Short Communication

http://www.jcpjournal.org
pISSN 2288-3649 · eISSN 2288-3657
https://doi.org/10.15430/JCP.24.023

JCP

Mitochondrial Ribosomal Protein S17 Silencing Inhibits Proliferation and Invasiveness of Lung Cancer Cells

Woo Rin Lee, Kook Sun Ha

Department of Chemistry, The University of Suwon, Hwaseong, Korea

Chromosomal alterations are frequent events in lung cancer progression. Although gains and losses of chromosomal position have been reported, the association between copy number alteration and lung cancer patient survival has not been extensively investigated. In this study, we performed a meta-analysis of public cBioPortal datasets spanning 25 lung cancer studies to identify putative cancer driver genes with copy number alterations associated with overall patient survival. Ten copy-number altered genes enriched in deceased lung cancer patients were identified. Seven of these putative driver genes were located in the 7p11.2 chromosomal location, and two were in the 9p21.3 cytoband. Among these genes, the mitochondrial ribosomal protein S17 (MRPS17) amplification was significantly associated with a lower patient survival rate (P = 1.47e-7). To investigate the functional role of MRPS17, small interfering RNA-mediated knockdown was performed in two non-small cell lung cancer cell lines, A549 and NCI-H460. MRPS17 knockdown significantly reduced cell proliferation, migration, invasion, and anchorage-independent growth in both cell lines. Furthermore, knockdown of MRPS17 decreased the activation of the phosphatidylinositol 3-kinase/protein kinase B signaling pathway, suggesting its role in driving lung cancer progression through this critical oncogenic pathway. Our findings highlight MRPS17 as a potential cancer therapy target and a prognostic biomarker that may improve the survival rates of lung cancer patients. Future studies should explore its inhibition as a therapeutic strategy as well as elucidate its molecular mechanisms in cancer progression.

Key Words Lung cancer, Mitochondrial ribosomal protein S17, Biomarker, Patient survival, Small interfering RNA

INTRODUCTION

Lung cancer is the second most commonly diagnosed cancer and the leading cause of cancer death for men and women in the United States [1]. Non-small cell lung cancer (NSCLC) accounts for 80% to 85% of all pulmonary malignancies [2]. According to the American Cancer Society, the 5-year relative survival rate of patients with localized NSCLC was 63% between 2010 and 2016 [3]. The previous study showed even lower survivability for patients with regional or distant NSCLC, yielding 5-year relative survival rates of just 35% and 7%, respectively [4]. Therefore, an analysis of which gene alterations are closely associated with the growth of NSCLC is crucial for both the early diagnosis of potential high-risk cancer patients and the development of new treatments to mitigate the spread of NSCLC.

Copy number alterations (CNAs) result from structural alterations of DNA that lead to gain/amplification or loss/deletion of DNA sections from a normal genome [5]. These CNAs have been shown to impact gene expression and appear to play a significant role in the pathogenesis of NSCLC [6]. Thus, there is an urgent need to identify CNA genes that are functionally associated with NSCLC development to support the development of alternative treatment options and improve the NSCLC therapeutic index.

Mitochondrial ribosomal protein S17 (*MRPS17*) belongs to a family of mammalian mitochondrial ribosomal protein genes, which encode proteins that comprise mitochondrial ribosomes (mitoribosomes) [7]. Mitoribosomes consist of a small 28S subunit and a large 39S subunit; *MRPS17* encodes a protein that, joining together with other proteins and rRNA, forms the small 28S subunit of the mitoribosomes [8]. Since MRPS17 is one of the mitoribosome's subunits, it may regulate protein synthesis in the mitochondria [9]. Therefore, alterations in these genes can potentially influence cellular respiration, a possible explanation for the correlation between MRPS17 amplification and reduced survival. The previous study indicated that MRPS17 is frequently overexpressed in

Received October 21, 2024, Revised January 10, 2025, Accepted January 15, 2025, Published on March 30, 2025
Correspondence to Kook Sun Ha, E-mail: holmium@suwon.ac.kr, https://orcid.org/0009-0001-3924-4761

gastric cancer by activating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway [10]. Also, this study found that MRPS17 promotes cell proliferation and metastasis in gastric cancer cells [10]. However, the functional role of MRPS17 in other malignancies, including lung cancer, remains unclear, and further investigation is required.

Understanding gene CNAs and their impact on overall survival in cancer patients is crucial for elucidating tumorigenesis mechanisms and developing novel therapeutic strategies [11]. This study investigates the MRPS17 gene, which, according to cBioPortal analysis, is frequently amplified in NSCLC patients. Our primary aim was to explore the effects of targeted small interfering RNA (siRNA) treatment on MRPS17 expression and to evaluate its functional role in NSCLC progression. Specifically, we assessed the consequences of MRPS17 knockdown on cell proliferation, migration, invasion, and colony formation using NSCLC cells.

MATERIALS AND METHODS

Reagents and antibodies

The primary antibody for MRPS17 (ab175207) was the product of Abcam. Phospho-AKT (# 9271), AKT (9272), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; #2118) were the products of Cell Signaling Technology. Antibodies against primary antibodies were purchased from Santa Cruz Biotechnology, Inc. The bicinchoninic acid (BCA) protein assay reagent was a product of Pierce Biotechnology. The PrestoBlue™ cell viability reagent was purchased from Invitrogen. BD Matrigel™ Matrix was purchased from BD Bioscience.

Analysis of copy number altered gene associated with patients' overall survival by cBioPortal

Altered genes associated with overall survival status in lung cancer patients were identified by meta-analysis in 25 studies provided with cBioPortal: Lung Adenocarcinoma (Broad, Cell 2012), Lung Adenocarcinoma (MSKCC, 2020), Lung Adenocarcinoma (MSKCC, Science 2015), Lung Adenocarcinoma (MSK, NPJ Precision Oncology 2021), Lung Adenocarcinoma (OncoSG, Nat Genet 2020), Lung Adenocarcinoma (TCGA, Firehose Legacy), Lung Adenocarcinoma (TCGA, Nature 2014), Lung Adenocarcinoma (TCGA, PanCancer Atlas), Lung Adenocarcinoma (TSP, Nature 2008), Lung Cancer (SMC, Cancer Research 2016), Lung Squamous Cell Carcinoma (TCGA, Firehose Legacy), Lung Squamous Cell Carcinoma (TCGA, Nature 2012), Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Non-Small Cell Cancer (MSKCC, Cancer Discov 2017), Non-Small Cell Lung Cancer (MSK, Cancer Cell 2018), Non-Small Cell Lung Cancer (MSKCC, J Clin Oncol 2018), Non-Small Cell Lung Cancer (TRACERx, NEJM & Nature 2017), Non-Small Cell Lung Cancer (University of Turin, Lung Cancer 2017), Non-Small Cell Lung Cancer (MSK, Science 2015), Pan-Lung Cancer (TCGA, Nat Genet 2016), Small Cell Lung Cancer (CLCGP, Nat Genet 2012), Small Cell Lung Cancer (Johns Hopkins, Nat Genet 2012), Small Cell Lung Cancer (U Cologne, Nature 2015), Small-Cell Lung Cancer (Multi-Institute, Cancer Cell 2017), Thoracic PDX (MSK, Provisional) [12]. cBioPortal group analysis was performed with the two groups of patients: living (2,929 samples/2,905 patients) vs. deceased (1,466 samples/1,425 patients). Then, the copy number altered genes associated with overall survival status in lung cancer patients were analyzed.

MRPS17 patient survival analysis by cBioPortal and Kaplan–Meier plotter

Patient survival was analyzed with MRPS17 amplified patients (n = 4,177) and MRPS17 non-amplified patients (n = 172). Using the Kaplan–Meier and log-rank test, the *P*-value was calculated for statistical significance. Kaplan–Meier provides a method for estimating the survival curve, and the log-rank test compares two groups statistically. Also, the Kaplan–Meier plotter, an online biomarker validation tool (kmplot.com/analysis), was used to estimate survival probabilities for 2,176 lung cancer patients based on MRPS17 gene expression.

Cell culture and maintenance

NSCLC cell lines (A549 and NCI-H460) were purchased from Korea Cell Line Bank. The cells were maintained in Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% FBS (Gibco BRL) and 1% penicillin and streptomycin (Gibco BRL). The cells were sub-cultured every two to three days to maintain the cells' health. The cells were placed in the 5% CO_2 incubator at 37°C.

siRNA transfection

The siRNA and transfection reagent complex were prepared by mixing the scramble RNA (siControl; cat# SN-1001; Bioneer) or siRNA oligonucleotide targeting MRPS17 (siMRPS17; cat# 51373-1; Bioneer) with RNAiMAX transfection reagent from Invitrogen. A549 cells were transfected with 10 nM of specific or scrambled siRNA oligonucleotides using Lipofectamine RNAiMAX according to the manufacturer's instruction Invitrogen.

Total RNA extraction

Total RNA Extraction kit (Intron) was used to extract total RNA from A549 samples. The protocol provided by the manufactured company was used to extract the RNA. Fifty μL of Elution buffer was added to the membrane to elute the RNA from the membrane. The RNA was finally eluted after incubating the elution buffer at room temperature for 1 minute and then centrifuged for 1 minute.

Synthesis of complementary DNA by reverse transcription

A mixture consisting of 1 μL reverse transcription (RT) buffer, 0.5 μL RT enzyme, 1 μL deoxynucleoside triphosphates mix, and 0.5 μL oligo dT primer was prepared (Enzynomics). Three μL of the mixture was added to 7 μL of extracted RNA from the cells. The mixtures were then put in a PCR Thermal Cycler machine (Bio-Rad) for a single cycle of 20°C for 5 minutes, 42°C for 1 hour, and 95°C for 5 minutes.

PCR of complementary DNA

A 20 μL PCR reaction was performed using complementary DNA from each cell sample to amplify the MRPS17 and GAPDH gene. The following primer sequences were used to amplify MRPS17 and GAPDH gene (MRPS17 forward, 5'-CGGAAAACCTACTTTGCTCACG-3', and MRPS17 reverse, 5'-TCTCAGCCAGTTCATGTTTCAC-3'; GAPDH forward, 5'-GGAGCGAGATCCCTCCAAAAT-3', GAPDH reverse, 5'- GGCTGTTGTCATACTTCTCATGG-3'). The following six steps were used to amplify the gene: 1) 95°C for 3 minutes, 2) 95°C for 30 seconds, 3) 61°C for 30 seconds, 4) 72°C for 13 seconds, 5) repeat steps 2 to 4 for 30 cycles, 6) 72°C for 5 minutes.

Western blot analysis

The cells were lysed in RIPA lysis buffer (150 mM NaCl, 0.5% Triton X-100, 50 mM Tri-HCl [pH 7.4], 25 mM NaF, 20 mM EGTA, 1 mM Na_3VO_4 and protease inhibitor cocktail tablets) for 20 minutes on ice. Afterward, the mixture was centrifuged at 13,000 *g* for 10 minutes to isolate the supernatant. The protein concentration of the supernatant was measured using the BCA reagent (Thermo Scientific). Twenty μg of protein was separated by running through an 12% SDS-PAGE gel and transferred to a polyvinylidene fluoride membrane (Pall Corperation). The blots were blocked with 5% nonfat dry milk/TBS with Tween 20 (TBST) buffer containing 0.1% Tween-20 for 1 hour at room temperature. The membranes were then incubated in a cold room for 12 hours with a 1:1,000 dilution of polyclonal antibody of MRPS17, Phospho-AKT, AKT (9272), and GAPDH. The blots were washed three times with TBST buffer for 5 minutes each. Washed blots were incubated with 1:10,000 dilutions of horseradish peroxidase-conjugated secondary antibody from Cell Signaling Technology for 1 hour and then washed again three times with TBST buffer. The transferred proteins were visualized using an enhanced chemiluminescence detection kit (Thermo Scientific) and ImageQuant™ LAS 4000 device (GE Healthcare).

Prestoblue cell proliferation assay

After A549 cells were seeded at 1×10^4 cells per well in a 96-well culture plate, cell proliferation was assessed by adding Prestoblue reagent Invitrogen to the cells. Prestoblue solution (10%) was added to each well and incubated for 1 hour. The absorbance of 470 nm and 600 nm were measured by a microplate reader (BioTek Instruments).

Cell migration (wound healing assay)

After the cells were seeded (1×10^6 cells) on the 6-well plate and incubated for 24 hours, a cross-shaped wound was scratched onto adhered cells with a 200 μL polypropylene micropipette tip. After the used RPMI-1640 media were removed, the cells were washed with 1 mL Dulbecco's phosphate buffered saline. Two mL of new RPMI-1640 were added to the wells. Brightfield microscopy (Nikon) with a 40 X lens was used to capture the cross-region of each attached cell sample right after the scratch and 16 hours after the wounds were initially made. After the image was loaded on the ImageJ program (1.48v), the cell-free area was analyzed by the Montpellier Resources Imagerie (MRI) wound healing tool. The rate of migration was calculated as the following equation: Area without cells at 0 hour after transfection / Area without cells at 16 hours after transfection.

Cell invasion assay

Tumor cell invasion activities were assayed using Transwell chambers (Corning). Briefly, BD Matrigel Matrix was coated on the upper surface of the Transwell filters (8 μm pore, Nucleopore), respectively. siRNA transfected A549 cells were cultured on the upper surface of Transwell filters for 48 hours and then suspended in RPMI-1640 supplemented with 0.1% bovine serum albumin (Gibco BRL). The live cells (2×10^5 in 0.1 mL) were placed in the upper compartment of a chamber and incubated for 7 hours in triplicate. After fixing and staining the filters, the lower surfaces of the filters were photographed with a microscope EVOS m5000 (Thermo Scientific). Four fields per filter, randomly chosen, were recorded at 10×, and then the number of cells was counted using the single-blind method.

Soft agar colony-forming assay

To prepare 1.2% agar gel, 0.24 g of agar (Thermo Scientific) was mixed with 20 mL water. To prepare 0.6% agar gel, 0.12 g of agar powder was mixed with 20 mL water. To create the bottom agar layer, 1.2% agar gel was mixed with RPMI-1640 at a 1:1 ratio, and 1.5 mL of the resulting mixture was added to each well in a 6-well plate. The 6-well plate was then incubated until the mixture solidified to form a bottom layer. Sample cells (either with transfected siControl or siMRPS17) were suspended in RPMI-1640 medium and mixed at a 1:1 ratio with 0.6% agar gel. The 1.5 mL of the resulting mixtures were added to the solidified agar layers in the 6-well plate. The triplicate samples were prepared for each condition. The samples were incubated in the 5% CO_2 incubator at 37°C for 20 days.

Statistical analysis

All measurements were conducted in triplicate. All values are expressed as the mean ± SD. Log rank test and one-way

ANOVA followed by Tukey post-hoc test was used to calculate the statistical significance using Prism 8 program (GraphPad). A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Association of MRPS17 gene amplification with a low lung cancer patient survival rate

To identify such gene alterations, we used cBioPortal, an analysis tool that allows users to explore large-scale genomic data sets [12]. To investigate the association between copy number altered genes and overall survival in patients, we divided the patient samples into living (n = 2,929) and deceased (n = 1,466) groups in multiple studies using the cBioPortal database (Table 1). Enrichment analysis was then performed to identify novel gene associations with overall survival status (Table 1). The top 10 significant copy number altered genes associated with overall survival status are presented in Table 1. Among these genes, seven (*MRPS17*, *ZNF713*, *EGFR*, *NIPSNAP2*, *PSPH*, *CHCHD2*, and *SEPTIN14*) were in the 7p11.2 chromosomal location, while two genes (*CDKN2B* and *CDKN2A*) were in the 9p21.3 cytoband. These results suggest that CNAs in these genes may play a critical role in lung cancer progression and could serve as prognostic markers for NSCLC patients.

To investigate the impact of *MRPS17* amplification on patient survival, we compared the overall survival of patients with amplified *MRPS17* to that of patients without amplification in cBioPortal [12]. The results showed that the *MRPS17*

Table 1. Top ten copy number altered genes enriched in deceased lung cancer patients, analyzed by meta-analysis across multiple studies using the cBioPortal database

Gene	Cytoband	Living number of patients (%)	Deceased number of patients (%)	Log ratio	*P*-value	Q-value	Major CNA type
MRPS17	7p11.2	82 (3.59)	112 (9.05)	–1.33	3.29E-11	6.390E-07	Amplification
ZNF713	7p11.2	83 (3.63)	112 (9.05)	–1.32	5.15E-11	6.390E-07	Amplification
EGFR	7p11.2	128 (4.44)	133 (9.65)	–1.12	9.40E-11	7.230E-07	Amplification
CDKN2B	9p21.3	443 (15.36)	324 (23.51)	–0.61	1.17E-10	7.230E-07	Deletion
NIPSNAP2	7p11.2	81 (3.55)	108 (8.73)	–1.30	1.80E-10	8.930E-07	Amplification
PSPH	7p11.2	81 (3.55)	107 (8.65)	–1.29	3.05E-10	1.261E-06	Amplification
CDKN2A	9p21.3	456 (15.81)	327 (23.73)	–0.59	4.94E-10	1.753E-06	Deletion
CHCHD2	7p11.2	79 (3.46)	104 (8.41)	–1.28	6.21E-10	1.927E-06	Amplification
SEPTIN14	7p11.2	92 (4.03)	114 (9.22)	–1.19	8.05E-10	2.219E-06	Amplification

The table shows the gene names, cytoband locations, and the number of patients with CNAs in both living and deceased groups. All genes were significantly enriched in the deceased group compared to the living group, as determined by a meta-analysis of large-scale genomic datasets (n = 1,466 for deceased patients and n = 2,929 for living patients). Statistical analysis was performed using Fisher's exact test to calculate *P*-values, followed by Q-value adjustments for multiple testing. MRPS17, mitochondrial ribosomal protein S17; CNA, copy number alteration.

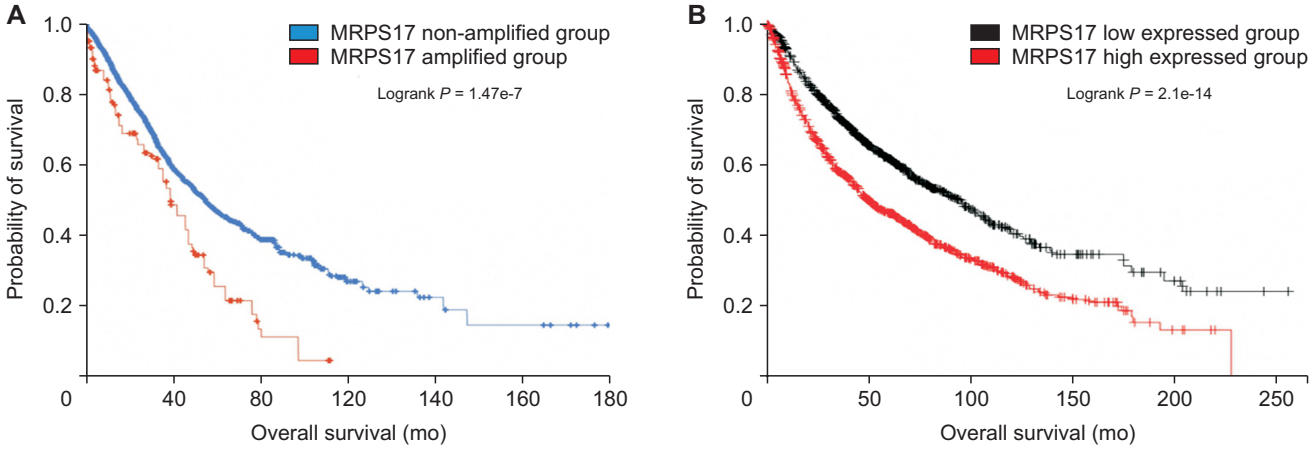

Figure 1. *MRPS17* amplified and highly expressed NSCLC patients were associated with lower survival rates. (A) The median month overall survival from *MRPS17* amplified group (n = 4,177): 38.50 (95% CI, 35.08-46.69), and from MRPS17 non-amplified group (n = 172): 54.40 (95% CI, 50.24-58.80). Log-rank *P*-value = 1.47e-7. (B) NSCLC patient survival analysis based on the mean expression value of the *MRPS17* gene expression (n = 2,165). Log-rank *P*-value = 2.1e-14. MRPS17, mitochondrial ribosomal protein S17; NSCLC, non-small cell lung cancer.

amplified group had a median survival of 38.50 months, which was significantly lower than the median survival of 54.40 months in the non-amplified group (Fig. 1A). The association between *MRPS17* amplification and decreased patient survival rate was statistically significant (P = 1.47e-7).

To examine the clinical importance of the *MRPS17* expression level in NSCLC progression, we analyzed the NSCLC patient survival rate based on the mean expression value of *MRPS17*. Analysis of a cohort of NSCLC patients revealed that high *MRPS17* gene expression led to a decreased survival rate compared with low *MRPS17* gene expression (Fig. 1B). These findings suggest that *MRPS17* amplification and high expression level may serve as a potential prognostic marker for NSCLC patients and further support the critical role of MRPS17 in NSCLC progression.

Effects of *MRPS17* knockdown on cell proliferation

To determine the functional role of *MRPS17* in lung cancer cell proliferation, we performed siRNA-mediated gene knockdown, and its effects on cell proliferation were analyzed in two NSCLC cancer cell lines: A549 and NCI-H460. RT-PCR

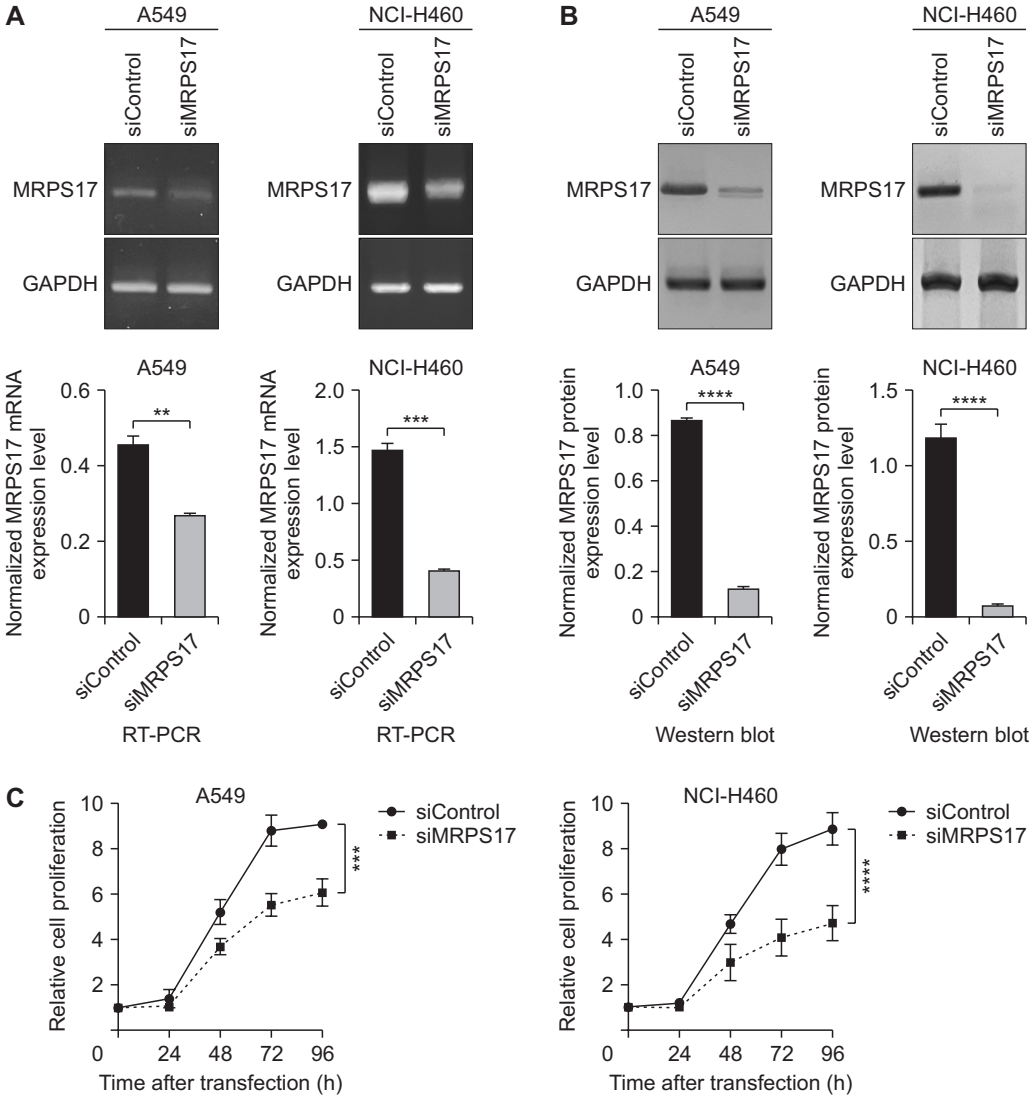

Figure 2. *MRPS17* knockdown decreased A549 and NCI-H460 cell proliferation. (A) Image of amplified bands of *MRPS17* and *GAPDH* analyzed on agarose gel electrophoresis. Quantification of the band intensity from agarose gel using ImageJ densitometer program. The mean and SD were plotted in the graph (n = 3). The normalized *MRPS17* expression level was calculated as follows: band intensity of *MRPS17* / band intensity of *GAPDH*. (B) Image of Western blot of MRPS17 and GAPDH. Quantification of the band intensity from Western blot using ImageJ densitometer program. The mean and SD were plotted in the graph (n = 3). (C) Quantification of relative cell proliferation after siMRPS17 transfection. Data were analyzed using one-way ANOVA followed by Tukey post-hoc test, **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001. siControl, scramble RNA; siMRPS17, small interfering RNA oligonucleotide targeting MRPS17; MRPS17, mitochondrial ribosomal protein S17; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT, reverse transcription.

results indicated a significant reduction in *MRPS17* mRNA expression in siMRPS17-transfected cells compared to the siControl group in both cell lines (Fig. 2A). This decrease in mRNA expression was corroborated by Western blot analysis, which confirmed a significant reduction in MRPS17 protein levels following siMRPS17 transfection (Fig. 2B). Furthermore, a cell proliferation assay revealed that *MRPS17* knockdown significantly reduced A549 and NCI-H460 cell growth over 96 hours (Fig. 2C). These results suggest that MRPS17 plays an essential role in the proliferation of NSCLC cells.

Effects of *MRPS17* knockdown on cell migration, invasion, and colony formation

Next, we analyzed the effect of MRPS17 on metastatic potential in lung cancer using cell migration, invasion, and colony formation assays. *MRPS17* knockdown significantly reduced these processes in both A549 and NCI-H460 cells. In the scratch wound assay, cells transfected with siMRPS17 exhibited a markedly lower migration rate than that in the siControl group, significantly reducing migration after 16 hours (Fig. 3A). Similarly, the invasion assay revealed that *MRPS17* knockdown substantially decreased the percentage of invading cells compared to controls (Fig. 3B). Furthermore,

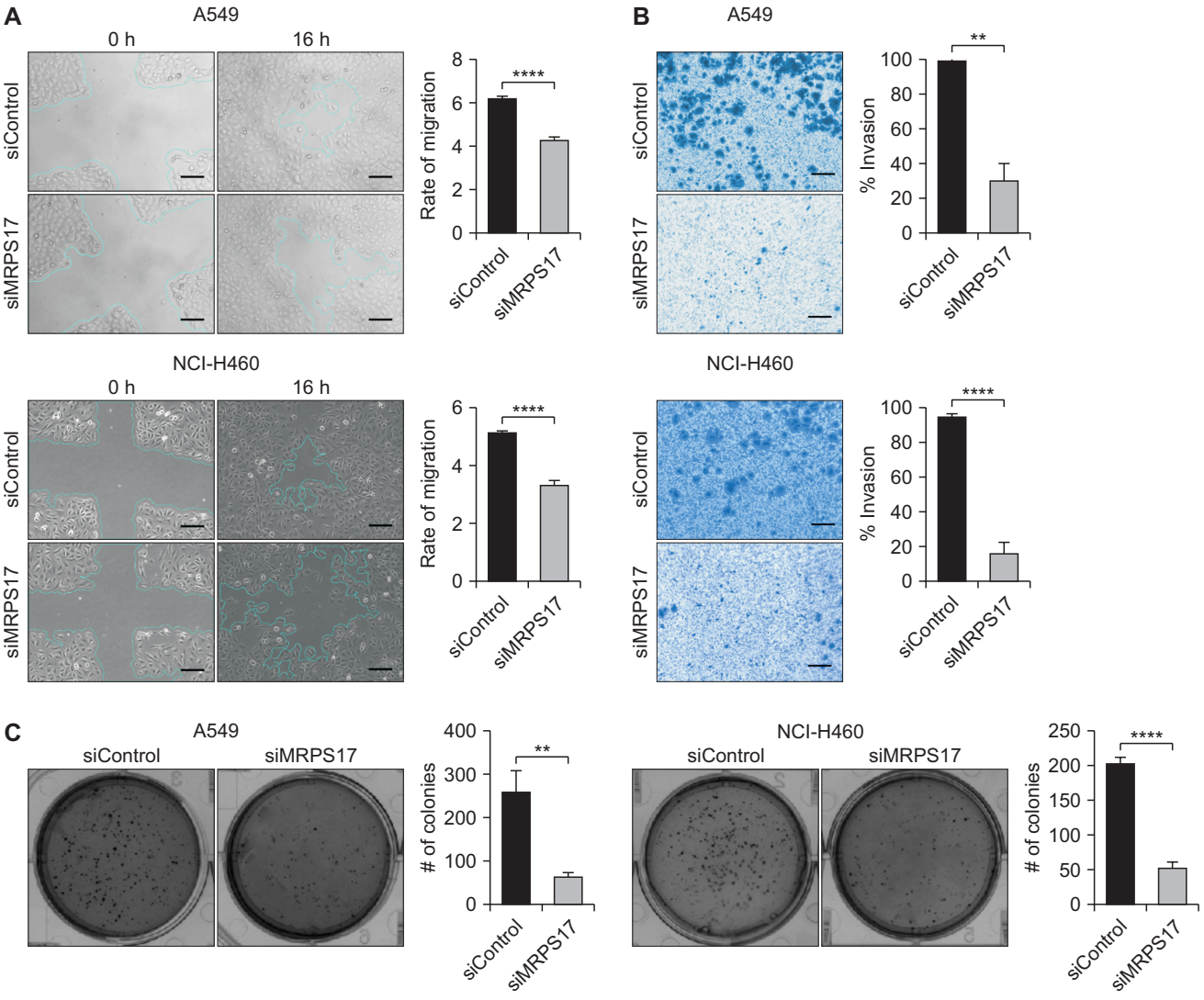

Figure 3. *MRPS17* knockdown reduced cell migration, invasion, and colony formation in A549 and NCI-H460 cell lines. (A) Representative images of cells before and after migration following a scratch wound assay. The mean migration rate and SD are presented in the accompanying graph (n = 3). (B) Representative images of cells after the invasion. The mean percentage of invaded cells and SD are shown in the graph (n = 3). (C) Representative images of colony formation following transfection with siControl and siMRPS17. Quantification of the number of colonies formed is displayed in the graph (n = 3). Data were analyzed using one-way ANOVA followed by Tukey post-hoc test, ***P*-value < 0.01, ****P*-value < 0.001, *****P*-value < 0.0001. Scale bar = 50 μM. siControl, scramble RNA; siMRPS17, small interfering RNA oligonucleotide targeting MRPS17; MRPS17, mitochondrial ribosomal protein S17.

MRPS17 knockdown led to a significant reduction in colony formation, with fewer colonies observed in the siMRPS17 group compared to the siControl group (Fig. 3C). These findings suggest that MRPS17 promotes migration, invasion, and colony formation in both NSCLC cells.

Impact of *MRPS17* knockdown on the AKT signaling pathway

Previous studies have shown that MRPS17 regulates the PI3K/AKT signaling pathway in gastric cancer [10]. To investigate this further, we examined the effects of *MRPS17* knockdown on the expression levels of phosphorylated AKT (P-AKT) and total AKT using Western blot analysis in A549 and NCI-H460 cells (Fig. 4A). The results demonstrated a significant reduction in both P-AKT and total AKT expression levels in these cell lines following *MRPS17* knockdown (Fig. 4B). These findings suggest that MRPS17 may contribute to cancer progression by modulating the PI3K/AKT signaling pathway.

DISCUSSION

Gene amplification is a typical genetic alteration in which the copy number of a gene is increased [13,14]. With genomic data collected from lung cancer patients, many oncogenes have been identified to be amplified in human lung cancer [15,16]. Our data analysis demonstrates that an amplification of the MRPS17 gene is negatively associated with the survival of lung cancer patients. In addition, the results of the in vitro study, which evaluated the effects of MRPS17 downregulation on cell proliferation, migration, invasion, and anchorage-independent growth, validate that *MRPS17* may be a proto-oncogene that affects cell proliferation, migration, and growth, likely by affecting cellular respiration.

Elevated MRPS17 expression has been previously linked to gastric cancer, where it facilitates tumor proliferation, migration, and metastasis [10]. This effect is mediated by activating oncogenic signaling pathways, such as the PI3K/AKT signaling pathway that plays a critical role in promoting cell survival and growth [10]. Our study suggests that MRPS17 may exert similar oncogenic effects in lung cancer by modulating cellular respiration and energy production. These findings highlight the potential of MRPS17 as both a prognostic biomarker and a therapeutic target, offering new avenues for improving outcomes in lung cancer patients.

Mammalian mitochondrial ribosomal small subunit genes

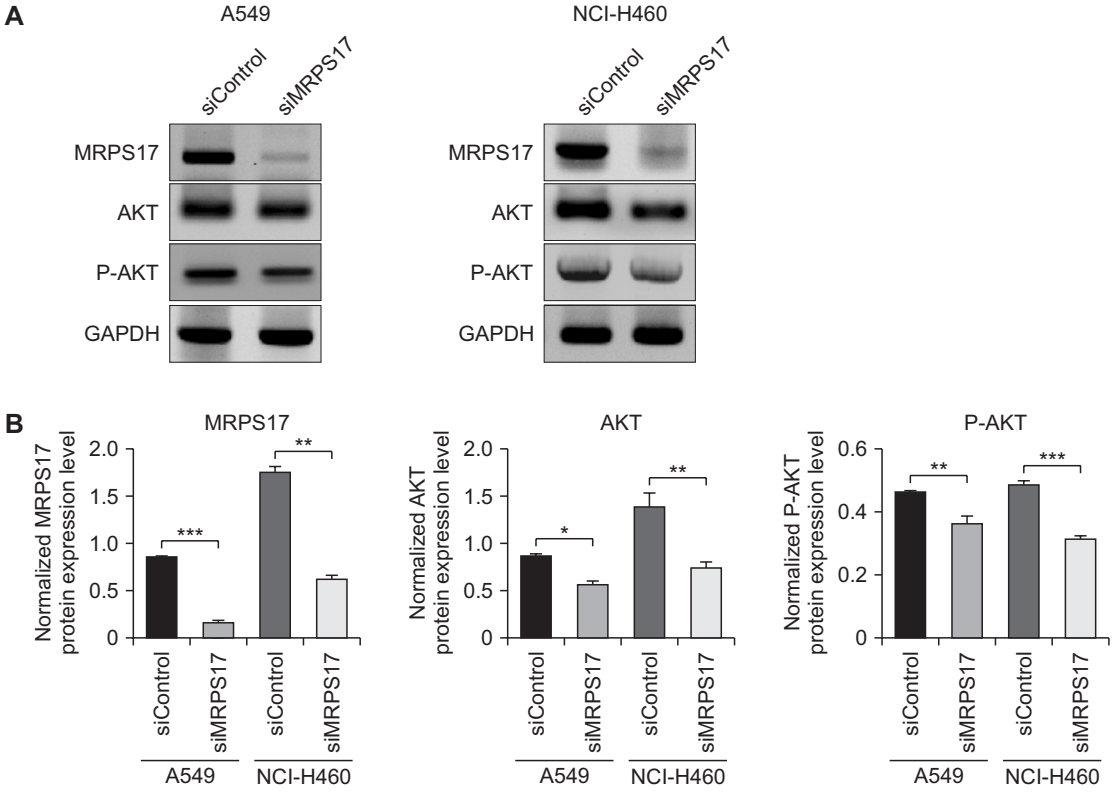

Figure 4. *MRPS17* knockdown reduced AKT and P-AKT protein expression levels. (A) Image of Western blot of MRPS17, AKT, P-AKT, and GAPDH. (B) Quantification of the band intensity from Western blot using ImageJ densitometer program. The mean and SD were plotted in the graph (n = 3). Data were analyzed using one-way ANOVA followed by Tukey post-hoc test, **P*-value < 0.05, ***P*-value < 0.01, ****P*-value < 0.001. siControl, scramble RNA; siMRPS17, small interfering RNA oligonucleotide targeting MRPS17; MRPS17, mitochondrial ribosomal protein S17; AKT, protein kinase B; P-AKT, phosphorylated AKT; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

encode essential components of the mitochondrial ribosome, which is responsible for protein synthesis within mitochondria [17]. As mitochondria play a pivotal role in energy production, apoptosis, and metabolic regulation, disruptions in mitochondrial functions can have significant implications for disease progression, including cancer [18]. In cancer cells, mitochondrial dysfunction is often observed, contributing to altered metabolic states that support rapid cell proliferation and survival [19].

MRPS genes, which encode the small subunit of the mitochondrial ribosome, have been increasingly recognized for their potential roles in cancer development and progression [20]. Aberrations in MRPS gene expression may lead to dysregulation of mitochondrial protein synthesis and oxidative phosphorylation, processes critical for maintaining the energy demands of rapidly dividing cancer cells [21]. As research continues to uncover the involvement of MRPS genes in cancer, they present promising targets for further investigation as potential biomarkers or therapeutic targets, with implications for understanding tumor metabolism and patient outcomes [22,23]. Additionally, because MRPS17 is a component of the mitochondrial ribosome, it may affect cellular respiration and energy production, processes often dysregulated in cancer cells to support rapid proliferation [9]. However, there have been no previous studies directly associating amplified MRPS17 with lung cancer, making MRPS17 a potential novel biomarker associated with decreased patient survival in lung cancer.

This study has several acknowledged limitations. One limitation of this study is the exclusive use of publicly available datasets from cBioPortal, which may limit the generalizability of the findings to broader lung cancer populations due to potential biases in the original studies included in the meta-analysis [24]. Additionally, the focus on CNAs in only ten genes may overlook other significant genetic changes or epigenetic factors that contribute to lung cancer progression and patient survival [25]. Furthermore, while *MRPS17* knockdown experiments in A549 and NCI-H460 cells provided functional insights, the study was limited to in vitro assays using two lung cancer cell lines, which may not fully represent the heterogeneity of NSCLC. Future studies should include animal tumor models and additional lung cancer cell lines to confirm the functional role of MRPS17 and validate its potential as a prognostic biomarker and therapeutic target. Lastly, while the knockdown experiments revealed the impact of MRPS17 reduction, the precise molecular mechanisms, particularly its role in modulating the PI3K/AKT signaling pathway and its influence on cancer progression, remain unclear and warrant further investigation.

The findings of our study demonstrate that targeted knockdown of *MRPS17* using siRNA significantly inhibits the proliferation, migration, and anchorage-independent growth of lung cancer cells. These results suggest that *MRPS17* plays a crucial role in the progression of lung cancer and support the observed association between *MRPS17* amplification and reduced patient survival rates. Mechanistically, *MRPS17* knockdown was found to reduce the activation of the PI3K/AKT signaling pathway, a critical regulator of cell survival, growth, and metastasis. This suggests that *MRPS17* promotes lung cancer progression, at least in part, through modulation of the PI3K/AKT pathway. Clinically, *MRPS17* amplification could serve as a valuable prognostic biomarker, helping to identify lung cancer patients at higher risk of poor outcomes. Importantly, our study highlights the potential of targeted siRNA treatments for reducing lung cancer metastasis and suggests that MRPS17 represents a promising therapeutic target for slowing tumor progression and improving patient survival. Future studies should focus on validating MRPS17's role as a biomarker and further explore its inhibition as a therapeutic strategy in clinical settings.

FUNDING

None.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ORCID

Woo Rin Lee, https://orcid.org/0000-0002-9109-3606
Kook Sun Ha, https://orcid.org/0009-0001-3924-4761

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin 2021;71:7-33.
2. Clark SB, Alsubait S. Non-small cell lung cancer. https://www.ncbi.nlm.nih.gov/books/NBK562307/ (Accessed December 23, 2024).
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
4. Torre LA, Siegel RL, Jemal A. Lung cancer statistics. Adv Exp Med Biol 2016;893:1-19.
5. Ried T, Meijer GA, Harrison DJ, Grech G, Franch-Expósito S, Briffa R, et al. The landscape of genomic copy number alterations in colorectal cancer and their consequences on gene expression levels and disease outcome. Mol Aspects Med 2019;69:48-61.
6. Qiu ZW, Bi JH, Gazdar AF, Song K. Genome-wide copy number variation pattern analysis and a classification signature for non-small cell lung cancer. Genes Chromosomes Cancer 2017;56:559-69.
7. Cheong A, Lingutla R, Mager J. Expression analysis of mammalian mitochondrial ribosomal protein genes. Gene Expr Patterns 2020;38:119147.
8. Kim HJ, Maiti P, Barrientos A. Mitochondrial ribosomes in cancer.

Semin Cancer Biol 2017;47:67-81.
9. Hanlon SE, Xu Z, Norris DN, Vershon AK. Analysis of the meiotic role of the mitochondrial ribosomal proteins Mrps17 and Mrpl37 in Saccharomyces cerevisiae. Yeast 2004;21:1241-52.
10. Zhou W, Ouyang J, Li J, Liu F, An T, Cheng L, et al. MRPS17 promotes invasion and metastasis through PI3K/AKT signal pathway and could be potential prognostic marker for gastric cancer. J Cancer 2021;12:4849-61.
11. Herceg Z, Hainaut P. Genetic and epigenetic alterations as biomarkers for cancer detection, diagnosis and prognosis. Mol Oncol 2007;1:26-41.
12. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.
13. Tang YC, Amon A. Gene copy-number alterations: a cost-benefit analysis. Cell 2013;152:394-405.
14. Hastings PJ, Lupski JR, Rosenberg SM, Ira G. Mechanisms of change in gene copy number. Nat Rev Genet 2009;10:551-64.
15. El-Telbany A, Ma PC. Cancer genes in lung cancer: racial disparities: are there any? Genes Cancer 2012;3:467-80.
16. Onozato R, Kosaka T, Kuwano H, Sekido Y, Yatabe Y, Mitsudomi T. Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. J Thorac Oncol 2009;4:5-11.
17. Lightowlers RN, Rozanska A, Chrzanowska-Lightowlers ZM. Mitochondrial protein synthesis: figuring the fundamentals, complexities and complications, of mammalian mitochondrial translation. FEBS Lett 2014;588:2496-503.
18. San-Millán I. The key role of mitochondrial function in health and disease. Antioxidants (Basel) 2023;12:782.
19. Luo Y, Ma J, Lu W. The significance of mitochondrial dysfunction in cancer. Int J Mol Sci 2020;21:5598.
20. Gopisetty G, Thangarajan R. Mammalian mitochondrial ribosomal small subunit (MRPS) genes: a putative role in human disease. Gene 2016;589:27-35.
21. Min S, Lee YK, Hong J, Park TJ, Woo HG, Kwon SM, et al. MRPS31 loss is a key driver of mitochondrial deregulation and hepatocellular carcinoma aggressiveness. Cell Death Dis 2021;12:1076.
22. Tao Z, Suo H, Zhang L, Jin Z, Wang Z, Wang D, et al. MRPL13 is a prognostic cancer biomarker and correlates with immune infiltrates in breast cancer. Onco Targets Ther 2020;13:12255-68.
23. Lin YX, Pan JY, Feng WD, Huang TC, Li CZ. MRPL48 is a novel prognostic and predictive biomarker of hepatocellular carcinoma. Eur J Med Res 2023;28:589.
24. Munafò MR, Clark TG, Flint J. Assessing publication bias in genetic association studies: evidence from a recent meta-analysis. Psychiatry Res 2004;129:39-44.
25. Taylor BS, Barretina J, Socci ND, Decarolis P, Ladanyi M, Meyerson M, et al. Functional copy-number alterations in cancer. PLoS One 2008;3:e3179.