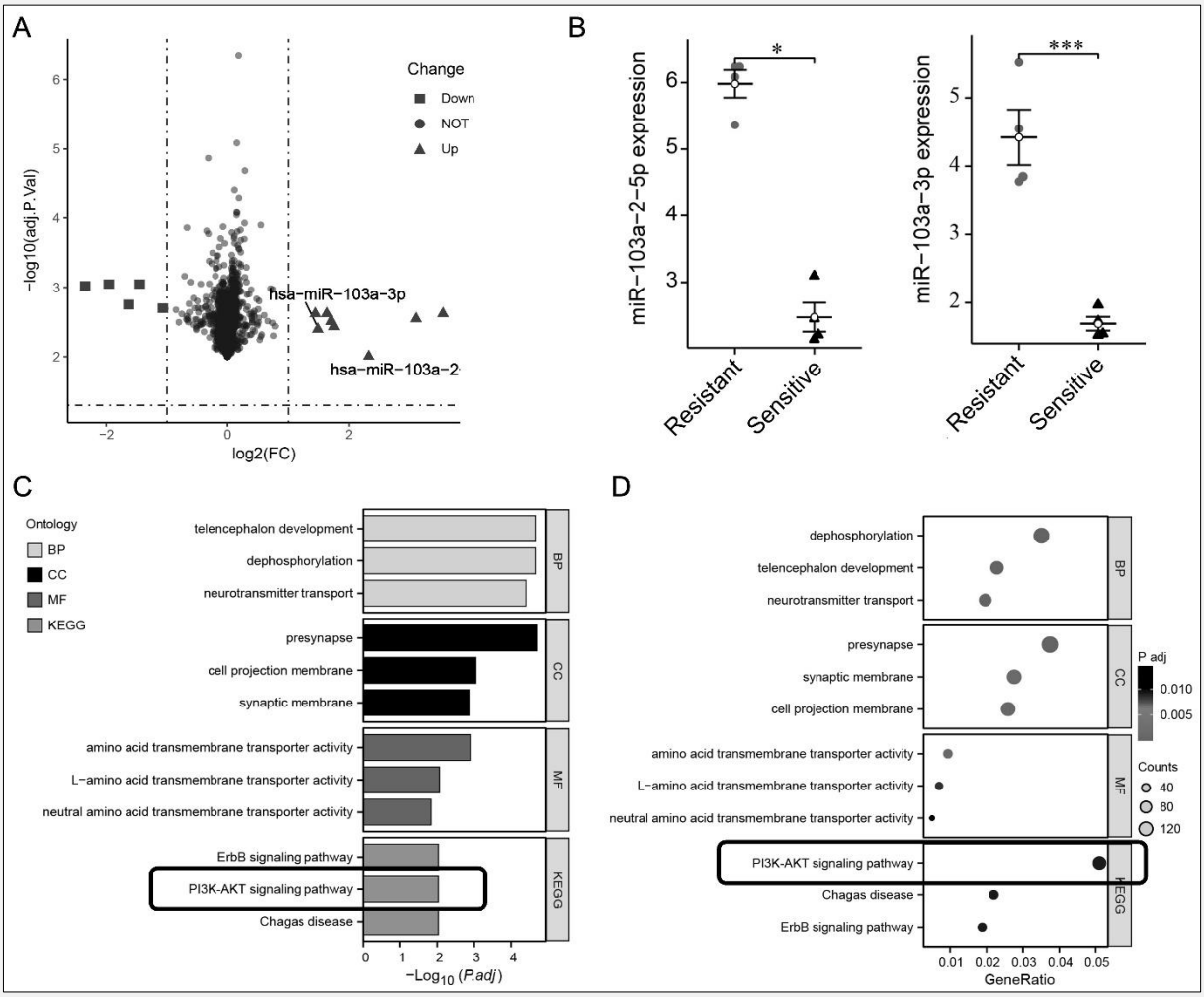

Figure 1. miR-103 expression in EGFR-TKI-resistant NSCLC tissues.

A) Volcano plot illustrating differentially expressed miRNAs between EGFR-TKI-resistant (n = 4) and sensitive (n = 4) NSCLC tissues from the GSE99143 dataset. B) Swarm plot showing miR-103 expression differences between EGFR-TKI-resistant (n = 4) and sensitive (n = 4) NSCLC tissues from the GSE99143 dataset. C, D) Enrichment analysis of differentially expressed genes between high- and low-miR-103 expression groups in the GSE99143 dataset. BP refers to biological process, CC refers to cellular component, MF refers to molecular function. * p < 0.01, ** p < 0.001, *** p < 0.0001.

at 0, 30, 60, 120, 180, and 240 minutes. Total RNA was extracted, and miR-103 expression levels were measured by qRT-PCR.

Statistical analysis

All experimental data were statistically analyzed using SPSS software version 21.0 (IBM, Armonk, NY, USA). Quantitative results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed for comparisons among multiple groups, followed by Tukey's post hoc test. Repeated-measures ANOVA was used to analyze group differences across multiple time points.

RESULTS

miR-103 expression is closely associated with EGFR-TKI resistance in NSCLC

To preliminarily investigate the role of miR-103 in NSCLC progression, we analyzed differentially expressed miRNAs between EGFR-TKI-resistant and -sensitive NSCLC tissues from the GEO database using the Limma package. As expected, miR-103 was significantly upregulated in EGFR-TKI-resistant NSCLC tissues compared to sensitive tissues (Figures 1A and 1B), suggesting its potential involvement in NSCLC progression and resistance.

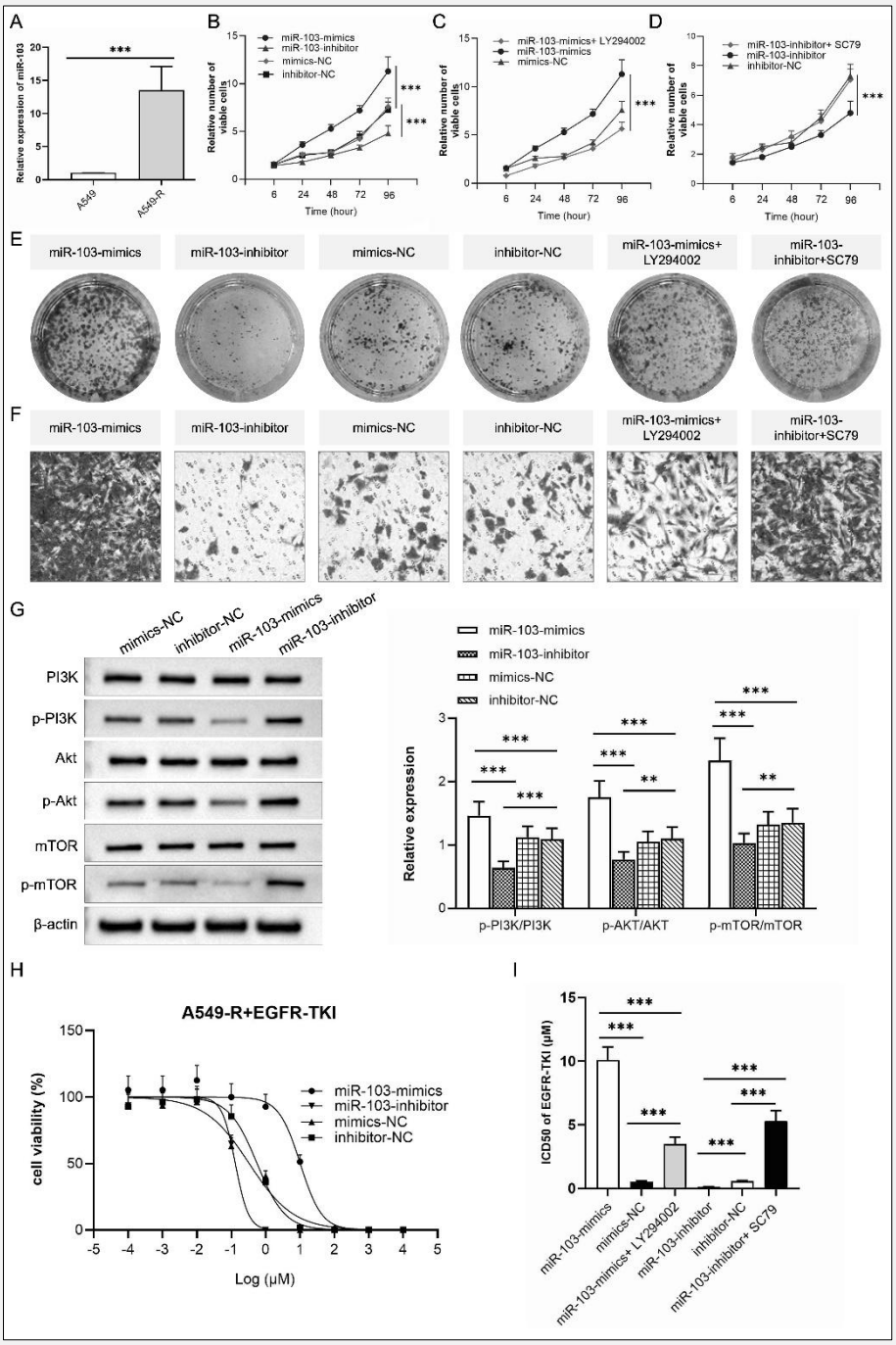

Figure 2. Effects of miR-103 on NSCLC proliferation and the PI3K/AKT/mTOR signaling pathway.

A) qRT-PCR detection of miR-103 expression in parental and EGFR-TKI resistant A549 cells (n = 3/group). B) CCK-8 assay to assess the effect of miR-103 overexpression or silencing on A549 cell proliferation (n = 3/time point). C, D) CCK-8 assay to examine the rescue effect of PI3K/AKT signaling inhibitors (treated with 10 μM LY294002 for 24 hours) or activators (treated with 10 μM SC79 for 24 hours) on miR-103 overexpression/silencing cells (n = 3/time point). E) Colony formation and Transwell assays to observe the effect of miR-103 overexpression (transfected with miR-103-mimics) and silencing (transfected with miR-103-inhibitor) on colony formation and invasion in A549-R cells. The mimics-NC and inhibitor-NC groups served as controls to exclude the effects of cell transfection. The miR-103-mimics + LY294002 and miR-103-inhibitor + SC79 groups were treated with 10 μM LY294002 and 10 μM SC79 for 24 hours after transfection, respectively, for rescue experiments (n = 3/group). G) Western blot analysis to detect the expression of the PI3K/AKT/mTOR pathway in A549 cells (n = 3/group). H) IC50 analysis of the effect of miR-103 overexpression and silencing on EGFR-TKI sensitivity in A549-R cells (n = 3/group). I) IC50 analysis of the effect of PI3K/AKT inhibitors and activators on EGFR-TKI sensitivity in A549-R cells (n = 3/group). * p < 0.01, ** p < 0.001, *** p < 0.0001. All experiments were performed in triplicate.

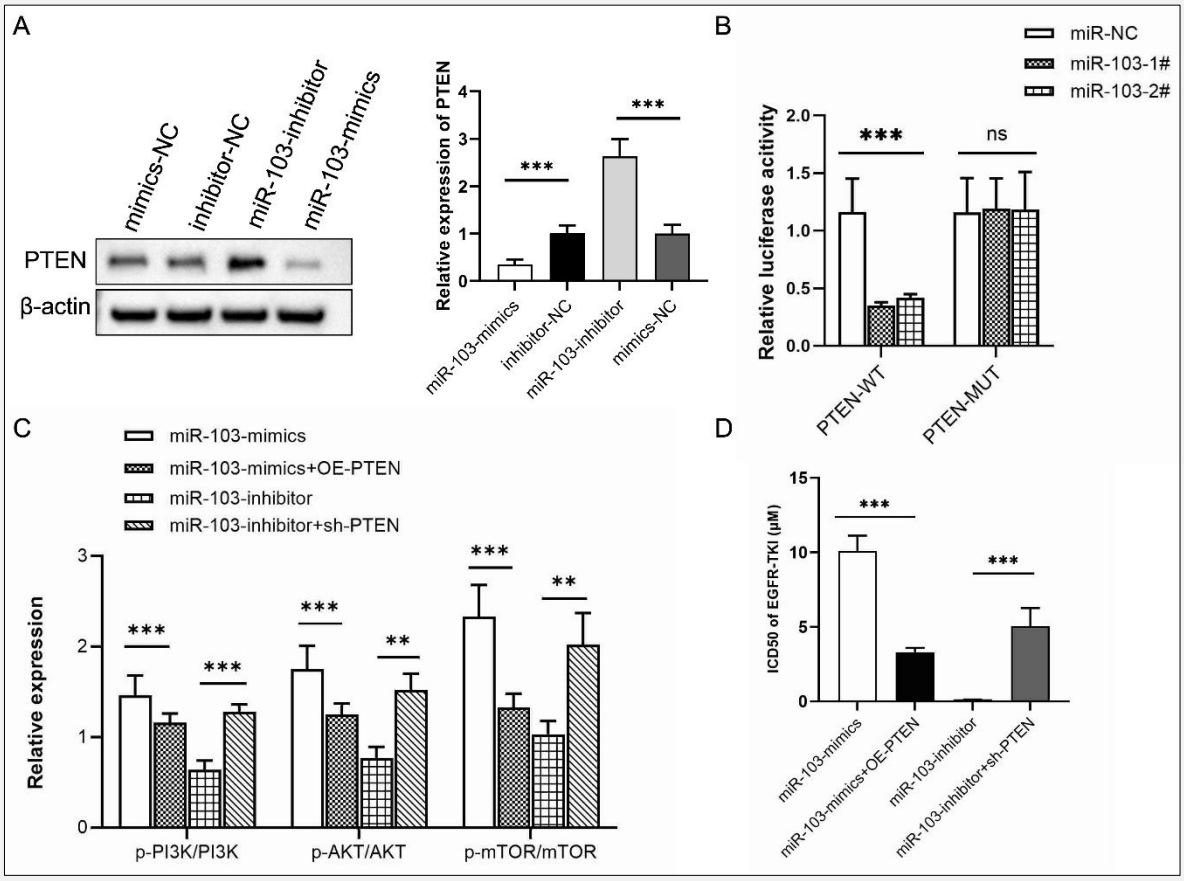

Figure 3. Regulatory mechanism of miR-103 on the PI3K/AKT/mTOR signaling pathway.

A, B) Western blot analysis of PTEN protein expression (n = 3/group). C) Dual-luciferase reporter gene assay (n = 3/group). D) Western blot analysis of the PI3K/AKT/mTOR pathway expression in A549 cells (n = 3/group). E) IC50 analysis of EGFR-TKI treatment sensitivity in A549-R cells (n = 3/group). * p < 0.01, ** p < 0.001, *** p < 0.0001. All experiments were performed in triplicate.

Next, we predicted potential target genes of miR-103 using the miRWalk database and performed KEGG pathway analysis. The results indicated that the PI3K/AKT signaling pathway was significantly enriched (Figures 1C and 1D), implying that miR-103 might promote NSCLC proliferation and resistance through activation of the PI3K/AKT/mTOR pathway.

miR-103 promotes NSCLC proliferation and EGFR-TKI resistance by regulating the PI3K/AKT/mTOR pathway

To explore the function and underlying mechanism of miR-103 in NSCLC, we established an EGFR-TKI (osimertinib)-resistant NSCLC cell line (A549-R) and assessed miR-103 expression. The results showed that miR-103 expression was significantly upregulated in A549-R cells compared to parental cells ($p < 0.001$, Figure 2A).

We then examined the effects of miR-103 overexpression and knockdown on cell behaviors. CCK-8 assays revealed that miR-103 overexpression significantly promoted proliferation, colony formation, and invasion of A549-R cells ($p < 0.001$, Figures 2A and 2E). Conversely, miR-103 knockdown markedly suppressed these behaviors ($p < 0.001$, Figures 2B and 2E). Western blot analysis demonstrated that p-PI3K, p-AKT, and p-mTOR levels were significantly increased in the miR-103 mimic group but decreased in the miR-103 inhibitor group ($p < 0.001$, Figures 2F and 2G).

To further confirm whether miR-103 acts through the PI3K/AKT/mTOR pathway, we performed rescue experiments. Treatment with the PI3K/AKT inhibitor LY294002 significantly abrogated miR-103 mimic-induced proliferation, colony formation, and invasion ($p < 0.001$, Figures 2C and 2E). Conversely, the PI3K/AKT activator SC79 restored proliferation, colony formation,

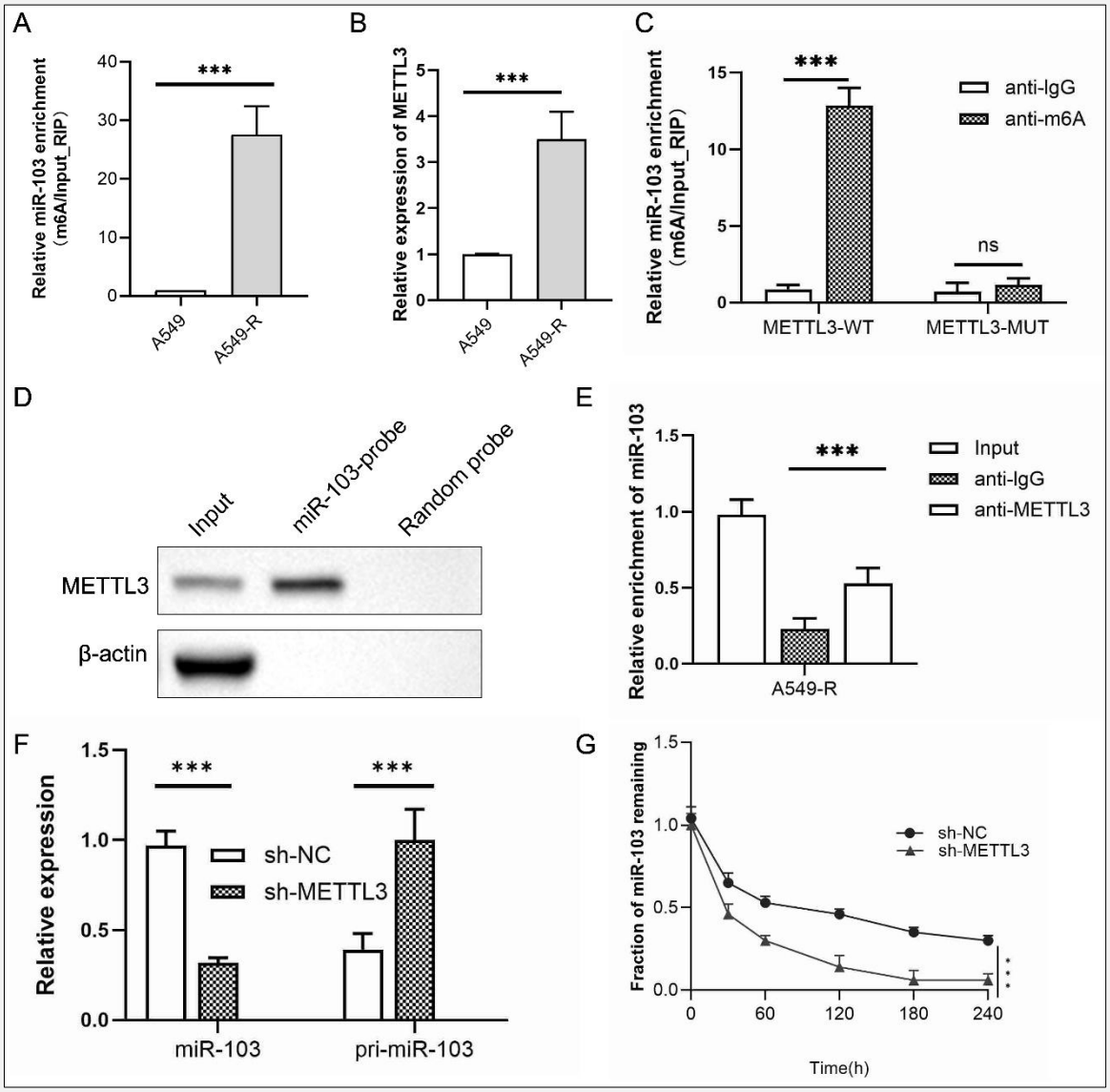

Figure 4. METTL3-mediated m6A modification of miR-103.

A) m6A co-IP assay to detect the m6A modification level of miR-103 in parental and EGFR-TKI resistant A549 cells (n = 3/group). B) Western blot analysis of METTL3 expression levels in parental and EGFR-TKI resistant A549 cells (n = 3/group). C) MeRIP-qPCR analysis of the m6A modification level of miR-103 in cells transfected with different METTL3 mutants (n = 3/group). D) RNA pull-down assay to examine the interaction between miR-103 and METTL3, using a random probe as a negative control (n = 3/group). E) RIP assay to detect the direct interaction between miR-103 and METTL3 protein (n = 3/group). F) qRT-PCR analysis of miR-103 expression levels in A549 and H1299 cells with METTL3 silencing (n = 3/group). G) qRT-PCR analysis of the remaining miR-103 levels in A549 cells at different time points after linezolid treatment (n = 3/time point). * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$. All experiments were performed in triplicate.

and invasion suppressed by miR-103 inhibition ($p < 0.001$, Figures 2D and 2E).

Finally, drug sensitivity assays indicated that miR-103 overexpression significantly reduced A549-R sensitivity to EGFR-TKI, while miR-103 inhibition increased sensitivity ($p < 0.001$, Figure 2H). Similarly, LY294002 reversed miR-103 mimic-induced resistance ($p < 0.001$, Figure 2I), and SC79 rescued the reduction in resistance caused by miR-103 inhibition ($p < 0.001$, Figure 2I). These findings suggest that miR-103 promotes NSCLC progression and resistance via activation of the PI3K/AKT/mTOR pathway.

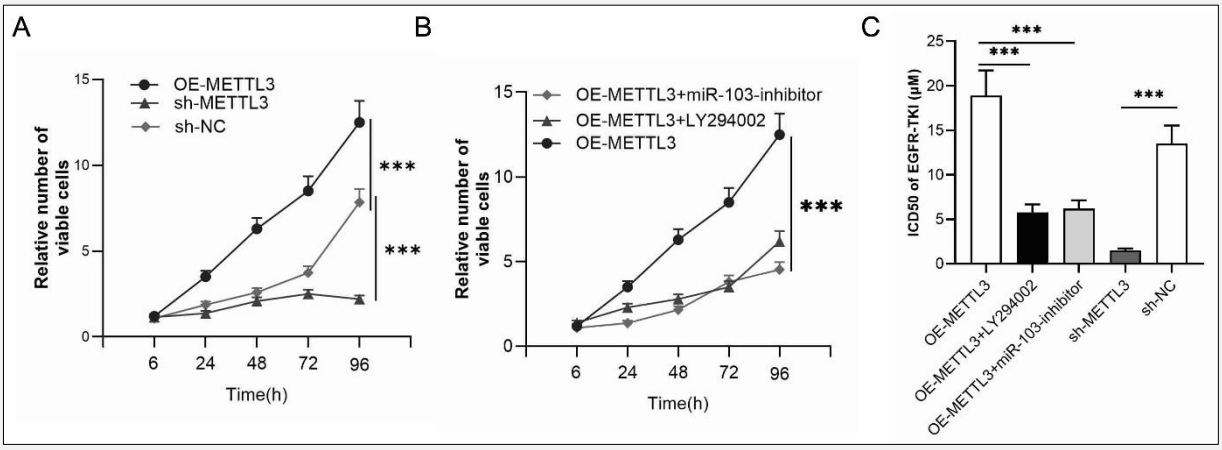

Figure 5. METTL3 regulation of NSCLC cell proliferation and EGFR-TKI resistance.

A) CCK-8 assay to assess the effect of METTL3 overexpression and silencing on A549 cell proliferation (n = 3/time point). B) CCK-8 assay to examine the rescue effect of the PI3K/AKT signaling inhibitor (treated with 10 μM LY294002 for 24 hours) on METTL3 overexpressing cells (n = 3/time point). C) IC50 analysis of the effect of METTL3 overexpression and silencing on EGFR-TKI treatment sensitivity in A549 cell lines (n = 3/group). * p < 0.01, ** p < 0.001, *** p < 0.0001. All experiments were performed in triplicate.

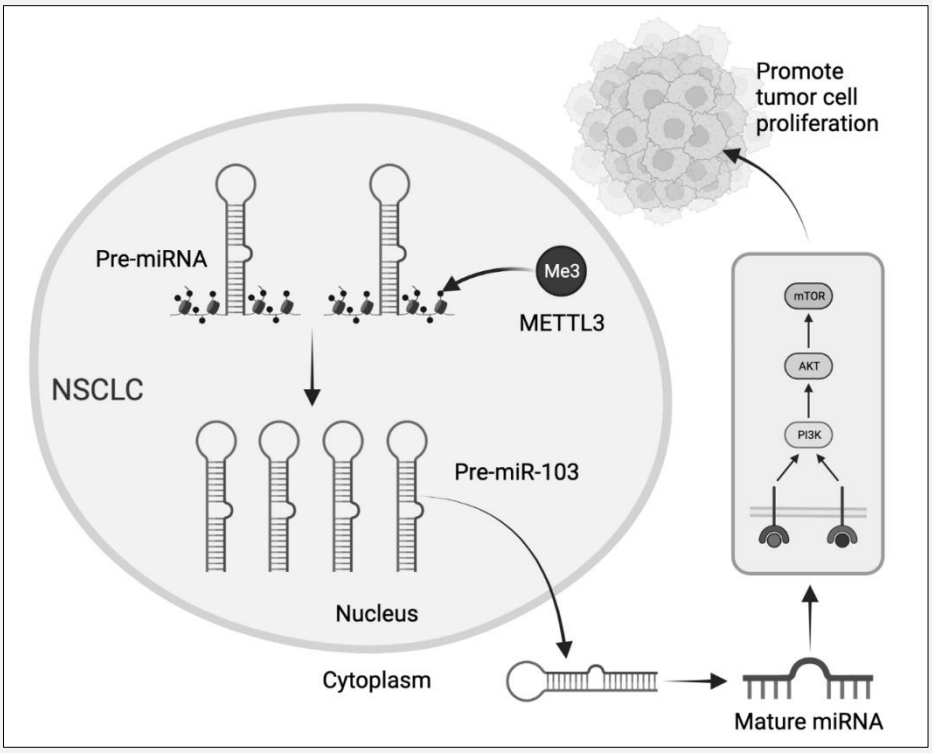

Figure 6. Schematic representation of the mechanism by which METTL3-mediated miR-103 maturation promotes NSCLC progression.

miR-103 regulates the PI3K/AKT/mTOR pathway by suppressing PTEN

To further elucidate the target of miR-103, we used the miRanda tool and identified PTEN as a potential target. Western blot analysis showed that miR-103 overexpression significantly reduced PTEN protein levels compared to the mimic NC group, while miR-103 inhibition markedly increased PTEN levels compared to the inhibitor NC group ($p < 0.001$, Figures 3A and 3B).

Dual-luciferase reporter assays confirmed that miR-103 significantly suppressed luciferase activity in PTEN-WT constructs but had no effect on PTEN-MUT constructs ($p < 0.001$, Figure 3C).

To verify whether miR-103 mediates PI3K/AKT/mTOR pathway activation via PTEN suppression, we conducted rescue experiments using PTEN overexpression and knockdown. PTEN overexpression significantly reduced p-PI3K, p-AKT, and p-mTOR levels in miR-103 mimic-transfected cells ($p < 0.001$, Figure 3D), whereas PTEN knockdown reversed the suppression effects induced by miR-103 inhibition ($p < 0.001$, Figure 3D).

Drug sensitivity assays further showed that PTEN overexpression significantly inhibited miR-103 mimic-induced resistance ($p < 0.001$, Figure 3E), while PTEN knockdown restored resistance reduced by miR-103 inhibition ($p < 0.001$, Figure 3E).

METTL3 enhances miR-103 maturation and stability via m6A modification

We next investigated whether METTL3-mediated m6A modification regulates miR-103 accumulation in resistant NSCLC cells. MeRIP assays revealed that both miR-103 m6A levels and METTL3 protein expression were significantly elevated in A549-R cells compared to parental cells ($p < 0.001$, Figures 4A and 4B).

To confirm the direct regulatory effect, we created a catalytically inactive METTL3 mutant (METTL3-MUT) by replacing lysine 144 with alanine (K144A) and transfected it into A549-R cells to assess its impact on miR-103's m6A modification. The results showed that only the wild-type METTL3 could significantly bind to miR-103, while the mutant did not exhibit this effect ($p < 0.001$, Figure 4C). RNA pull-down assays demonstrated specific enrichment of METTL3 in the miR-103 probe group (Figure 4D), and RIP assays further confirmed the interaction between METTL3 and miR-103 (Figure 4E). qRT-PCR analysis showed that METTL3 knockdown (sh-METTL3) significantly reduced mature miR-103 levels and increased pri-miR-103 expression compared to the sh-NC group ($p < 0.001$, Figure 4F).

Furthermore, actinomycin D chase assays indicated that miR-103 half-life was significantly shortened in sh-METTL3 cells ($p < 0.001$, Figure 4G). Overall, these results demonstrate that METTL3 promotes miR-103 maturation and stability through m6A modification.

Targeting METTL3 and the downstream miR-103/PI3K/AKT/mTOR axis inhibits NSCLC progression and EGFR-TKI resistance

Finally, we assessed the therapeutic potential of targeting METTL3 and its downstream miR-103/PI3K/AKT/mTOR axis in NSCLC. Upregulation of METTL3 significantly increased cell proliferation, whereas METTL3 knockdown markedly reduced proliferation compared to the sh-NC group ($p < 0.001$, Figure 5F).

Drug sensitivity assays showed that METTL3 overexpression increased IC50 values (indicating enhanced resistance), while METTL3 knockdown decreased IC50 values ($p < 0.001$, Figure 5C).

Rescue experiments revealed that treatment with LY294002 or miR-103 inhibitor significantly reduced proliferation and resistance in OE-METTL3 cells ($p < 0.001$, Figures 5B and 5C). These findings suggest that targeting METTL3 and its downstream miR-103/PI3K/AKT/mTOR axis effectively suppresses NSCLC progression and resistance.

DISCUSSION

In this study, we provide the first comprehensive evidence that METTL3-mediated maturation of miR-103 plays a critical role in promoting both progression and drug resistance in non-small cell lung cancer (NSCLC). Analysis of the GEO database revealed that miR-103 is significantly upregulated in EGFR-TKI-resistant NSCLC tissues and likely contributes to therapeutic resistance by modulating the PI3K/AKT/mTOR signaling pathway. Through extensive cellular and mechanistic investigations, we confirmed that miR-103 enhances NSCLC cell proliferation and resistance by directly suppressing PTEN expression and activating the PI3K/AKT/mTOR pathway. Furthermore, we identified METTL3-mediated m6A methylation as a key molecular mechanism underlying the increased maturation and expression of miR-103 in drug-resistant A549-R cells. miR-103, a member of the miR-15/107 family, was initially discovered in the HeLa cervical cancer cell line [14]. Extensive research has since shown that miR-103 is widely expressed in both peritumoral and normal tissues and is involved in various physiological and pathological processes [15]. Beyond its role in cancer, miR-103 has been implicated in several non-malignant conditions, including glucose and lipid metabolism, pulmonary arterial hypertension, and neurological disorders [15-17]. Notably, miR-103 is consistently upregulated in most disease contexts, with downregulation reported only rarely, supporting its characterization as a putative oncogene [18]. Recent evidence indicates that miR-103 is also involved in NSCLC progression. In line with these findings, Fathinavid et al. [19] demonstrated aberrant miR-103 expression in NSCLC through microarray-based miRNA expression profiling. Despite these insights, the specific regulatory functions and mecha-

nisms of miR-103 in NSCLC, particularly in drug resistance, remain poorly understood.

To our knowledge, this is the first study to thoroughly elucidate the mechanistic role of miR-103 in NSCLC resistance. Our results confirm that miR-103 promotes resistance by directly inhibiting PTEN and activating the PI3K/AKT/mTOR axis, consistent with previous reports. For instance, Tan et al. [20] found that miR-103 overexpression significantly enhances proliferation and migration in hepatocellular carcinoma (HCC) cells by suppressing PTEN and activating the PI3K/AKT pathway. Similarly, miR-103 has been shown to act as an oncogene in bladder cancer by targeting the 3′ untranslated region (UTR) of PTEN mRNA, resulting in PI3K/AKT pathway activation. Additionally, miR-103 has been implicated in the development of polycystic ovary syndrome (PCOS) via regulation of the PI3K/AKT pathway [21]. Although mTOR was not directly addressed in these studies, its role as a downstream effector of PI3K/AKT is well established [8,22]. Collectively, activation of the PI3K/AKT/mTOR signaling cascade by miR-103 plays a pivotal role in NSCLC progression and resistance, highlighting its potential as a therapeutic target.

With advancements in molecular biology, RNA modifications such as m6A methylation have garnered increasing attention [23]. Among these, N6-methyladenosine (m6A) is one of the most common types of reversible methylation modification. It is primarily catalyzed by a complex consisting of RNA methyltransferases, such as METTL3 and METTL14 [24]. The role of METTL3 in tumors has been well established, where it mainly promotes RNA maturation through m6A methylation, contributing to the initiation and progression of various cancers [10,25]. Additionally, m6A has been shown to regulate RNA processes such as localization, translation, and metabolism, including those involving circular RNA (circRNA) [26,27]. Furthermore, as research advances, m6A modification has been demonstrated to interact with other epigenetic modifications, such as histone/DNA modifications, to affect chromatin states and precisely regulate gene expression, playing essential roles in both physiological and pathological processes [28]. Notably, METTL3 has also been found to regulate immune responses through an m6A-independent pathway [29]. Therefore, research on METTL3 and its mediation of m6A modification is expected to offer valuable insights into tumor pathogenesis and provide important strategies for the development of therapeutic interventions. Indeed, accumulating evidence supports the key role of METTL3 in NSCLC. In a comprehensive review, Qiu et al. [30] summarized the functions of m6A methyltransferases in NSCLC and concluded that METTL3 predominantly acts as an oncogene. Thus, METTL3 and its associated signaling pathways represent promising targets for early diagnosis and treatment of NSCLC. Numerous studies have demonstrated that silencing METTL3 expression significantly inhibits NSCLC cell proliferation, drug resistance, and metastasis, while promoting apoptosis. These oncogenic effects are believed to be mediated through enhanced phosphorylation of PI3K/AKT pathway components [31-33]. Moreover, METTL3 has been shown to facilitate NSCLC progression through both m6A-dependent and m6A-independent mechanisms [30]. Our findings demonstrate that METTL3 upregulation significantly promotes miR-103 maturation, which in turn activates the miR-103/PI3K/AKT/mTOR pathway, thereby enhancing malignant behaviors and resistance in NSCLC. These results provide novel insights into the multifaceted role of METTL3 in NSCLC and underscore its potential as a promising therapeutic target.

Despite these significant findings, our study has certain limitations. First, due to financial and time constraints, in vivo animal experiments were not conducted, and further in vivo validation is necessary to confirm the proposed mechanisms. Second, larger-scale clinical studies involving more NSCLC patient samples are required to further validate the regulatory role of the METTL3-miR-103/PI3K/AKT/mTOR axis. Ultimately, the clinical translation of targeting the METTL3-miR-103 axis remains a long-term goal that requires extensive further investigation. Future studies should focus on validating its therapeutic potential using robust in vivo models and rigorously designed clinical trials.

CONCLUSION

In conclusion, our findings demonstrate that METTL3 accelerates miR-103 maturation via an m6A-dependent mechanism, thereby activating the PI3K/AKT/mTOR signaling pathway and promoting NSCLC progression and EGFR-TKI resistance. Targeting key nodes within the METTL3-miR-103/PI3K/AKT/mTOR axis holds promise as a novel strategy for early diagnosis and therapeutic intervention in NSCLC.

Declaration of Interest:

The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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