European Review for Medical and Pharmacological Sciences 2019; 23: 3383-3389

Long noncoding RNA MIAT promotes the growth and metastasis of non-small cell lung cancer by upregulating TDP43

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Abstract. – OBJECTIVE: Recent researches have proved that long noncoding RNAs (lncRNAs) act and have an important role in many diseases. In this research, lncRNA MIAT was explored to identify how it functions in the development of non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: Real-time quantitative polymerase chain reaction (RT-qPCR) was utilized to detect MIAT expression in NSCLC patients. Next, we conducted cell counting kit-8 (CCK-8) assay, colony formation assay, ethynyl deoxyuridine (EdU) incorporation assay, wound healing assay and transwell assay to identify its biological function. Further experiments were performed to explore the potential mechanism.

RESULTS: By comparing with MIAT expression in adjacent tissues, MIAT expression level was significantly higher in NSCLC samples. Moreover, functional assays showed that cell growth ability of NSCLC cells was inhibited after MIAT was knocked down. In addition, the migrated and invaded ability of NSCLC cells inhibited after MIAT was knocked down. Furthermore, the expression of TDP43 was downregulated by knockdown of MIAT. Meanwhile, it was found that TDP43 expression positively correlated to MIAT expression in NSCLC tissues.

CONCLUSIONS: Results above suggest that MIAT could enhance cell proliferation and metastasis of NSCLC by upregulating TDP43, which suggests that MIAT may be a potential therapeutic target in NSCLC.

Key Words:
Long noncoding RNA, MIAT, Non-small cell lung cancer, TDP43.

Introduction

Lung cancer is one of the most frequent malignancies in the world, which is also the leading cause of tumor-related deaths globally, accounting for almost 1.3 million deaths annually[1]. Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancer cases[2]. Despite technology develops in the molecular pathogenesis and therapeutic treatment of NSCLC, most of NSCLC patients are unfortunately diagnosed at advanced stage without the opportunity to take curable surgery, which contributes to the poor survival rate[3]. Therefore, it is urgent to realize the underlying mechanism and find a new treatment strategy. Long non-coding RNAs (lncRNAs) are defined as non-protein-coding RNAs, which are longer than 200 nucleotides. Numerous studies have showed that lncRNAs are a new frontier in the research of malignant diseases. For instance, the upregulation of PVT1 promotes proliferation, cell progression and metastasis of melanoma cells[4]. lncRNA-SNHG7 regulates proliferation, apoptosis and invasion of bladder cancer cells[5]. lncRNA MALAT1 accelerates cell migration and invasion *via* targeting miR-204 in hepatocellular carcinoma[6]. However, the function of lncRNA MIAT in NSCLC and the potential molecular mechanism haven't been studied so far. In this study, we found out that the expression of MIAT was remarkably higher in NSCLC tissues. Moreover, knockdown of MIAT inhibited the proliferation and metastasis of NSCLC cells. Moreover, our further experiment explored the underlying mechanism how MIAT functioned in NSCLC development.

Patients and Methods

Tissue Samples

A total of 56 NSCLC patients were obtained from NSCLC patients who underwent surgery at

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Harbin Medical University Cancer Hospital. No radiotherapy or chemotherapy was performed before the surgery. All fresh tissues got from surgery were stored immediately at -80°C. This study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital. Signed written informed consents were obtained from all participants before the study.

Cell Culture

Human NSCLC cell lines (A549, PC-9 and H358) and normal human bronchial epithelial cell line (16HBE) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). Culture medium consisted of 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) as well as penicillin. Besides, cells were cultured in an incubator containing 5% CO_2 at 37°C.

Cell Transfection

The cDNA oligonucleotides specifically targeting MIAT (sh-MIAT) was synthesiz GenePharma (Shanghai, China) and ed into the shRNA expression vector pC Neo. sh-MIAT was then used for transfec in NSCLC cells. 48 h later, Real-time quantita tive polymerase chain reaction (RT CR) was used to monitor the transfection y.

RNA Extraction and RT-q

TRIzol reagent (Invitrog , CA, USA) was utilized to se rate RNA. Through reverse Transcr n Kit (Biotechnology Co., Ltd., an, China), t tal RNA was reverse-tra complementary deoxyribose nucleic s (Following are the primers used for RT-qPC primers forward: 5′-GC GTTCACAACC ACTG-3′, reverse: 5′- CACTTTGGCATTCTAGG-3′; GAPDH p ers ward: 5′-CCAAAAT-CAGATGC CTGC and reverse: 5′-TGATGG CTG GTCATTCA-3′. Thermal cycle wa 0 s at 95°C, 5 s for 40 cy °C, 35 s C.

Ce oun (CCK-8) Assay

vi ol of CCK8 assay (Dojin atories, Kumamoto, Japan), cell growth f transfected cells in 96-well plates was d at 24, 48, and 72 hours. Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was u asure the absorbance at 450 nm.

Colony Formation A

H358 cells were place well p for 10 days. Then, colo s were 10% formaldehyde for 2 in and sta 5 min with 0.5% crysta olet. e Image ro Plus 6.0 (Silver Spri MD SA) as used for data analysis.

Ethynyl D yuridi Incorpo n Assay

Acco the manufacturer's manual, an EdU K Ro nheim, Germany) was utilized to monitor t liferation of transfected ed . Zeiss Axiop Photomicroscope (Carl , Oberkochen, Germany) was performed to the representative images.

nd Hea Assay

ed into 6-well plates were cultur ecco's Modified Eagle Medium (DMEM) medium overnight. After scratched ith a plastic tip, cells were cultured in serum-free ound closure was viewed at 48 h. Each s independently repeated in triplicate.

ranswell Assay

For detecting the migrating ability of these ated cells, 5×10^4 cells in 200 µL serum-free MEM were transformed to the top chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA). For detecting the invading ability of these treated cells, 5×10^4 cells in 200 µL serum-free DMEM were transformed to top chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA) coated with 50 µg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). And the bottom chamber was added with DMEM and FBS. The top surface of chambers was wiped by cotton swab 48 h later and immersed for 10 min with precooling methanol. Then, they were stained in crystal violet for 30 min. Three fields were used to count the data for invasion membrane.

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA). Student *t*-test method was performed to the data. Data were presented as mean ± SD (standard deviation). $p<0.05$ was considered as statistically significant.

Results

MIAT Expression Level in NSCLC Tissues and Cells

Firstly, MIAT expression was detected *via* RT-qPCR in 56 NSCLC patients' tissues and 3 NSCLC cell lines. Results showed that MIAT was significantly upregulated in tumor tissue samples (Figure 1A). MIAT expression level of NSCLC cells was higher than that of 16HBE (Figure 1B).

Knockdown of MIAT Suppressed Cell Proliferation of NSCLC Cells

In this study, we chose A549 NSCLC cell line for the silence of MIAT. MIAT expression was detected by RT-qPCR (Figure 2A). Results of CCK8 assay showed that silence of MIAT inhibited cell growth ability of NSCLC cells (Figure 2B). The outcome of colony formation assay also revealed that the number of colonies was remarkably decreased after MIAT was silenced in NSCLC cells (Figure 2C). Moreover, results of EdU incorporation assay also revealed that the percentage of EdU positive cells reduced after silence of MIAT in A549 cells (Figure 2D).

Knockdown of MIAT Suppressed Cell Migration and Invasion of NSCLC Cells

To detect the function of MIAT in NSCLC metastasis, wound healing assay and transwell assay were performed. Results of wound assay showed that knockdown of MIAT inhibited cell migrated ability of NSCLC cells (Figure 3). Outcome of transwell assay also revealed that the number of migrated and invaded cells was remarkably decreased after MIAT was knocked-down in NSCLC cells (Figure 3).

Interaction Between TDP43 and MIAT in NSCLC

Starbase v2.0 (http://starbase.sysu.edu.cn/starbase2/rbpLncRNA.php) was used to predict the target proteins of MIAT, among which TDP43 was selected for the following experiments. RT-qPCR results showed that the expression level of TDP43 in NSCLC tissues was remarkably lower in sh-MIAT group compared that in control group (Figure 4A). Besides, we found that TDP43 expression in NSCLC cells was significantly higher when compared with 16HBE (Figure 4B). Furthermore, we found that TDP43 expression in NSCLC tissues was remarkably higher when compared with that of adjacent tissues (Figure 4C). Correlation analysis demonstrated that TDP43 expression level was positively correlated with MIAT expression in cancer tissues (Figure 4D).

Discussion

lncRNAs are important regulators in lung cancer initiation and progression. For example, up-regulated FGF1 by overexpression of lncRNA HOXB1A-2 could promote NSCLC development and lead to poor prognosis[7]. LncRNA PRNCR1 functions as an oncogene in NSCLC and promotes tumor progression through regulating miR-488/HEY2 signal network[8]. LncRNA MIAT, as a novel molecular, has been found to participate in numerous diseases, including the development of

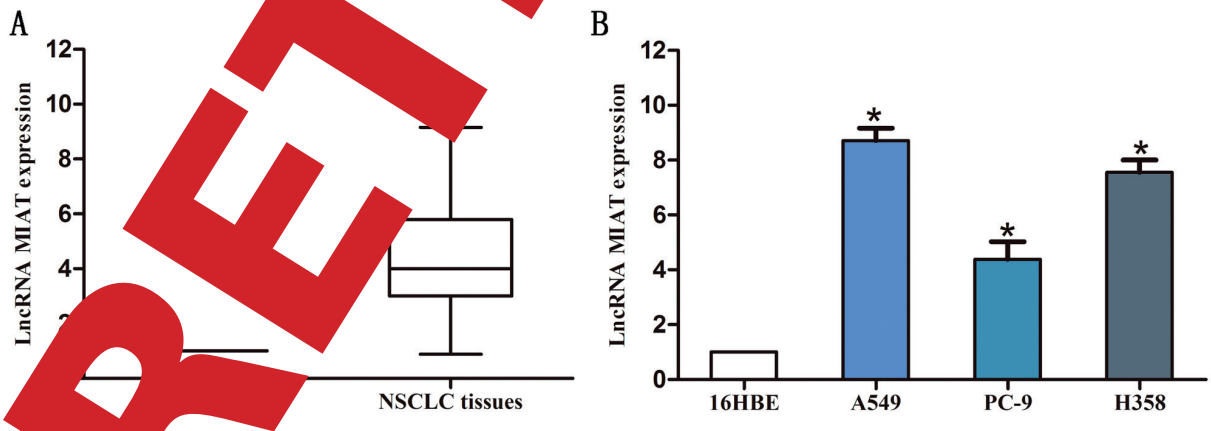


Figure ...sion levels of MIAT were increased in NSCLC tissues and cell lines. ***A,*** MIAT expression was significantly increased in ...C tissues compared with adjacent tissues. ***B,*** Expression levels of MIAT relative to GAPDH were determined in the hu... SCLC cell lines and 16HBE (normal human bronchial epithelial cell) by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

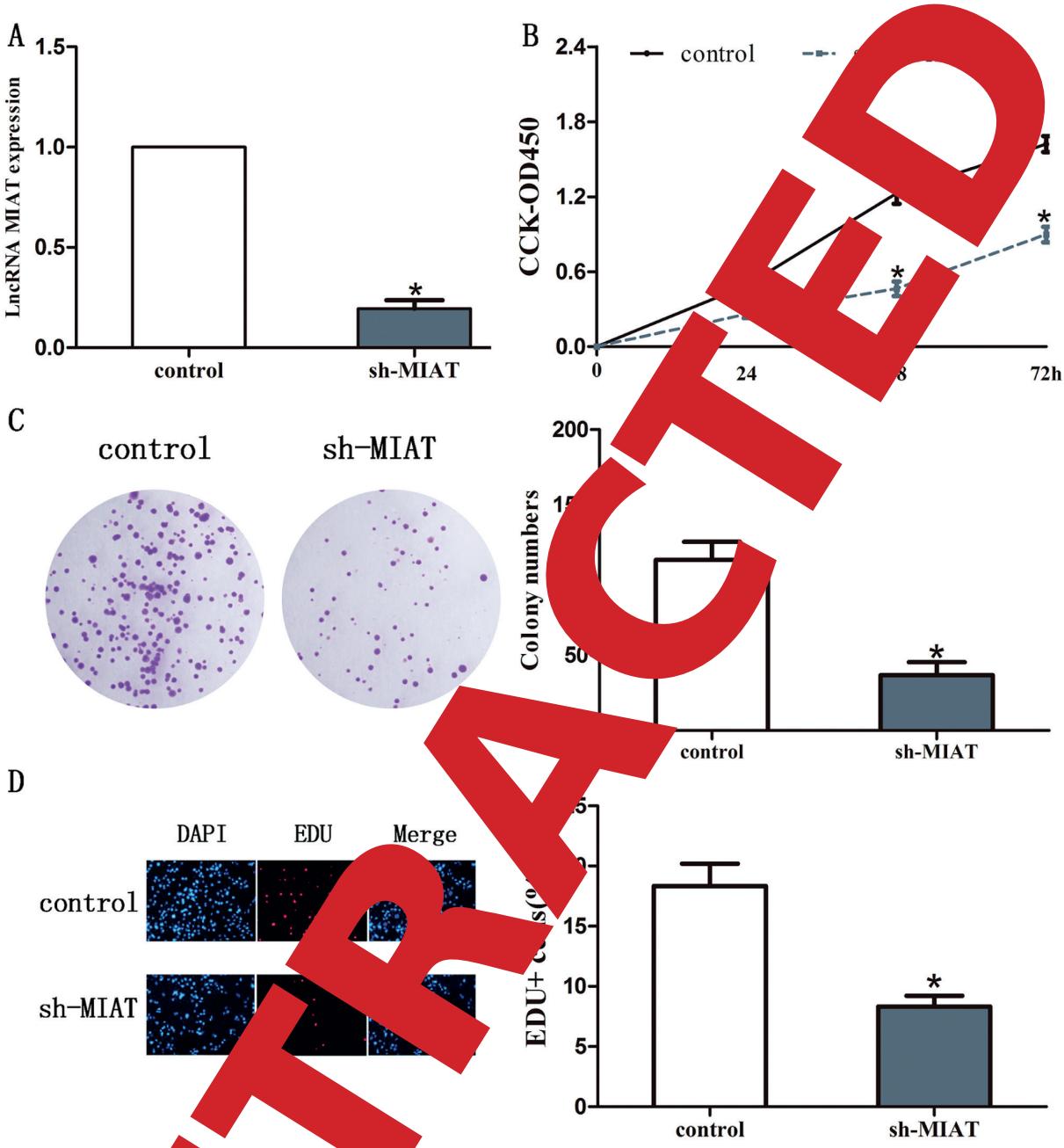
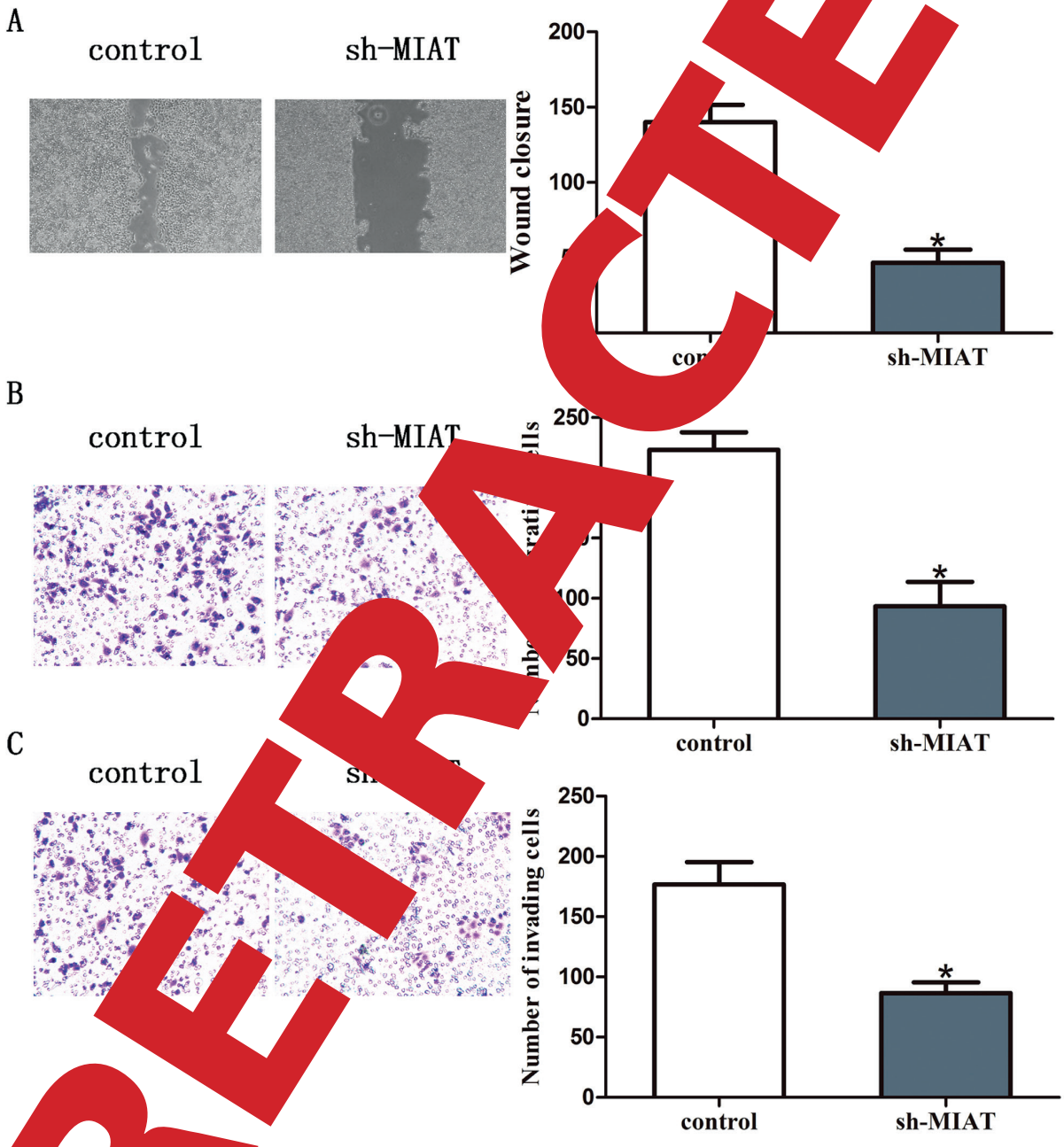


Figure 2. CCK8 a… showed knockdow… MIAT repressed NSCLC cell proliferation. ***A,*** MIAT expression in NSCLC cells transfected … sh-MI…T and control vector was detected by RT-qPCR. GAPDH was used as an internal control. ***B,*** CCK8 assay sho… that kn… own of MIAT significantly repressed cell proliferation in NSCLC cells. ***C,*** Colony formation assay showed … umbe… olonie… s significantly decreased *via* knockdown of MIAT in NSCLC cells (magnification: 40×). ***D,*** EdU … assay s… d that number of EdU positive cells was significantly decreased *via* knockdown of MIAT in NSCLC … resul… present the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$ as co… … control cells.

c… M… … the progression of breast can… rving as a ceRNA for miR-155-5p[9]. MIAT … colorectal cancer growth and tumor meta… y targeting Derlin-1[10]. In this study, we found that MIAT was upregulated both in NSCLC samples. Besides, silence of MIAT repressed cell proliferation of NSCLC cells. Above results indicated that MIAT promotes tumorigenesis of NSCLC and might act as an oncogene. To further identify the underlying mechanism of how

MIAT affects NSCLC, we used Starbase v2.0 to predict the target proteins of MIAT and picked TDP43 as the potential targets of MIAT. TDP43 is aberrantly expressed in breast cancer and is associated with patients' prognosis[11]. TDP43 acts as an oncogene in melanoma and participates in regulation of melanoma path... Knockdown of TDP43 suppresses t... prog... *via* SRSF3 in triple-negative b... st cancer[13]. ...43 enhances cell proliferati... d invasion ... targeting HDAC6 in glioblas... A recent ...dy[15] reveals that TDP43/MALA... ...nces



Fi... B. W... ay and transwell assay showed that knockdown of MIAT inhibited NSCLC cell migration and in... nd-healing assay showed that knockdown of MIAT significantly repressed cell migration in NSCLC cells (magni... 0×). ***B,*** Transwell assay showed that number of migrated cells was significantly decreased *via* knockdown of MIAT i... lls (magnification: 40×). ***C,*** Transwell assay showed that number of invaded cells was significantly decreased *via* kno... of MIAT in NSCLC cells (magnification: 40×). The results represent the average of three independent experiments (mean ... standard error of the mean). *$p<0.05$, as compared with the control cells.

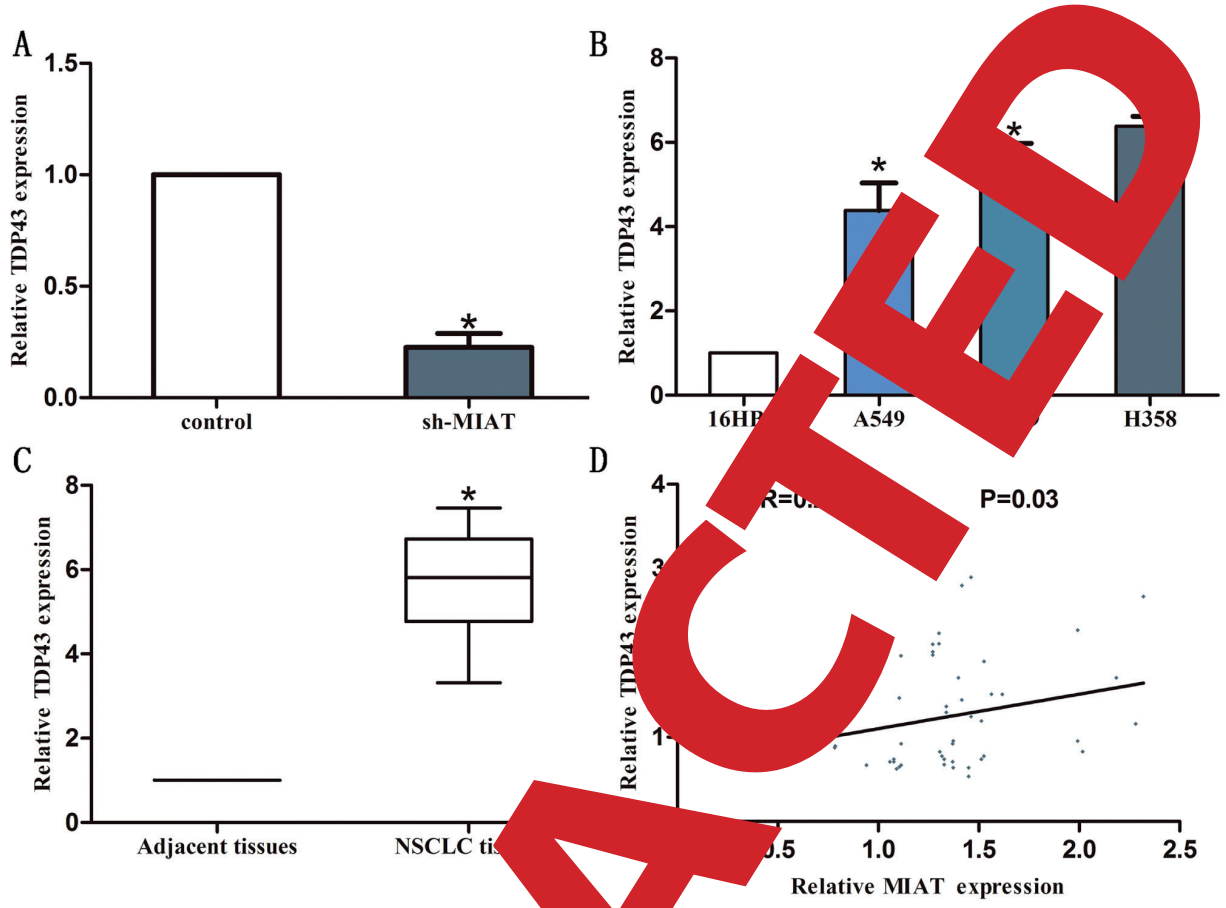


Figure 4. Interaction between MIAT and TDP43. ***A,*** [illegible] results showed that TDP43 expression was decreased in sh-MIAT compared with the control group. ***B,*** Expression l[illegible] of TDP43 relative to GAPDH were determined in the human NSCLC cell lines and 16HBE by RT-qPCR. [illegible]43 was sig[illegible]ly upregulated in NSCLC tissues compared with adjacent tissues. ***D,*** The linear correlation betwe[illegible]ssion level [illegible]DP43 and MIAT in NSCLC tissues. The results represent the average of three independent expe[illegible]nts. D[illegible]ented as the mean ± standard error of the mean. *$p<0.05$.

development and meta[illegible]s of NSCLC. [illegible]the present study, we fir[illegible]ered the interaction between TDP[illegible]nd [illegible]TDP43 could be downregulated after knoc[illegible]f MIAT. Besides, TDP43 [illegible]pression was h[illegible]er in NSCLC cell lines. [illegible]rthermore, TDP43 expression in NSCLC ti[illegible]s was [illegible]itively related to MIAT expression. [illegible]lts ab[illegible] indicated that MIAT might [illegible] tumo[illegible]nesis of NSCLC through upregulat[illegible]

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[illegible]strated that MIAT could facilitate cell pro[illegible] in NSCLC through upregulating TDP43, pro[illegible] candidate target for NSCLC treatment.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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