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Original Paper

Musashi1 Promotes Non-Small Cell Lung Carcinoma Malignancy and Chemoresistance via Activating the Akt Signaling Pathway

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Key Words
Msi1 • NSCLC • Chemoresistance • Akt • MiR-181a-5p

Abstract
Background/Aims: Lung cancer is one of the leading causes for cancer mortality. The poor therapeutic outcome of non-small cell lung carcinoma (NSCLC) is mainly due to late diagnosis and chemoresistance. In this study, we investigated the role of Musashi1 (MSI1) in NSCLC malignancy and chemoresistance. ***Methods:*** Colony formation, MTT, glucose uptake and lactate production assays were employed to study lung cancer cell malignancy and chemoresistance. RT-PCR and Western blotting were performed to detect mRNA and protein expressions of genes. We used immunohistochemistry and Pearson correlation analysis to study the relationship of gene expression. ***Results:*** We demonstrated that MSI1 was able to promote the proliferation and glucose metabolism of NSCLC cells, and to mediate the sensitivity to chemotherapy drugs in NSCLC cells. Importantly, we found that MSI1 could regulate the activity of Akt signaling. The regulation of NSCLC proliferation, glucose metabolism and chemoresistance by MSI1 was dependent on the modulation of the activity of the Akt signaling pathway. We also found that MSI1 was a target of miR-181a-5p, a microRNA involved in the regulation of cancer development. The expression levels of MSI1 and miR-181a-5p were negatively correlated in NSCLC. ***Conclusion:*** MSI1 promotes non-small cell lung carcinoma malignancy and chemoresistance via activating the Akt signaling pathway, which provides a new strategy for the therapy of NSCLC.

Introduction

Lung cancer is one of the leading causes for cancer mortality in both men and women [1]. Histologically, lung cancer can be categorized into small cell lung carcinoma (SCLC) and

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non-small cell lung carcinoma (NSCLC). NSCLC encompasses a number of cancer subtypes with heterogeneous cell origin and morphology, including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. With these subtypes combined, NSCLC accounts for nearly 85% of all lung cancer cases [2]. Although advancement of scientific and clinical research has been made recently, the therapeutic outcome of NSCLC remains poor. This situation is primarily due to the fact that a large proportion of patients diagnosed with NSCLC are found to be in advanced stages, and therefore often suffered from metastasis and recurrence [3]. Chemotherapy with platinum-based drugs, such as cisplatin, is one of the most effective treatment regimens for NSCLC [3]. Unfortunately, many NSCLC patients develop resistance to cisplatin during the course of treatment, leading to failure of chemotherapy. Extensive evidence has demonstrated that modulation in signaling pathways supporting the growth, proliferation and survival of cells contributes to the development of chemoresistance [4].

Recently, a series of studies have suggested the involvement of Musashi1 (MSI1) in the growth and proliferation of multiple cancer types. High expression levels of MSI1 were found in gallbladder [5], gastric [6], colorectal [7] cancers and glioblastoma [8]. In addition, increased levels of MSI1 expression in breast cancer were correlated with cancer metastasis and poor outcome [9]. MSI1 was initially identified in *Drosophila melanogaster* to regulate asymmetric cell division during the development of sensory organs [10], and was later found in mammalian tissues as a stem cell marker [11-13]. As an RNA-binding protein, MSI1 has been shown to modulate the translation activities of a number of target genes involved in cancer development, including Numb, a negative regulator of the Notch signaling pathway [14], $p21^{WAF1}$, a negative regulator of cyclin-dependent kinases [15], and APC, a negative regulator of Wnt signaling [16].

In particular, a recent study has demonstrated that MSI1 was also involved in the development of NSCLC, and that it could serve as a diagnostic marker for NSCLC [17]. However, the mechanism of action of MSI1 in the regulation of cancer development is still poorly understood. In this study, we demonstrated that MSI1 was able to promote the proliferation and glucose metabolism of NSCLC cells, and to mediate the sensitivity to chemotherapy drugs in NSCLC cells. Importantly, we found that MSI1 could regulate the activity of Akt signaling. The regulation of NSCLC proliferation, glucose metabolism and chemoresistance by MSI1 was dependent on the modulation of the activity of the Akt signaling pathway. We also found that MSI1 was a target of miR-181a-5p, a microRNA involved in the regulation of cancer development. The expression levels of MSI1 and miR-181a-5p were negatively correlated in NSCLC.

Materials and Methods

Patients and clinical samples

Clinical samples were surgically obtained from a total of 79 NSCLC patients in the Harbin Medical University Cancer Hospital. Samples were snap frozen and stored in liquid nitrogen. The stage of NSCLC was categorized according to surgical and pathological findings based on the guidelines described by the sixth edition of AJCC/UICC. Written informed consents were obtained from all patients. This study was approved by the ethic committee of the Harbin Medical University Cancer Hospital.

Cell lines and CDDP-resistant induction

The human lung cancer cell lines A549 and H522 were obtained from the American Type Culture Collection. Cells were grown in Dulbecco's Modified Eagle medium supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin, and 100 U/ml streptomycin. Cells were incubated at 37°C in a humidified incubator containing 5% CO_2. The CDDP-resistant A549 and H522 cells were established by 3-months intermittent exposure to 2μg/ml CDDP every other day.

Cell transfection

The human MSI1 gene was amplified from A549 cell cDNA by PCR and constructed into pcDNA3 vector (Invitrogen). MSI1 siRNA and control siRNA were purchased from Santa Cruz Biotechnology. Transfections

of plasmid or siRNA into cells were performed using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. The 3'UTR of MSI1 was PCR-amplified from A549 genomic DNA and cloned downstream of luciferase gene in pGL vector (Promega). For the reporter assay, cells were cultured in 96 well plates and transfected with luciferase reporters (50 ng), and 50 nM of miR-control or miR-181a-5p. After 48 h, luciferase activity was measured using dual-luciferase reporter system (Promega). The Renilla activity was used as an internal control.

Protein isolation and Western blot

Total proteins were extracted with RIPA lysis buffer with proteinase/phosphatase inhibitors (Thermo Scientific). Lysate was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the gel was blotted onto PVDF membrane (Millipore). The membrane was blocked in 1% BSA, and then incubated with one of the following antibodies: anti-MSI1 (Abcam) or anti-GAPDH (Santa Cruz Biotechnology). Horse radish peroxidase-conjugated anti-mouse and anti-rabbit IgG were incubated as the secondary antibodies. The image was acquired using the ECL plus Kit.

Cell proliferation assay

NSCLC cells were seeded into 96-well culture plates. At different time points a volume of 20 μl MTS (Sigma–Aldrich) was added into each well and the cells were incubated for additional 4 h. Cell growth was measured at wavelength of 490 nm.

Colony formation assay

The cells were seeded into six-well plates at a density of 200 cells/well after transfection and maintained in DMEM containing 10% FBS. The medium was replaced after 24 h and changed every three days. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were counted using NIH Image J software.

Measurement of oxidative phosphorylation and glycolysis

oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse XFe96 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA, USA). Briefly, NSCLC cells (1×10^4) were seeded into a XFe96 cell culture microplate overnight. The cells were switched into the Seahorse XF Base Medium supplemented with 2 mM sodium pyruvate prior to the beginning of the assay and maintained at 37°C in a CO_2 free incubator. OCR was reported in the unit of picomoles per minute and ECAR was reported in milli-pH units (mpH) per minute.

After baseline measurements, OCR and ECAR were measured by sequentially adding to each well with 20μl oligomycin, 0.5 or 0.25 μM carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP).

Immunohistochemistry

Immunohistochemistry was performed on 5mm thick sections obtained from formalin-fixed tissue embedded in paraffin. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave. Anti-MSI1 antibody (Abcam) was used as primary antibody at 1:100 dilution and incubated at 4°C overnight, and then were incubated with secondary antibody.

RNA extraction and real time PCR (RT-PCR)

Total RNA was extracted with Trizol reagent (Invitrogen) from NSCLC samples and cell lines according to the manufacturer's instructions. Total RNA (2 μg) was reverse transcribed to cDNA with first strand cDNA Synthsis Kit (Takara). The expression of mRNA was examined by RT-PCR with SYBR Premix Taq and Applied Biosystems 7500 Sequence Detection system. The relative expression levels of mRNA were normalized to GAPDH expression PCR reaction was performed using MSI1 primers: 5′-CTCCAAAACAATTGACCCTA-3′ (forward), 5′- GCTCAAAATATTGCTTCACG-3′(reverse); GAPDH primers: 5′-GTGGACATCCGCAAAGAC-3′ (forward), and 5′-AAAGGGTGTAACGCAACTA-3′(reverse). The expression level of miR-181a-5p was quantified using miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems) and normalized by U6 small nuclear RNA. The amplification results for RT-PCR was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data were analyzed using SPSS software 19.0. Results were represented as mean ± SEM. analyzed by *t*-test or one-way ANOVA. $P < 0.05$ in the *t*-test or one-way ANOVA was considered to be statistically significant.

Results

MSI1 promotes NSCLC cell proliferation

In order to examine the function of MSI1 in the development of NSCLC, we utilized two independent NSCLC cell lines, A549 and H522 cells. MSI1 expression levels in both cell lines

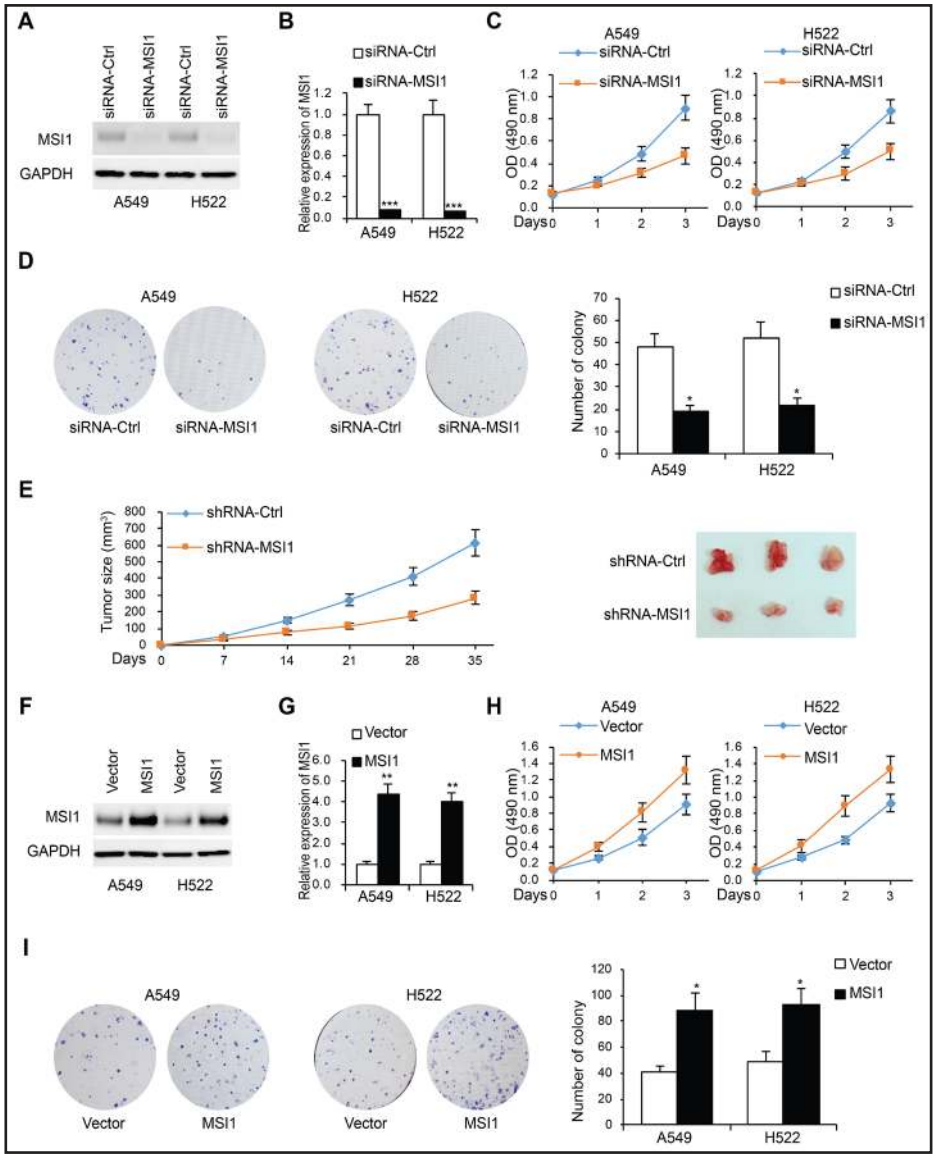

Fig. 1. MSI1 promotes NSCLC cell proliferation. A. Western blot analysis of MSI1 expression in MSI1 knockdown A549 and H522 cells. B. MSI1 mRNA expression was examined by RT-PCR in A549 and H522 cells transfected with MSI1 control (siRNA-Ctrl) or MSI1 siRNA (siRNA-MSI1). C. MTS assays of A549 and H522 cells with MSI1 knockdown. D. Representative micrographs (left) and quantification (right) of colony number in A549 and H522 cells transfected with Control or MSI1 siRNA. *p<0.05 versus cells transfected with siRNA-Ctrl. E. Subcutaneous tumor volumes of nude mice were measured at different time points. F. Western blot analysis of MSI1 expression in MSI1 overexpression A549 and H522 cells. G. MSI1 mRNA expression was examined by RT-PCR in A549 and H522 cells transfected with vector or MSI1 plasmid. H. MTS assays of A549 and H522 cells with MSI1 overexpression. I. Representative micrographs (left) and quantification (right) of colony number in A549 and H522 cells transfected with vector or MSI1 plasmid. *p<0.05 versus cells transfected with empty vector.

were efficiently reduced by siRNA transfection, as demonstrated by Western blot and RT-PCR analysis (Fig. 1A and 1B). We then performed MTS assay to study cell proliferation in response to reduced MSI1 expression in these cells. We found that decreased MSI1 levels in both A549 and H522 cells led to significantly reduced viable cells in MTS assay, suggesting suppressed proliferation of cancer cells (Fig. 1C). Colony formation assay also revealed inhibited cell growth in both NSCLC cell lines, as evidenced by many less colony counts (Fig. 1D). We next examined the effect of MSI1 knockdown on tumor growth *in vivo* by establishing subcutaneous xenografts in nude mice. As shown in Fig. 1F, knockdown of MSI1 reduced the growth of subcutaneous tumors (Fig. 1E). Conversely, MSI1 expression levels in A549 and H522 cells were increased by transfection of plasmids overexpressing MSI1 (Fig. 1F and 1G). As expected, both MTS assay (Fig. 1H) and colony formation assay (Fig. 1I) demonstrated that overexpression of MSI1 significantly promoted cell proliferation in NSCLC cells.

MSI1 promotes glucose metabolism in NSCLC cells

It has been well documented that cancer cells differ from normal cells in that they prefer to use glucose as their energy source [18]. To investigate whether MSI1 regulates glucose

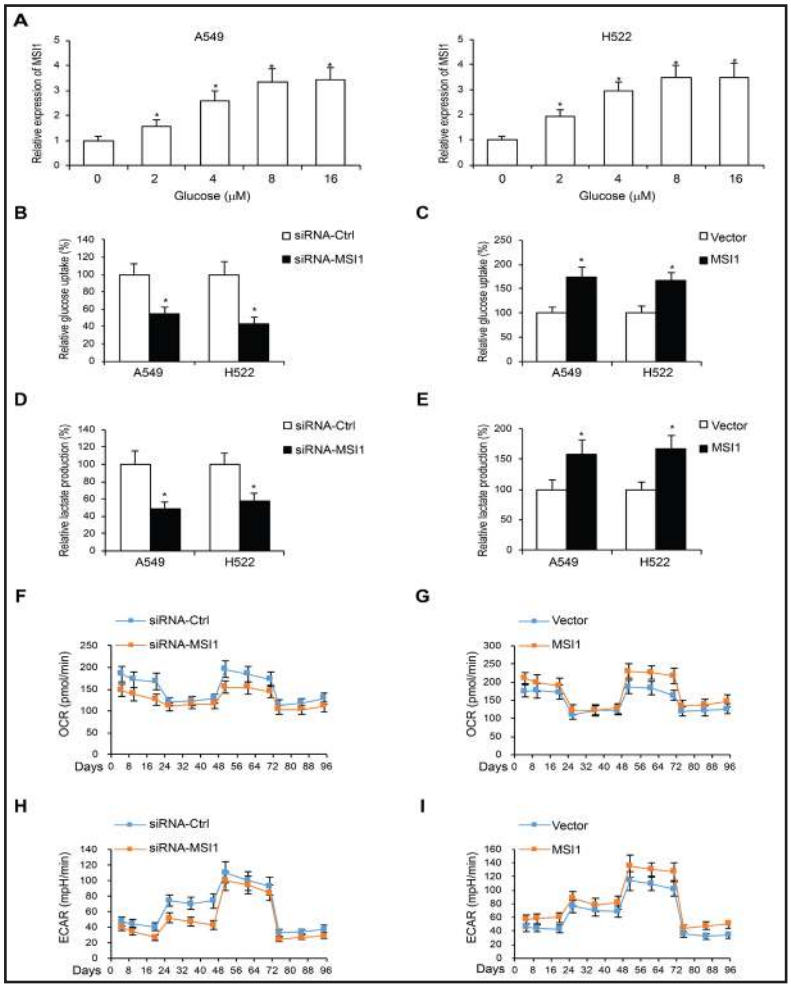

Fig. 2. MSI1 promotes NSCLC cell glucose metabolism. A. RT-PCR analyses of MSI1 levels in A549 and H522 cells treated with different doses of glucose (0, 2, 4, 8 and 16μM). *p<0.05 versus cells treat with 0μM glucose. B. Glucose uptake analysis in A549 and H522 cells transfected with siRNA-Ctrl or siRNA-MSI1. *p<0.05 versus cells transfected with siRNA-Ctrl. C. Glucose uptake analysis in A549 and H522 cells transfected with vector or MSI1 plasmid. *p<0.05 versus cells transfected with empty vector. D. Lactate production in A549 and H522 cells transfected with siRNA-Ctrl or siRNA-MSI1. *p<0.05 versus cells transfected with siRNA-Ctrl. E. Lactate production in A549 and H522 cells transfected with vector or MSI1 plasmid. *p<0.05 versus cells transfected with empty vector. F. Real-time mitochondrial oxygenconsumption rate (OCR) was measured in A549 cells transfected with siRNA-Ctrl or siRNA-MSI1. G. OCR was analyzed in A549 cells transfected with vector or MSI1 plasmid. H. Extracellular acidification rate (ECAR) was measured in A549 cells transfected with siRNA-Ctrl or siRNA-MSI1. I. ECAR was analyzed in A549 cells transfected with vector or MSI1 plasmid.

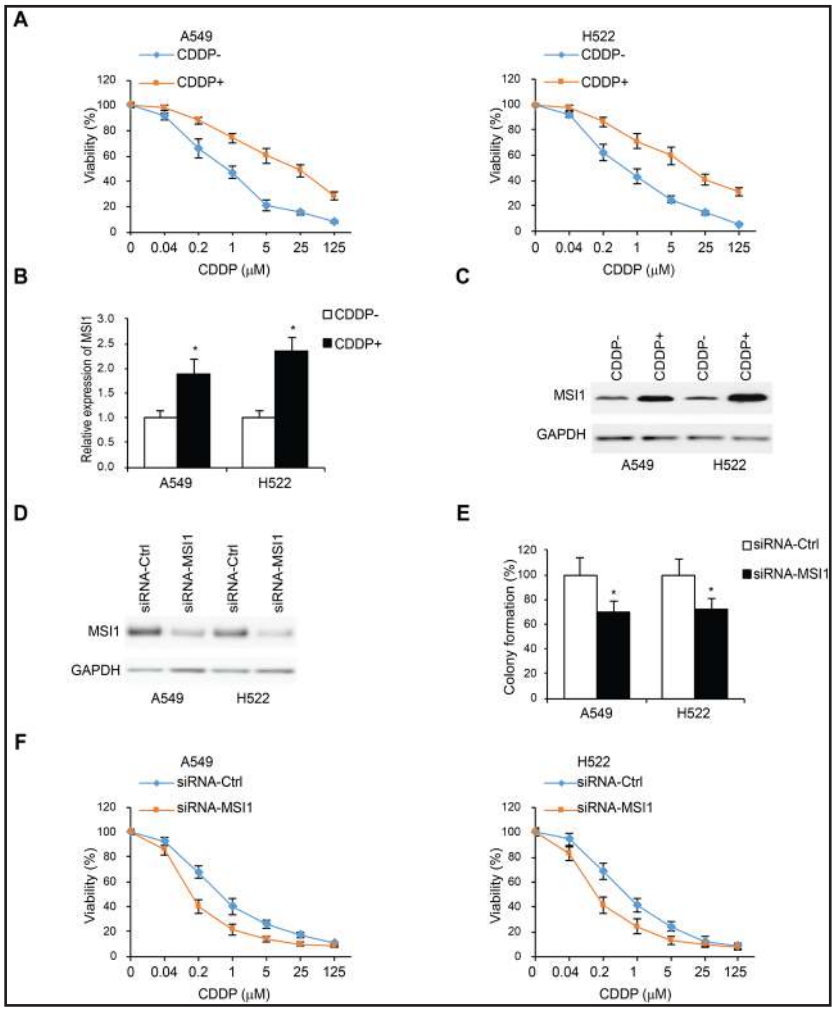

Fig. 3. MSI1 enhances NSCLC cell chemoresistance. A. Cell viability assays in CDDP resistant A549 and H522 cells (CDDP+). B. RT-PCR analysis of MSI1 levels in CDDP resistant A549 and H522 cells. *p<0.05 versus wild type cells (CDDP-). C. Western blot analysis of MSI1 levels in CDDP+ A549 and H522 cells. D. Western blot analysis of MSI1 levels in CDDP+ A549 and H522 cells with MSI1 knockdown. E. Colony formation rate in MSI1 knockdown CDDP+ A549 and H522 cells treated with 0.2μM CDDP. *p<0.05 versus cells transfected with siRNA-Ctrl. F. Cell viability assays in MSI1 knockdown CDDP+ A549 and H522 cells treated with different doses of CDDP (0, 0.04, 0.2, 1, 5, 25 and 125μM).

metabolism of NSCLC cells, we first examined the expression levels of MSI1 in response to different glucose concentrations in culture medium. We found that in both A549 and H522 cells, the expression levels of MSI1 were potentiated in a dose dependent manner by the increasing glucose concentrations (Fig. 2A). Knocking down MSI1 led to less glucose uptake in NSCLC cells, suggesting reduced glucose utilization and metabolic rate (Fig. 2B). In contrast, overexpression of MSI1 led to stimulation of glucose uptake (Fig. 2C). The major metabolic product of glucose in cancer cells is lactate [18]. We monitored the amount of lactate released into the media in NSCLC cell with modified expression of MSI1. We found that knock down of MSI1 resulted in less lactate output (Fig. 2E), while overexpression of MSI1 led to significantly increased production of lactate (Fig. 2F). Furthermore, we examined the effects of MSI1 expression on mitochondrial respiration and aerobic glycolysis. As shown in Fig. 2F to 2I, knockdown or overexpression of MSI1 decreased or increased cell oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in A549 and H522 cells. Taken together, these results indicated that MSI1 promoted glucose metabolism in NSCLC cells.

MSI1 mediates the sensitivity to chemotherapy drug in NSCLC cells

Since MSI1 participated in the regulation of proliferation and glucose metabolism of NSCLC cells, we next sought to determine whether MSI1 expression was associated with the sensitivity of NSCLC cells to platinum based chemotherapy. We first established

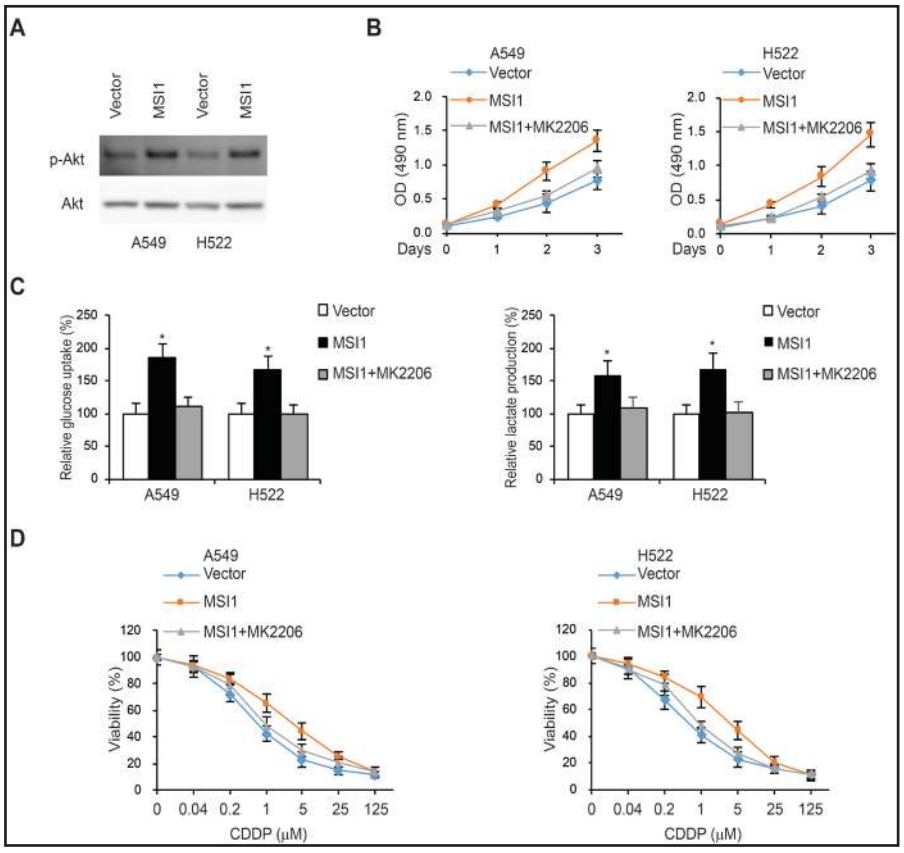

Fig. 4. MSI1 regulates NSCLC cells proliferation and chemoresistance through the Akt signaling pathway. A. Western blot analysis of total and phosphorylated (S473) Akt expression in A549 and H520 cells transfected with empty vector or plasmid overexpressing MSI1. B. MTT cell proliferation, C. glucose uptake, D. lactate production, and E. Cell viability assays performed in A549 and H522 cells transfected with empty vector or plasmid overexpressing MSI1, in the absence or the presence of MK2206. *p<0.05 versus cells transfected with vector.

CDDP-resistant A549 and H522 NSCLC cells. The ability of drug resistance was confirmed by cell viability assays (Fig. 3A). A549 and H522 cells were then treated with platinum based chemotherapy drug CDDP, and we found that the expression levels of MSI1 were significantly increased in response to this treatment, as evidenced by both quantitative real time PCR analysis for mRNA (Fig. 3B) and by Western blot analysis for protein (Fig. 3C). We next sought to determine whether the expression of MSI1 was required for the resistance to chemotherapy in NSCLC cells. We knocked down the expression of MSI1 in both A549 and H522 cells (Fig. 3D), and then subjected these cells to CDDP treatment. Compared to control cells, cells with reduced MSI1 expression formed significantly less colonies (Fig. 3E), suggesting decreased resistance to chemotherapy drug treatment. We also performed cell viability assay on A549 and H522 cells with reduced MSI1 expression levels following the treatment of a series of concentrations of CDDP (0.04, 0.2, 1, 5, 25, 125 μM). We found that while increasing concentrations of CDDP led to decreased cell viability in both A549 and H522 cells, knockdown of MSI1 further reduced cell viability in response to CDDP treatment (Fig. 3F). These data suggested that in addition to regulating cell proliferation and glucose metabolism, MSI1 also contributed to the regulation of the sensitivity of NSCLC cells to the treatment of chemotherapy drug.

MSI1 regulated NSCLC proliferation and chemoresistance via the Akt signaling pathway

The involvement of the Akt signaling pathway in many cancer types, including NSCLC, has been demonstrated by previously studies [19, 20]. In order to investigate whether MSI1 could regulate the proliferation and chemoresistance of NSCLC via the Akt signaling pathway, we first examined the levels of phosphorylated Akt in A549 and H522 cells with increased

MSI1 expression levels. We found that while the total levels of Akt did not appear to change with increased MSI1 expression, the levels of phosphorylated Akt were significantly elevated in MSI1 overexpressing cells (Fig. 4A), suggesting that MSI1 could activate the Akt signaling pathway. We postulated that if MSI1 regulated NSCLC malignancy and chemoresistance via the Akt signaling pathway, then loss of Akt activities would abolish the regulatory ability of MSI1. Therefore, we treated NSCLC cells with MK2206, a specific inhibitor of Akt signaling. Significantly, in both A549 and H522 cells, MK2206 treatment completely reversed the promotion of cell proliferation (Fig. 4B), glucose uptake (Fig. 4C) and lactate production (Fig. 4C) induced by MSI1 overexpression. In addition, in NSCLC cells treated with MK2206, MSI1 overexpression was no longer able to increase cell viability in response to CDDP treatment (Fig. 4D). In summary, these results were consistent with our hypothesis that MSI1 promoted NSCLC proliferation and chemoresistance by activating the Akt signaling pathway.

MSI1 is a target of miR-181a-5p

The involvement of microRNA miR-181a-5p in cancer development has been previously reported. It appears that depending on cancer type, miR-181a-5p could serve both as an oncogene [21-24] and as a tumor suppressor [25, 26]. With publicly available complementarity based algorisms, we identified that MSI1 could a potential target of miR-181a-5p (Fig. 5A). To validate this prediction, we overexpressed miR-181a-5p in both A549 and H522 cells (Fig. 5B). We found that overexpression of miR-181a-5p led to reduced expression of MSI1 in both mRNA (Fig. 5C) and protein levels (Fig. 5D). Based on the predicted targeting sites of miR-181a-5p, we generated MSI1 3'-UTR wild type (WT) and mutant (MUT) luciferase reporter plasmids (Fig. 5E). We then performed luciferase reporter assays by co-transfecting

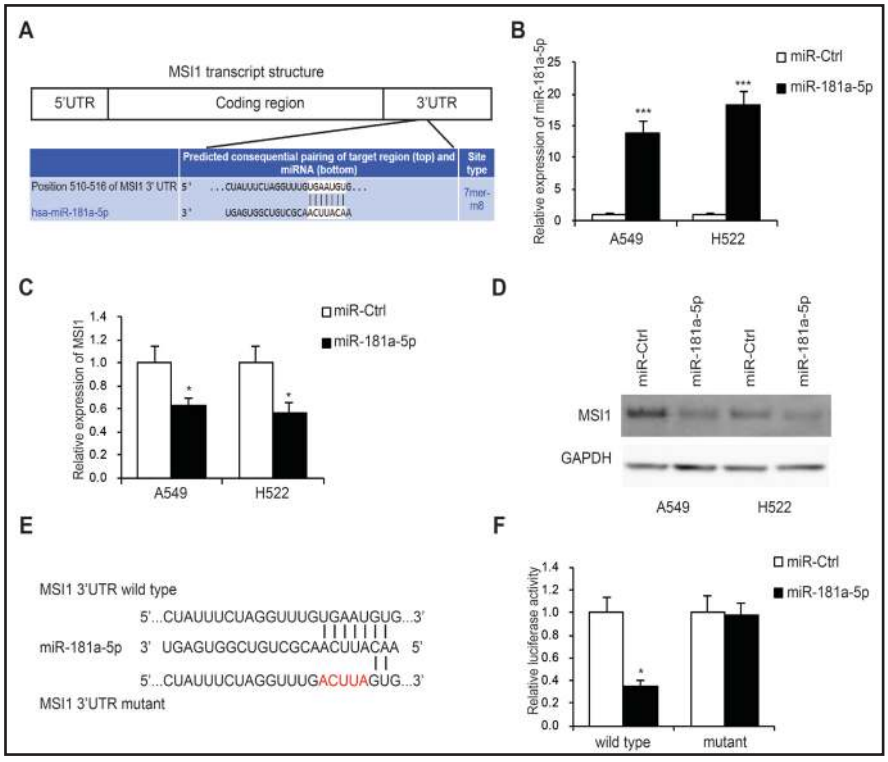

Fig. 5. MSI1 is targeted by miR-181a-5p. A. Sequences of the putative miR-181a-5p binding sites in the 3'-UTR of MSI1. B. miR-181a-5p expression levels in A549 and H522 cells transfected with miR-181a-5p. ***p<0.001 versus cells transfected with miR-Ctrl. C. RT-PCR analysis of MSI1 expression in A549 and H522 cells transfected with miR-181a-5p. *p<0.05 versus cells transfected with miR-Ctrl. D. Western blot analysis of MSI1 expression in A549 and H522 cells transfected with miR-181a-5p. E. MSI1 mutated sequences of potential miR-181a-5p binding site. F. The luciferase reporter constructs of MSI1 3'UTR wild type and mutant were generated. Luciferase activity assays were performed in A549 cells co-transfected with wild type and mutant MSI1 3'UTR luciferase reporter constructs with miR-Ctrl and miR-181a-5p. *p<0.05 versus cells transfected with miR-Ctrl.

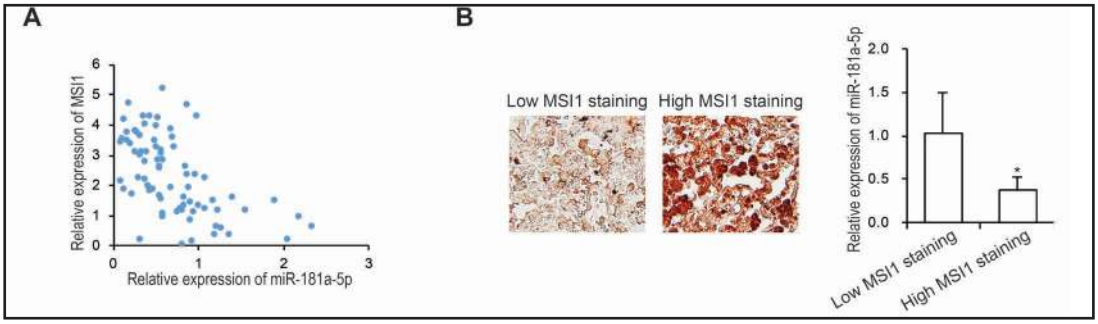

Fig. 6. Negative correlation between MSI1 and miR-181a-5p in NSCLC. A. Pearson correlation between MSI1 and miR-181a-5p expression in NSCLC samples. r = -0.565, p<0.001. B. miR-181a-5p expression in high and low MSI1 immunohistochemical staining samples. *p<0.05 versus low MSI1 staining samples.

luciferase reporter plasmids with plasmids overexpressing miR-181a-5p in A549 cells. We found that the overexpression of miR-181a-5p decreased the luciferase activity driven by the wild type 3'-UTRs of MSI1, but not by the mutant 3'-UTRs of MSI1 (Fig. 5C). In sum, MSI1 is a target of miR-181a-5p and could therefore mediate the effects of miR-181a-5p in cancer development.

Negative correlation between MSI1 and miR-181a-5p expression in NSCLC

To further investigate the functional connection between MSI1 and miR-181a-5p in NSCLC development, we examined the expression levels of MSI1 and miR-181a-5p in tumor tissues collected from patient surgery. We found that that MSI1 expression and miR-181a-5p expression were negatively correlated (Fig. 6A). This was consistent with the role of MSI1 as a target for miR-181a-5p. Furthermore, since MSI1 was not expressed at a uniform level between NSCLC patients, we performed immunohistochemistry staining on patient tissue samples. Remarkably, the expression of miR-181a-5p was significantly higher in NSCLC tissue with lower expression levels of MSI1 (Fig. 6B). These data collectively demonstrated a negative correlation between MSI1 and miR-181a-5p expression in NSCLC.

Discussion

In the current study, we have investigated the functional association between MSI1 and the proliferation and chemoresistance of NSCLC. We demonstrated that MSI1 was able to promote the proliferation and glucose metabolism of NSCLC cells. Importantly, we found that MSI1 also contributed to the resistance against chemotherapy drugs in NSCLC cells. In addition, we determined that the Akt signaling pathway, which played important roles during cancer development, was regulated by MSI1 in NSCLC cells, and this regulation is essential for the mechanism of action of MSI1 to regulate the proliferation, glucose metabolism and chemoresistance of NSCLC cells. In addition, we also found that MSI1 was a target of miR-181a-5p, a microRNA involved in the regulation of cancer development. The expression levels of MSI1 and miR-181a-5p were negatively correlated in NSCLC.

It has been shown by numerous recent studies that elevated MSI1 expression was associated with malignant development and poor outcome in multiple cancer types [6-9, 27]. In addition to its utility as a biomarker for cancer, MSI1 could also be a potential therapeutic target. Reduction of MSI1 expression in breast cancer [9, 28] induced by lentivirus-mediated shRNA delivery led to inhibition of xenograft growth and tumor proliferation. In consistency with these studies, out study confirmed the relationship between MSI1 expression and the development of NSCLC [17]. Interestingly, the result that the expression levels of MSI1 were higher in patients with cancer in advanced stage suggested that MSI1 played important roles not only during the onset of NSCLC, but also during the progression of the cancer.

In investigating the functional roles of MSI1, we utilized two independent NSCLC cell lines, in order to exclude the possibility that any phenotype observed in this study was limited to a single cell lines. We found that MSI1 was capable of inducing the proliferation and glucose metabolism of NSCLC cells, suggesting that MSI1 was likely involved in the growth of NSCLC cells. However, the role of MSI1 in NSCLC metastasis remains to be investigated. Metastasis is a complex process that contributes to the most common cause of mortality in NSCLC patients. During metastasis, a series of molecular and cellular events occur, including degradation of base membrane, cell migration and invasion and cell adhesion at new location [29]. It is important to note that, it is possible that MSI1 also functions in other processes that facilitated metastasis of NSCLC cells.

In this study, we established the relationship between MSI1 expression and resistance to chemotherapy drug CDDP. We provided convincing evidence that modulation of MSI1 expression would change CDDP sensitivity in NSCLC cell lines. A large body of literature has attributed important roles in various aspects of cancer development to the Akt signaling pathway. It has been shown that Akt signaling is involved in the regulation of cell proliferation, cell cycle progression, apoptosis and the interaction between cancer cells and the extracellular matrix [19, 20]. In addition, it has been shown that the activation of the Akt signaling pathways was associated with chemoresistance and malignancy in lung cancer [30-32]. Therefore, it is reasonable to postulate that MSI1 regulated NSCLC malignancy and chemoresistance via Akt signaling. In our study, we found that the expression levels of MSI1 were positively correlated with the activities of the Akt signaling pathway. Importantly, when Akt signaling was inhibited, the regulation of MSI1 on NSCLC malignancy and chemoresistance was completely abolished. These results served to confirm our hypothesis that MSI1 regulated NSCLC malignancy and chemoresistance via modulating the activity of the Akt signaling pathway. Since MSI1 is an RNA-binding protein and functions primarily through regulating protein expression, it is unlikely that MSI1 directly regulated the phosphorylation status of Akt. There are probably other factors between MSI1 and Akt that have yet not identified by this study.

The involvement of miR-181a-5p in cancer development has been confirmed by many previous studies, but the actual role of miR-181a-5p was controversial and appeared to be caner type specific. MiR-181a-5p was found to be up-regulated in breast cancer [24] and pancreatic cancer [22], and other miR-181 family members were up-regulated in hepatocellular cancer stem cells [21, 23]. However, miR-181a was also demonstrated by other studies to be down-regulated in aggressive chronic lymphocytic leukemia [26] and gliomas [25]. In this study, our data appeared to support a tumor suppressor role of miR-181a-5p in NSCLC. MSI1 was identified as targets of miR-181a-5p by bioinformatics prediction. The prediction was then confirmed by several lines of evidence. First, miR-181a-5p was able to inhibit the 3'-UTR activity of MSI1 genes in a luciferase assay. But when the putative binding sites of miR-181a-5p were mutated at the 3'-UTR of MSI1, miR-181a-5p had no effect on the luciferase activities. Second, miR-181a-5p overexpression reduced the expression of endogenous MSI1. Third, in NSCLC tissues, the expression levels of MSI1 and miR-181a-5p were negatively correlated. Collectively, the lines of evidence consistently supported that MSI1 was a target gene for miR-181a-5p.

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YL and SX conceived, coordinated and designed the study, performed the statistical analysis and drafted the manuscript. XK, CH, FW, BL, SZ and JN participated in the design of the study. All authors participated in writing of the manuscript and revised it critically. All authors have read and approved the final version of the manuscript.

Disclosure Statement

The authors indicate that there is no Disclosure Statement involved in this study.

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