High Metastasis-Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) Expression Promotes Proliferation, Migration, and Invasion of Non-Small Cell Lung Cancer via ERK/Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

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Background: In present study, we explored the function of the metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) gene in the development of non-small cell lung cancer (NSCLC).

Material/Methods: qRT-PCR was used to detect the *MALAT1* mRNA expression level in cancer tissues and adjacent normal tissues of 115 NSCLC patients and in cell lines. *MALAT1*-mimic, *MALAT1*-inhibitor, and corresponding negative controls (NC) were utilized to transfect the H460 cells. Proliferation, migration, and invasion of H460 cells were evaluated by MTT method and Transwell assay. Expression levels of proteins in the ERK/MAPK signaling pathway were assessed by Western blot analysis.

Results: *MALAT1* mRNA was upregulated in NSCLC tissues and cell lines compared to that in adjacent tissues and normal human bronchial cell line (BEAS-2B), respectively. Overexpression of *MALAT1* significantly strengthened the proliferation, migration, and invasion ability of H460 cells. In comparison with the NC group, expression levels of *CXCL5* and p-*JNK* proteins were elevated, while p-*MAPK* and p-*ERK* proteins were decreased in the *MALAT1*-mimic group. *MALAT1* targets the 3'- untranslated region (UTR) fragment of the *CXCL5* gene and inhibits its translation. Disturbance of the *CXCL5* gene can reduce the protein expression of *MAPK*, p-*MEK*1/2, p-*ERK*1/2, and p-*JNK*, and inhibit the proliferation, migration, and invasion of *MALAT1*-mimic cells.

Conclusions: High *MALAT1* expression promotes the proliferation, migration, and invasion of non-small cell lung cancer via the ERK/MAPK signaling pathway.

MeSH Keywords: Adenocarcinoma • Lung Neoplasms • MAP Kinase Signaling System • Small Cell Lung Carcinoma

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Background

Non-small cell lung cancer (NSCLC) is a malignant tumor with high incidence and mortality rates [1]. Various factors have been found act as risk factors for the development of NSCLC [2,3]. DNA damage is the primary cause for cancer development [4]. Deficiency in DNA repair also leads to abnormal physiological processes of NSCLC [5]. Abnormal inflammation, formation of blood vessels, and hypoxia were found to play important roles in the development of cancer [6–8]. Therefore, any factors, especially genetic factors, involved in these processes may contribute to the development of NSCLC.

Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is a long noncoding RNA (lncRNA) that is highly conserved in mammals [9]. *MALAT1* is involved in numerous physiological processes, including transcription, splicing, translation, and epigenetic modulating of gene expression [10,11]. Numerous studies suggest that abnormal expression of *MALAT1* is significantly correlated with different pathological processes [12,13]. Deficiency of *MALAT1* can reduce the formation of blood vessels and blood flow perfusion in mice [14]. A recent study found that *MALAT1* participates in inflammation pathways, cell cycle, cell death, and other pathways in brain injury [15]. Sallé-Lefort et al. found that *MALAT1* expression level was obviously increased in hypoxia condition [16]. Many researches indicated that *MALAT1* is increased in various cancers and predicts poor overall survival [17,18]. It acts as an oncogene in several cancers [19]. While, Kwok and colleagues suggested that *MALAT1* acts as a tumor suppressor in colon and breast cancers [20]. Therefore, *MALAT1* might play a crucial role in the development of NSCLC [21]. However, the exact mechanism of *MALAT1* still not clear.

In the present study, we analyzed the mechanism of *MALAT1* in NSCLC development and assessed the expression level and function of *MALAT1* in NSCLC. The target gene and signaling pathway of *MALAT1* were also assessed in this study.

Material and Methods

Sample collection

We recruited 115 NSCLC patients from the PLA General Hospital between January 2017 and December 2017. These patients did not receive any radiotherapy or chemotherapy before the operation. All the patients received radical lobectomy, and the cancer tissues and adjacent normal tissues (≥5 cm away from tumor tissues) were collected as samples. All tissue specimens were diagnosed by pathological examinations, and the results were confirmed by 2 pathologists. This study was approved by Ethics Committee of the PLA General Hospital. All patients signed informed consent before being enrolled in this study.

Cell culture

NSCLC cell lines (A549, H661, andH460) and a normal human bronchial cell line (BEAS-2B) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. These cell lines were cultured in RPMI-1640 or DMEM medium with 10% fetal bovine serum (FBS). Cultural conditions were: saturated humidity with 5% CO_2 at 37°C, replacing medium every 2 days until the cells fused into a single layer. Cells were digested by 0.25% trypsin, then subcultured at a 1: 3 ratio.

MALAT1 mRNA expression analysis

qRT-PCR (quantitative real-time reverse transcriptional polymerase chain reaction) was used to detect *MALAT1* mRNA expression in tissues and cells. NSCLC cells were collected in exponential growth phase. Total RNA was extracted from these cells and tissues by Trizol. The PrimerScript RT reagent kit (Takara, Dalian, China) was used in the reverse transcription of *MALAT1* cDNA, then the ABI 7900HTSequence Detection System was utilized for the detection of *MALAT1* expression.

Transfection of *MALAT1* into H460 NSCLC cells

Logarithmic cells with good growth state were digested for transfection. Overexpression vector (*MALAT1*-mimic), silent vector (*MALAT1*-inhibitor), and corresponding negative controls (NC) were diluted and mixed with Lipofectamine™ 2000, then cultured at room temperature for 20 min. This mixture (50 µmol/ml) was placed into 6-well plates with H460 cells (concentration $1×10^5$/ml), fully mixed, and cultured for 6 h, then we replaced the medium and continued cultivated for 48 h. Transfection results were verified by qRT-PCR.

Cell proliferation analysis

(4,5-dimethylthiazol-2-yl)-2,5-diphenyltet-razolium bromide (MTT) was used for the cell proliferation analysis. We added 200 µl transfected H460 cells ($1×10^4$/ml) added into 96-well plates, cultured in a 37°C incubator with 5% CO_2, and we replaced the medium every day. The medium was discarded at 24 h, 48 h, and 72 h. After washing in PBS, 20 µl MTT solution was added to every well and incubated at 37°C for 4h. Then, we discarded the solution, added 150 µl DMSO, oscillated in the dark for 10 min. The OD value of every well was assessed by enzyme-linked immunoassay at 490 nm.
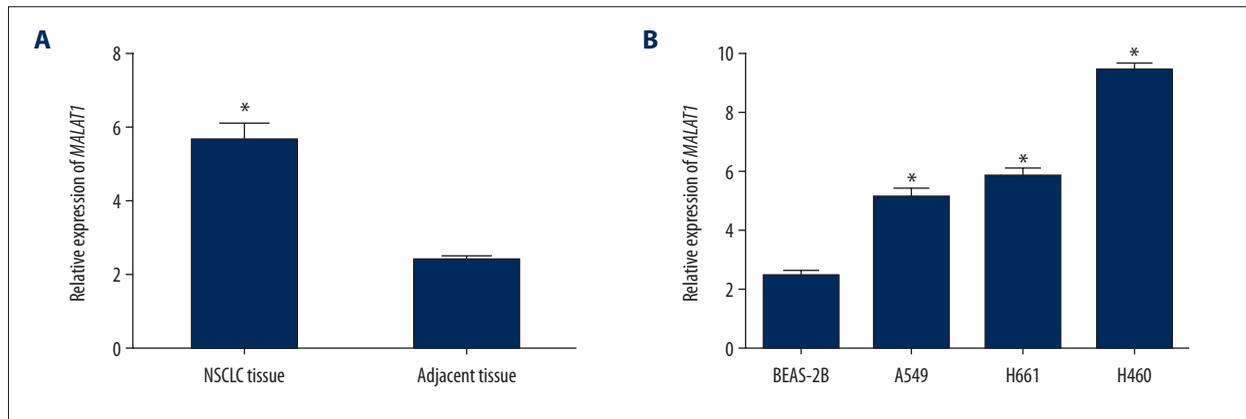
Figure 1. *MALAT1* expression level in NSCLC tissues and cell lines. (**A**) Expression level of *MALAT1* was significantly higher in NSCLC tissues than in adjacent tissues. (**B**) Expression level of *MALAT1* was significantly higher in A549, H661, and H460 cell lines than that in normal bronchial cell line (BEAS-2B). * *P*<0.001.

Assay of invasion and migration

Invasion analysis was performed in Transwell chambers with Matrigel, and migration analysis was performed in Transwell chambers without Matrigel.

In 24-well plates, we placed 50 µl DMEM medium with 10% fetal bovine serum (FBS) and 200 µl cell suspension into the upper Transwell chamber, and we placed 500 µl DMEM medium (including 10% FBS) into the lower chamber, followed by incubation at 37°C for 48 h. The upper chambers were stained by crystal violet. We randomly selected 10 views, and a 200× inverted microscope was used to count the cells. Every experiment was performed in in triplicate.

Western blot analysis

Total proteins were extracted from the logarithmic growth phase cells. We used 12% SDS-PAGE electrophoresis to separate the proteins. After being transferred to polyvinylidene difluoride (PVDF) membranes, the proteins were blocked by 10% fat milk, and incubated by primary and secondary antibodies associated with the ERK/MAPK signaling pathway: CXCL5 antibody, MAPK antibody, p-MEK1/2 antibody, p-ERK1/2 antibody, and p-JNK antibody. Finally, the results were observed by use of an imager.

Dual-luciferase reporter analysis

To verify whether *CXCL5* is the target gene of the *MALAT1* gene, wide- and mutant-3' untranslated regions (UTR) of the *CXCL5* gene were transferred into dual-luciferase reporter vector (pmirGLO) and examined by direct sequencing. Transfection of *CXCL5*-pmirGLO into H460 cells were performed with Lipofectamine™ 2000 (Invitrogen). After being cultured at 37°C for 48 h, the cells were detected by a Dual-Luciferase Reporter Assay System.

Statistical analysis

All figures were plotted using GraphPad Prism. SPSS 18.0 was used to perform the data analysis. Continuous variables between different groups are presented by mean±SD and assessed by *t* test. Results had statistical significance when *P*<0.05.

Results

MALAT1 was upregulated in NSCLC tissues and cell lines

Expression levels of *MALAT1* were significantly higher in NSCLC tissues than in adjacent normal tissues (Figure 1A, *P*<0.001), and it was also significantly higher in A549, H661, and H460 cell lines than in the normal bronchial cell line (BEAS-2B) (Figure 1B, *P*<0.001). In the 3 NSCLC cell lines, *MALAT1* expression level was significantly higher in H460 cells and lower in A549 cells. Therefore, H460 cells were selected for further analysis.

Transfection of *MALAT1* in H460 cells

qRT-PCR was used to assess the transfection results of H460 cells. We found that *MALAT1* was overexpressed in *MALAT1* mimic cells and reduced in *MALAT1* inhibitor cells (Figure 2, *P*<0.05), suggesting that the transfection of *MALAT1* was successful.

Overexpressed *MALAT1* promoted the proliferation, invasion, and migration of H460 cells

MALAT1-mimic, *MALAT1*-inhibitor, and relative NC were transferred into H460 cells. We assessed the effects of *MALAT1* expression levels on biological behaviors of H460 cells. We found that overexpressed *MALAT1* promoted the proliferation, invasion, and migration of H460 cells; but low *MALAT1* expression

levels inhibited the proliferation, invasion, and migration of H460 cells (Figure 3, *P*<0.001).

Effects of *MALAT1* for proteins associated with ERK/MAPK signaling pathway

Western blot analysis showed that *CXCL5 and* p-*JNK* expressions were significantly increased, and p-*MAPK and* p-*ERK* were distinctly decreased in the *MALAT1*-mimic group. In the *MALAT1*-inhibitor group, *CXCL5*, p-*JNK*, and p-*MEK1/2* expression levels were significantly lower than in the NC group (Figure 4, *P*<0.001).

CXCL5 acts as the target gene of *MALAT1*

Dual-luciferase reporter analysis result indicated that low expression of *MALAT1* could suppress the expression of luciferin, but when mutant 3'-UTR was combined with luciferin, the suppression was lost, suggesting that the 3'-UTR of *CXCL5* acts as the target of *MALAT1* and inhibits its expression (Figure 5).
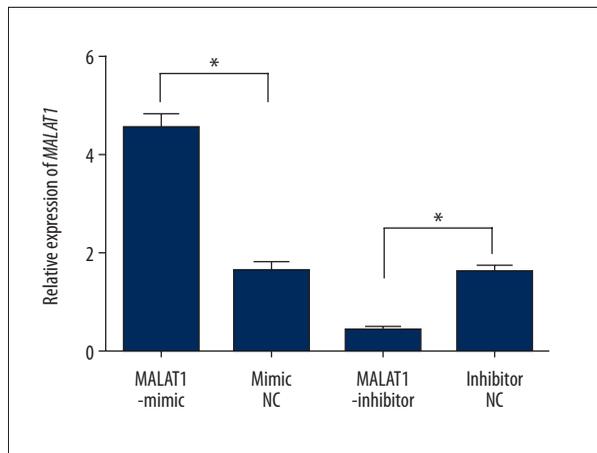
Effects of *CXCL5* on proteins associated with ERK/MAPK signaling pathway

CXCL5 was silenced by *CXCL5* siRNA (siCXCL5) in *MALAT1*-mimic H460 cells, then we examined the *CXCL5*, *MAPK*, p-MEK1/2, p-*ERK1/2*, and p-*JNK* protein levels by Western blot analysis. The results showed that p-*MEK1/2*, p-*ERK1/2*, and p-*JNK* proteins were significantly lower in the *MALAT1*-mimic +si*CXCL5* group compared to the than in *MALAT1*-mimic group (Figure 6, *P*<0.05).

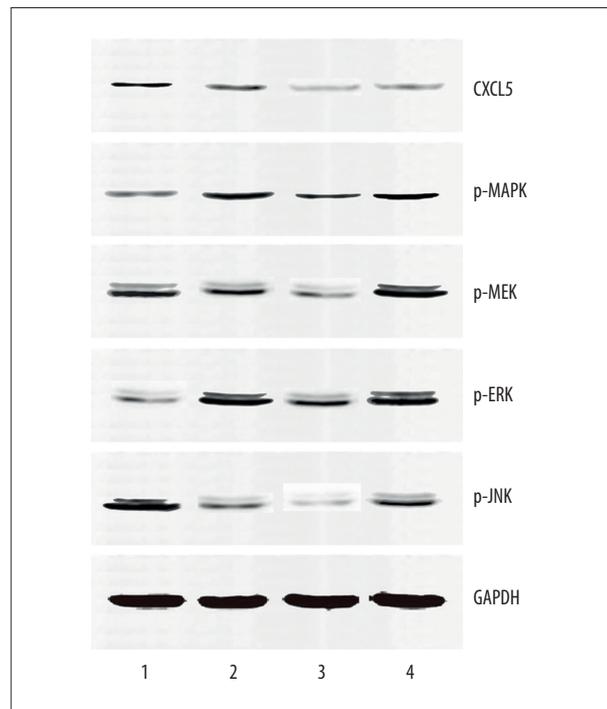**Figure 2.** *MALAT1* transfection in H460 cells. * *P*<0.05.

Figure 4. Effects of *MALAT1* for proteins associated with the ERK/MAPK signaling pathway. 1: *MALAT1*-mimic; 2: Mimic NC; 3: *MALAT1*-inhibitor; 4: Inhibitor NC.
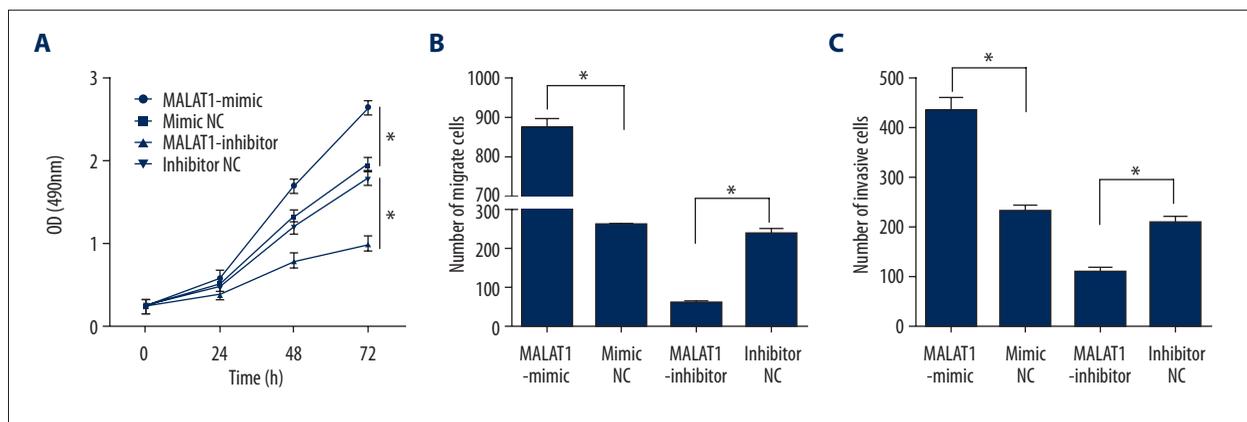

Figure 3. Effects of *MALAT1* for biological behaviors of H460 cells. (**A**) Effects of *MALAT1* for H460 proliferation. (**B**) Effects of *MALAT1* for H460 migration. (**C**) Effects of *MALAT1* for H460 invasion. * *P*<0.001.

Silenced *CXCL5* inhibited the proliferation, migration, and invasion of H460 cells

We also detected the alteration of H460 biological behaviors in *MALAT1*-mimic+si*CXCL5,* finding that knockout of *CXCL5* inhibited the proliferation, migration, and invasion of H460 cells, which was caused by the *MALAT1* overexpression (Figure 7).

Discussion

lncRNA *MALAT1* is significantly associated with several diseases. *MALAT1* is upregulated in numerous tumors, and its abnormal expression causes many pathological alterations of tumors [22].

In the present study, we explored the expression of *MALAT1* in tissues and different cell lines, showing that the expression level of *MALAT1* was significantly increased in NSCLC tissues and cell lines, and the H460 cell line had the highest *MALAT1* level. This agrees with a previous study performed by Guo et al., who suggested that *MALAT1* was upregulated in NSCLC cells and tissues [23]. Elevated *MALAT1* has also been discovered in esophageal squamous cell carcinoma (ESCC) tissues and influences tumor development [24]. We explored the effects of *MALAT1* on the biological behaviors of H460 cells, finding that upregulated *MALAT1* in H460 cells accelerates the biological behaviors of cells, including proliferation, invasion, and migration activity. Guo and colleagues indicated that knockout of *MALAT1* inhibits the invasion and migration of NSCLC cells [23]. Proliferation and migration of prostate cancer (PC) cells were shown to be promoted by increased *MALAT1* levels [25].
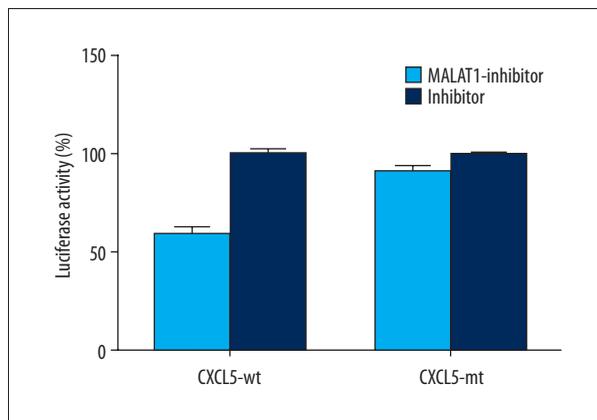
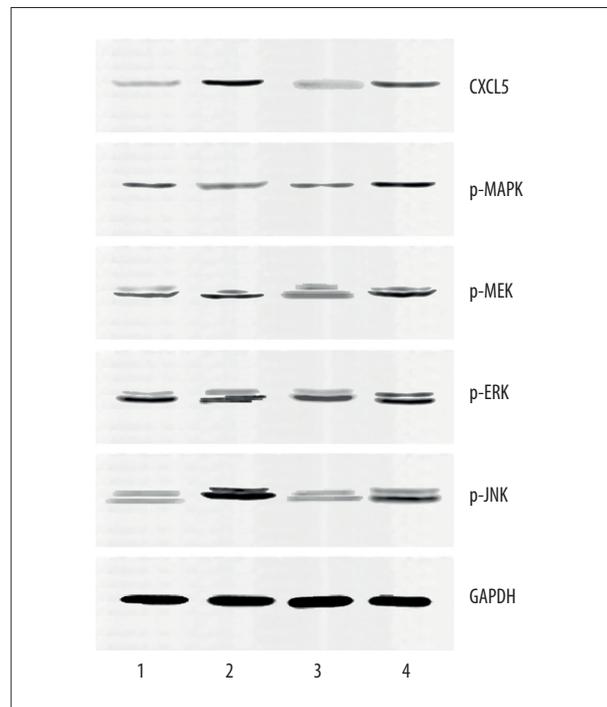**Figure 5.** Dual-luciferase reporter analysis result.

Figure 6. Effects of *CXCL5* siRNA for proteins associated with the ERK/MAPK signaling pathway. 1: *MALAT1*-mimic +si*CXCL5*; 2: *MALAT1*-mimic; 3: Mimic NC+si*CXCL5*; 4: Mimic NC.
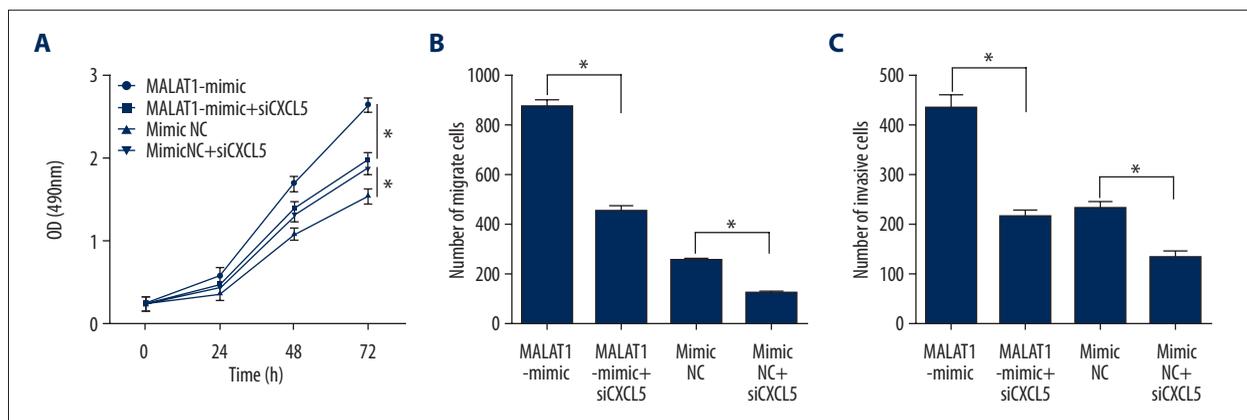
Figure 7. Knockout of *CXCL5* in *MALAT1*-mimic cells inhibits the proliferation, migration, and invasion of H460 cells. (**A**) Effects of *CXCL5* for H460 proliferation. (**B**) Effects of *CXCL5* for H460 migration. (**C**) Effects of *CXCL5* for H460 invasion. * $P<0.001$.

We found that the 3'-UTR of *CXCL5* is the target gene of *MALAT1*. In the *MALAT1*-mimic group, *CXCL5 and* p-*JNK* were upregulated, while p-*MAPK and* p-*ERK* were downregulated, which is in accordance with results in different tumors. Guo and coworkers found that knockout of *MALAT1* downregulates *CXCL5* expression [23]. Leti et al. suggested that knockout of *MALAT1* suppresses the *CXCL5* expression in hepatic stellate cells [26]. Chen and colleagues found that knockdown of *MALAT1* inhibits the activity of *JNK* [27]. However, Lei et al. indicated that low *MALAT1* inhibits p-*ERK* expression and had no effects for p-*JNK* expression [28]. These results suggested that *CXCL5* is the target of *MALAT1*, and that it plays an important role in tumor development via the ERK/MAPK signaling pathway.

When we knocked out the *CXCL5* gene, p-*MEK1/2*, p-*ERK1/2*, and p-*JNK* proteins were distinctly lower in the *MALAT1*-mimic +si*CXCL5* group than in the *MALAT1*-mimic group. We also assessed the biological behaviors of H460 cells in different groups. In the *MALAT1*-mimic+si*CXCL5* cells, we found that the proliferation, migration, and invasion activities of H460 cells were reduced by silencing *CXCL5*. Guo and colleagues showed that knockout of *CXCL5* in A549 cells inhibits migration and invasion [23]. Wang and colleagues suggested that *CXCL5* facilitates the proliferation of H460 cells via activation of MAPK/ERK1/2 and PI3K/AKT pathways [29]. These results suggested that the function of *MALAT1* for NSCLC biological behaviors was performed by the ERK/MAPK signaling pathway via

the *CXCL5* gene. A similar function has been found in other cancers. Du et al. suggested that *MALAT1* acts as a suppressor for glioma through the ERK/MAPK signaling pathway [30,31].

Certain limitations of the present study must be considered. First, the function of *MALAT1* for NSCLC development was only verified in the H460 cell line, which might have influenced the accuracy of our results. However, published articles have reported the oncogenic function of *MALAT1* in other lung cancer cell lines, such as A549 [32] and H1299 [33], which was in accordance with our results. Secondly, effects of *MALAT1* for other signaling pathways associated with tumor occurrence were did not assessed in the present study. Finally, the mechanism of *MALAT1* in NSCLC development was not assessed in animal models.

Conclusions

We found that *MALAT1* was upregulated in NSCLC tissues and cell lines, and it promotes the proliferation, invasion, and migration of H460 cells. *CXCL5* acts as the target gene of *MALAT1* and mediates the function of *MALAT1* for the biological behaviors of H460 cells. Further studies focused on the association of *MALAT1* with other signaling pathways should be performed in the future, so as to verify the mechanism of *MALAT1* in NSCLC development.

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