Effect of metastasis suppressor 1 on H1299 cells and its clinical significance in non-small cell lung cancer

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Abstract. The present study aimed to investigate the effect of metastasis suppressor 1 (MTSS1) on the proliferation, migration and invasion of human H1299 non-small cell lung cancer cells and its clinical significance in non-small cell lung cancer. The target gene MTSS1-overexpressing lentivirus (LV-MTSS1) was transfected into H1299 cells and expression of MTSS1 was detected at the mRNA and protein levels. Cell Counting Kit-8, wound healing and Transwell assays revealed that the migration and invasion activities were significantly suppressed by MTSS1, but that it had no effect on cell proliferation. In addition, MTSS1 expression in tissue microarrays including samples from 223 cases of non-small cell lung cancer was tested by immunohistochemistry to explore the correlation between MTSS1 and clinicopathological characteristics and prognosis. MTSS1 suppressed H1299 cell migration and invasion, and its expression level can be used as a new independent factor for determining the prognosis of non-small cell lung cancer.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the majority of countries worldwide and causes 1 million deaths each year (1-5). According to the pathological diagnosis, lung cancer can be divided into small cell lung cancer and non-small cell lung cancer (NSCLC), of which the latter accounts for ~85% of all diagnosed cases. Although great progress has been made in the prevention and early diagnosis of lung cancer by the development of medical technology such as surgery, radiotherapy, chemotherapy and molecular-targeted therapy, the overall prognosis of lung cancer is poor and its 5-year survival rate is only ~15% (6).

The molecular level of tumor occurrence, development and invasion has become a hotspot of scientific research, and increasing attention is being focused on the correlation between tumor metastasis genes and patient prognosis. Numerous studies have shown that the occurrence and development of lung cancer is a complex process of multiple gene-factor interactions. Researchers are seeking molecular markers that may influence the prognosis of lung cancer.

Metastasis suppressor 1 (MTSS1), also known as missing in metastasis (MIM), MIM-B, basal cell carcinoma-enriched gene 4 (BEG4) or KIAA0429 identified by Lee *et al* as a transcript missing in the metastasis of bladder cancer (7), is a newly identified actin-binding protein that is mainly involved in cytoskeletal remodeling, signal transduction and transcriptional activation, and closely associated with tumor growth and invasion.

Numerous scholars have thoroughly investigated MTSS1; Loberg *et al* found that reduced MIM-A gene expression in prostate and other cancers may contribute to tumor growth and development as well as metastasis (8). In 2011, Mustafa *et al* also reported that MTSS1 overexpression clearly inhibited prostate cancer cell metastasis, growth and adherence (9). In a study of breast cancer, Parr and Jiang reported that patients with reduced MTSS1 levels had poorer prognosis. High MTSS1 levels correlated with increased patient overall and disease-free survival. Furthermore, MTSS1 overexpression significantly suppressed the invasive, migratory, growth and adherence properties of a human breast cancer cell line. In contrast, MTSS1 knockdown dramatically enhanced these properties (10).

The prognostic value of MTSS1 has also been demonstrated in esophageal squamous cell carcinoma, and patients with high MTSS1 expression levels had a favorable prognosis compared to those with reduced MTSS1 expression levels (11). A gastric cancer study in a Chinese population also revealed that the

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Abbreviations: MTSS1, metastasis suppressor 1; MIM, missing-in-metastasis; NSCLC, non-small cell lung cancer; BEG4, basal cell carcinoma-enriched gene 4; SCC, squamous cell carcinoma; ADCA, adenocarcinoma; GFP, green fluorescent protein; OD, optical density; CCK-8, Cell Counting Kit-8

Key words: MTSS1, non-small cell lung cancer, H1299, squamous cell carcinoma, adenocarcinoma

MTSS1 expression levels were increased in tissues adjacent to carcinoma, but clearly reduced in cancer tissues, and MTSS1 expression levels were gradually reduced as histological differentiation degree decreased (12). In the hematopoietic system, MTSS1-knockout mice had an increased propensity to develop aggressive B cell lymphomas (13,14). These studies suggested that MTSS1 acts as a tumor metastasis suppressor gene in these malignancies, that low MTSS1 expression levels confer a poorer prognosis and that higher expression levels correlate with improved overall survival rates. However, in colorectal cancer, high MTSS1 expression was recently shown to correlate with poor prognosis (15) and disease progression in a subset of human melanoma cases (16).

Therefore, what is the effect of MTSS1 on NSCLC? In the present study, we chose the NSCLC cell line H1299 and 223 patients with NSCLC [136 with squamous cell carcinoma (SCC) and 87 with adenocarcinoma (ADCA), the two main types of NSCLC] as the research subjects. The target gene LV-MTSS1 was transfected into H1299 to analyze the effect of MTSS1 on proliferation, migration and invasion. The tissue microarray was stained to explore the correlation between MTSS1 and NSCLC clinicopathological characteristics and prognosis by statistical analysis.

Materials and methods

Cell culture. NSCLC cell line H1299 was obtained from GeneChem Co., Ltd. (Shanghai, China). We maintained H1299 in Roswell Park Memorial Institute (RPMI)-1640 culture medium (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, UT, USA) at 37˚C with 5% CO_2 in a humidified atmosphere. All experiments were conducted in accordance with the Ethical Standards of the Declaration of Helsinki and according to national and international guidelines, and have been approved by the Ethics Committee of the Affiliated Hospital of Nantong University.

Cell transfection. MTSS1-overexpressing lentivirus (LV-MTSS1) and the negative control lentivirus (LV-NC) were purchased from GeneChem Co., Ltd. The highest transfection efficiency was evident when the virus titer was $1x10^9$ TU. A total of $2x10^5$ H1299 cells were planted and maintained on a 6-well plate (Corning Inc., Corning, NY, USA) to achieve 80-90% confluency and then transfection was carried out according to the manufacturer's instructions (GeneChem Co., Ltd.). After transfection, cells were starved for green fluorescent protein (GFP) and fluorescence detection was performed using an EVOS FL Auto (Invitrogen Life Technologies).

Quantitative real-time polymerase chain reaction. Cells in the different groups were cultured in 6-well plates with expansion medium (2 ml/well) at 80-90% confluency, and total RNA was isolated using a UNIQ-10 Spin Column RNA Purified kit (Sangon, Shanghai, China). The first strand of complementary DNA (cDNA) was synthesized using a RevertAid™ First Strand cDNA Synthesis kit (Fermentas, Burlington, Canada). and was subsequently subjected to processing by a Corbett RG-6000 polymerase chain reaction system (Qiagen, Dusseldorf, German) using FastStart Universal SYBR-Green Master Mix (Roche, Basel, Switzerland). The reactions were optimized by varying the annealing temperatures at 52-55˚C, and the sense and antisense primers were synthesized as follows: glyceraldehyde 3-phosphate dehydrogenase, 5'-GCA AGTTCAACGGCACAG-3' and 5'-GCCAGTAGACTCCAC GACAT-3'; MTSS1, 5'-GAGGAGATGGAGGCTTGTGA-3' and 5'-TGGTTGTCTGGGTGCTGTAG-3'. Fold-changes in mRNA expression were determined using the $2^{-\Delta\Delta Ct}$ method (17).

Western blot analysis. H1299 cells were lysed by RIPA lysis buffer on ice and total protein was extracted using a protein extraction kit (both from Beyotime, Jiangsu, China) according to the manufacturer's instructions. A bicinchoninic kit (Thermo Fisher Scientific, Pittsburgh, PA, USA) was used to detect the concentration of extracted protein. A total of 50 μg of protein was vertically electrophoresed through sodium dodecylsulfate-polyacrylamide gels and the separated proteins were electronically transferred to polyvinylidene difluoride membranes. Next, the non-specific interactions were blocked by 5% defatted milk-Tris-buffered saline and Tween-20 solution and incubated at 37˚C for 1 h. After being washed, specific antibodies against MTSS1 (1:50; Abcam, San Francisco, CA, USA) and β-actin (1:2,000; Beyotime) were applied to incubate the membranes at 4˚C for 12 h to detect corresponding proteins. The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (Bioss, Woburn, MA, USA) for 1 h at 37˚C. Immunoblots were then detected by enhanced chemiluminescence reagents (Invitrogen Life Technologies) and finally exposed to X-ray films. Software Quantity One (Bio-Rad, Hercules, CA, USA) was used to analyze the relative protein expression levels, while β-actin was introduced as the internal reference.

Cell proliferation assay. Cells in the different groups were seeded onto 96-well plates in 100 μl of RPMI-1640 with 10% FBS at a density of 2,000 cells/well. Cell viability was determined after 24, 48 and 72 h using a Cell Counting Kit-8 (CCK-8) (Beyotime) according to the manufacturer's instructions. The optical density (OD) at 450 nm was detected using a Synergy 2 enzyme mark instrument (BioTek, Winooski, VT, USA).

Wound healing assay. After transfection, confluent monolayers of cells were scratched with a 1,000 μl pipette tip to induce a wound. The wound edges were imaged using a Leica DM IRB inverted microscope (Leica, Solms, Germany). Images were collected at 0, 24 and 48 h after wounding. Images were quantified by measuring the number of cells across the initial wounded edge (black line) at each time-point.

Cell migration and invasion assays. The migration and invasion abilities of the H1299 cells were assessed using Transwell plates with 8-μm pore membranes (Corning Inc.) according to protocols previously described (18). Briefly, the H1299 cells were cultured in RPMI-1640 medium for 24 h and the medium was collected as conditioning medium. For the invasion assay, Matrigel (0.1 mg/ml; GeneChem Co., Ltd.) was coated on the top surface of the Transwell chambers. The treated cells were seeded into the chambers and incubated in a humidified

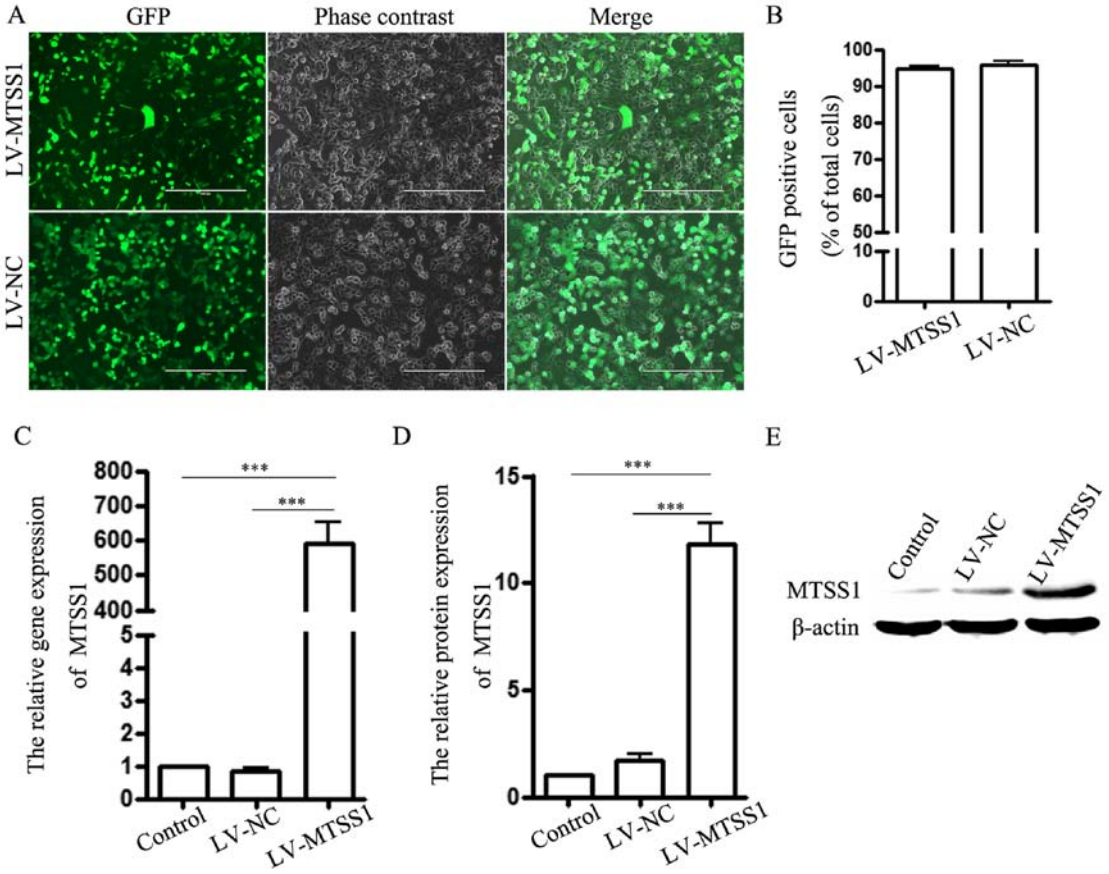

Figure 1. Transfection efficiency. (A) Green fluorescence was found in the metastasis suppressor 1 (MTSS1)-overexpressing lentivirus (LV-MTSS1) and negative control lentivirus (LV-NC) groups (magnification, x100). (B) Approximately 95% of the cells in the LV-MTSS1 and LV-NC groups expressed green fluorescent protein (GFP) fluorescence, but the difference between the two groups was not statistically significant. MTSS1 gene (C) and protein (D and E) expression levels were higher in the LV-MTSS1 group than in the other two groups (***P<0.001).

environment with 5% CO_2 at 37˚C for 48 h. The cells that passed through the polyethylene membrane (migrated cells) or through the Matrigel (invaded cells) were fixed with methanol. Crystal violet staining was then used to indicate migration or invasion under the EVOS FL Auto.

Patients and tissue samples. NSCLC tissues were collected from surgical resection specimens of 223 patients who had not undergone radiotherapy or chemotherapy in the Affiliated Hospital of Nantong University between September 2006 and December 2010. Tumor tissues including 136 SCC and 87 ADCA were used to construct a tissue microarray. Briefly, the tumor of each patient was represented by 2.0-mm cores. Histotypes were confirmed using hematoxylin and eosin staining. The Tumor-Node-Metastasis (TNM) staging was according to the 7th edition of the TNM Staging System for Lung Cancer (19). Written consent was obtained from all patients before sample analysis, and all investigations were conducted in accordance with the Ethical Standards according to the Declaration of Helsinki and according to national and international guidelines and were approved by the Ethics Committee of the Affiliated Hospital of Nantong University.

Immunohistochemistry and evaluation of staining. The immunohistochemical analysis was performed as previously described (20). Rabbit anti-MTSS1 polyclonal (1:50; Abcam) antibody was used for detection and Leica microscopy was used to capture the images. The immunostaining of these sections was evaluated by two independent experienced pathologists who were unaware of the clinicopathological data and patient outcomes. The MTSS1 labeling score was defined by multiplying the percentage of positive cells and staining intensity (21). The percentages of MTSS1-positive cells were scored and placed into four categories according to staining proportion: <10%=1; 10-50%=2; >50-75%=3; and >75%=4. The MTSS1 staining intensities were divided into four grades: no staining, 0; weak staining 1; moderate staining 2; and strong staining 3. MTSS1 positivity was determined using the following formula: Overall scores = percentage score x intensity score. Overall scores of <3 and ≥3 were defined as negative and positive, respectively. All samples were evaluated at a magnification of x200.

Statistical methods. The data were subjected to Student's t-test or one-way analysis of variance test. Associations between clinicopathological variables and MTSS1 were examined by χ^2 tests. Survival curves were calculated by the Kaplan-Meier method and analyzed by the log-rank test. The multivariate analysis was performed using a Cox regression model. P-values <0.05 were considered statistically significant. Data were analyzed using SPSS 22.0 for Windows (SPSS, Inc., Chicago, IL, USA).

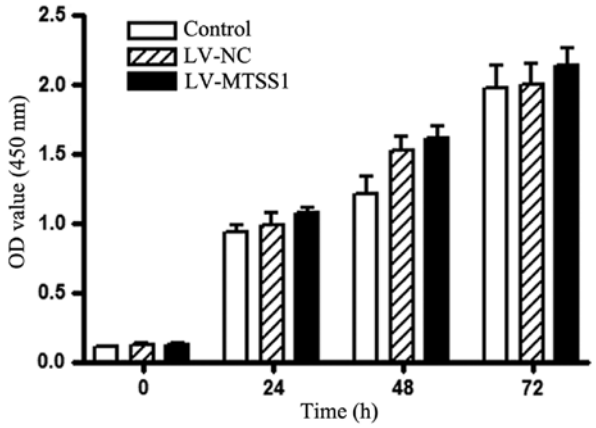

Figure 2. Metastasis suppressor 1 (MTSS1) had no effect on cell proliferation. In the cell proliferation assays, the optical density value (450 nm) in the MTSS1-overexpressing lentivirus (LV-MTSS1), negative control lentivirus (LV-NC) and control groups did not significantly differ at 24, 48 or 72 h.

Results

Efficiency of transfection and MTSS1 expression level detection. Transfection efficacy was detected using an immunofluorescence technique. H1299 cells stably expressing MTSS1-GFP (LV-MTSS1 group) or GFP alone (LV-NC group) were obtained after lentivirus transfection (Fig. 1A). The transfection efficiency was ~95% (Fig. 1B). Next, real-time polymerase chain reaction and western blotting were utilized to detect the relative expression of MTSS1 in each group. The results indicated that MTSS1 expression in H1299 cells (control group) was low and the mRNA and protein levels of MTSS1 were significantly increased in the LV-MTSS1 group compared to the LV-NC and the control groups (Fig. 1C-E). However, there was no difference between the latter two groups.

Effect of MTSS1 on cell proliferation. We examined cell proliferation following MTSS1 overexpression in the cells using the CCK-8 assay. The OD value (450 nm) did not significantly differ among the three groups at 24, 48 or 72 h (Fig. 2). MTSS1 had no effect on H1299 proliferation.

Effect of MTSS1 on cell migration and invasion in H1299 cell lines. The Transwell results displayed that the number of cells that penetrated the membrane in the LV-MTSS1 group was significantly lower than those of the other two groups in both the cell migration (Fig. 3A and C) and cell invasion assays (Fig. 3B and D). The same result was obtained with the wound healing assay at 24 and 48 h in the LV-MTSS1 group (Fig. 3E and F). This finding implied that MTSS1 suppressed cell migration and invasion.

MTSS1 expression of tissue microarray. Representative tissue microarray immunohistochemical staining in NSCLC observed for MTSS1 are shown in Fig. 4. The MTSS1 protein was mainly located in the cytoplasm and strong staining was detected in well-differentiated tissues, moderate staining was seen in medium-differentiated tissues, and weak staining was seen in poorly differentiated tissues (both SCC and ADCA).

Table I. Correlation of MTSS1 expression with clinicopathological characteristics of the NSCLC cases.

Clinicopathological characteristics	No.	MTSS1		P-value
		Negative	Positive	
Age (years)				0.879
<60	84	39	45	
≥60	139	66	73	
Gender				0.678
Male	137	63	74	
Female	86	42	44	
Histological type				0.415
SCC	136	67	69	
ADCA	87	38	49	
Differentiation				0.010
Well	28	8	20	
Medium	87	33	54	
Poorly	108	64	44	
Tumor size (cm)				0.351
<3	80	41	39	
≥3	143	64	79	
Lymphatic metastasis				0.000
N0	145	48	97	
N1+2	78	57	21	
TNM				0.024
I	94	36	58	
II	51	23	28	
III+IV	78	46	32	
Smoking history				0.079
Smoker	147	63	84	
Non-smoker	76	42	34	

MTSS1, metastasis suppressor 1; NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; ADCA, adenocarcinomas; TNM, tumor-node-metastasis; P<0.05 was considered statistically significant.

The overall MTSS1 expression level scores changed from high to low as the differentiation degree changed from high to low.

Correlation between MTSS1 expression and clinicopathological parameters in NSCLC. An overview of MTSS1 expression and clinicopathological parameters is shown in Table I. MTSS1 expression was significantly associated with tumor tissue differentiation degree (P<0.05), TNM stage (P<0.05) and lymph node metastases (P<0.001), respectively. There was no significant correlation between MTSS1 and variables such as age, gender, tumor histology, tumor size or smoking history (all P>0.05).

Survival analysis. Univariate survival analysis of these 223 patients revealed that only 27 of the 105 (26%) patients in the MTSS1-negative group were alive vs. 48 of 118 (38%) in the

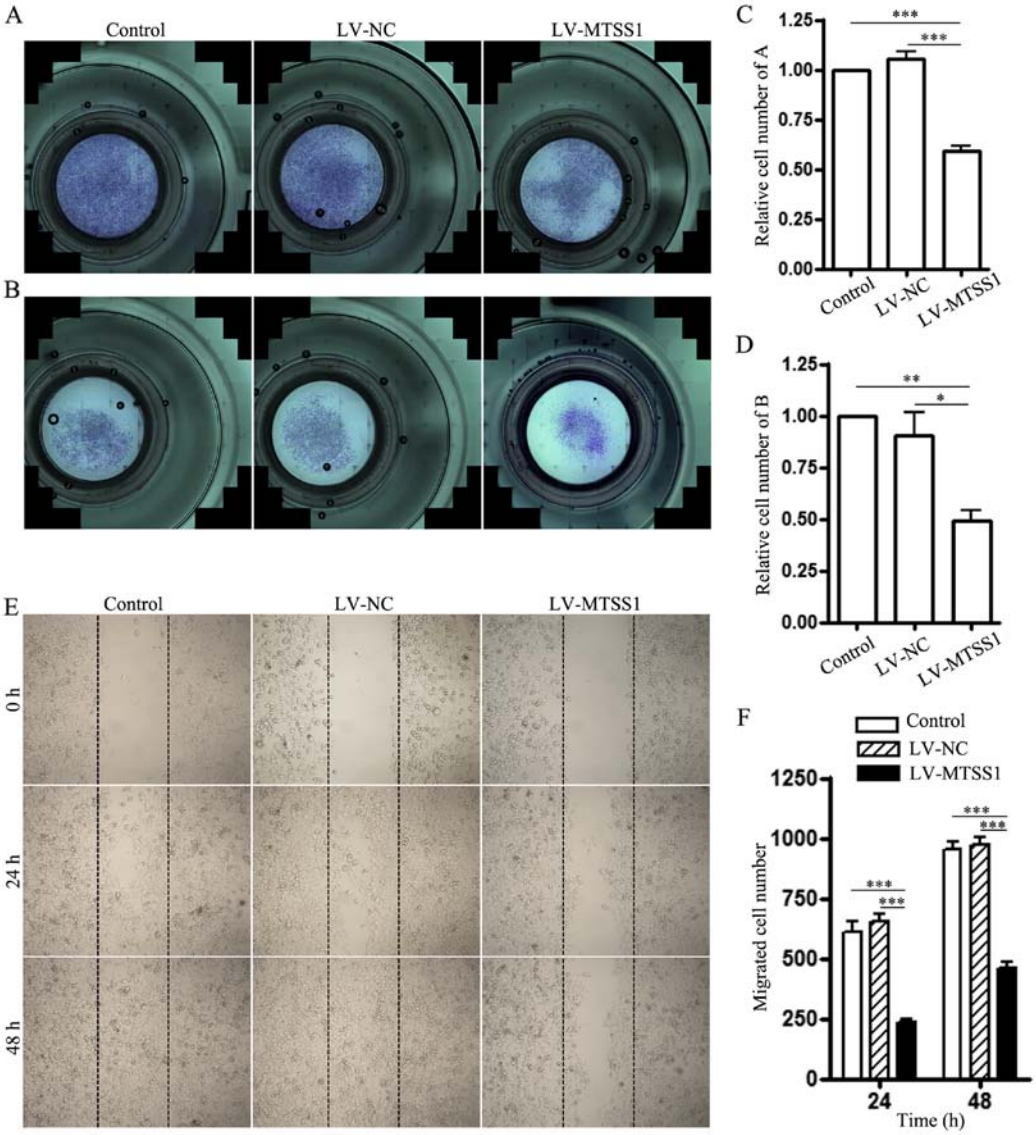

Figure 3. Metastasis suppressor 1 (MTSS1) suppresses H1299 cell migration and invasion *in vitro*. In the cell migration (A and C) and invasion (B and D) assays, significantly fewer cells in the LV-MTSS1 group than in the other two groups penetrated the membrane. (E) Confluent monolayers of the H1299 cells were scratched and imaged at 0, 24 and 48 h. The black line represents the position of the wounded edges (magnification, x100). (F) The number of cells across the line at 24 or 48 h (shown in E) are shown as histograms (*P<0.5, **P<0.01, ***P<0.001).

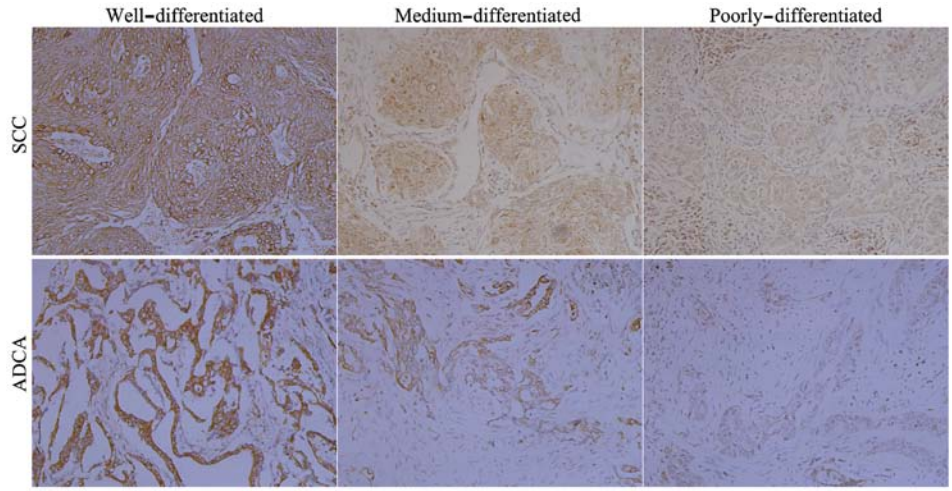

Figure 4. Immunohistological staining of non-small cell lung cancer (NSCLC) cells for metastasis suppressor 1 (MTSS1). Strong MTSS1 staining in well-differentiated squamous cell carcinoma (SCC) and adenocarcinoma (ADCA) cells is visible, while moderate staining is observed in medium-differentiated SCC and ADCA cells and weak staining is observed in poorly-differentiated SCC and ADCA cells (magnification, x200).

Table II. Survival status and clinicopathological parameters in 223 NSCLC specimens.

Clinicopathological parameters	No.	Survival status		P-value
		Alive	Dead	
Age (years)				0.941
<60	84	28	56	
≥60	139	47	91	
Gender				0.139
Male	137	41	96	
Female	86	34	52	
Histological type				0.830
SCC	136	45	91	
ADCA	87	30	57	
Differentiation				0.000
Well	28	18	10	
Medium	87	35	52	
Poorly	108	22	86	
Tumor size (cm)				0.003
<3	80	37	43	
≥3	143	38	105	
Lymphatic metastasis				0.000
N0	145	65	80	
N1+2	78	10	68	
TNM				0.000
I	94	52	42	
II	51	16	35	
III+IV	78	17	71	
MTSS1				0.018
Negative	105	27	78	
Positive	118	48	70	
Smoking history				0.287
Smoker	147	53	94	
Non-smoker	76	22	54	

NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; ADCA, adenocarcinomas; TNM, tumor-node-metastasis; MTSS1, metastasis suppressor 1; P<0.05 was considered statistically significant.

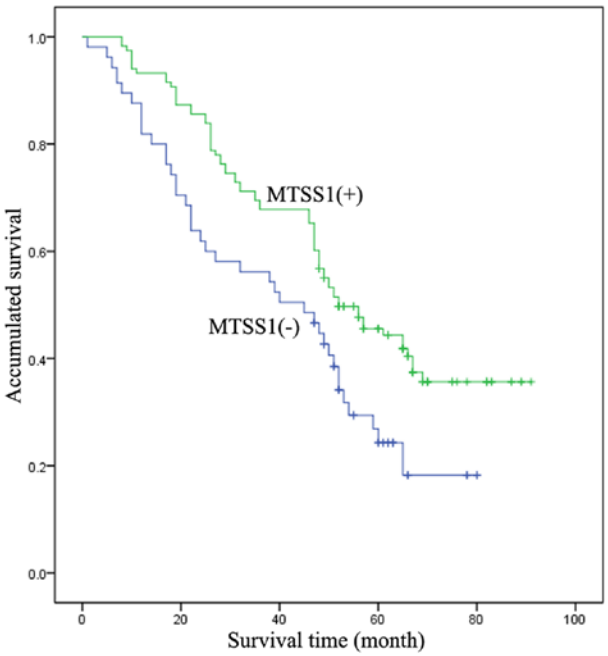

Figure 5. Correlation of metastasis suppressor 1 (MTSS1) and overall survival as shown by Kaplan-Meier survival curves. Patients with poor prognosis had decreased or absent MTSS1 expression.

Table III. Contribution of various potential prognostic factors to survival by Cox regression analysis in 223 NSCLC specimens.

Characteristics	Hazard ratio	95% CI	P-value
Age	1.282	0.891-1.845	0.180
Gender	0.990	0.682-1.437	0.958
Histological type	1.256	0.871-1.812	0.223
Differentiation	0.698	0.530-0.919	0.010
Tumor size	1.615	1.115-2.338	0.011
Lymphatic metastasis	1.889	1.280-2.788	0.001
TNM	2.014	1.619-2.506	0.000
MTSS1	0.669	0.466-0.960	0.029
Smoking history	0.937	0.652-1.347	0.725

NSCLC, non-small cell lung cancer; CI, confidence interval; TNM, tumor-node-metastasis; MTSS1, metastasis suppressor 1; P<0.05 was considered statistically significant.

MTSS1-positive group (Table II). The Kaplan-Meier survival curves showed that decreased or absent MTSS1 expression was significantly correlated with poor survival (Fig. 5). When all variables were compared separately with survival status, only differentiation stage (P<0.001), TNM stage (P<0.001), lymph node metastases (P<0.001), MTSS1 (P<0.05) and tumor size (P<0.01) significantly affected postoperative outcome (Table II). The Cox proportional hazards regression model proved that MTSS1 (P<0.05), differentiation stage (P<0.05), TNM stage (P<0.001), lymph node metastases (P<0.01) and tumor size (P<0.05) were independent prognostic factors in patients with NSCLC (Table III).

Discussion

Non-small cell lung cancer (NSCLC) remains the leading cause of death in patients suffering from this malignant disease, and its cure is rare due to the lack of an effective screening method and typical early symptoms and as such, many patients have distant metastases at the time of diagnosis (22). In the present study we aimed to investigate the effect of MTSS1 on the proliferation, migration and invasion of H1299 cells as well as the clinical significance of MTSS1 in NSCLC.

The exact role of MTSS1 in tumor progression remains under debate. Most of the research to date into gastric, breast, and prostate cancer has implicated MTSS1 as a tumor

suppressor and that low MTSS1 expression levels confer poorer prognosis while higher expression levels are correlated with improved overall survival rates. Our results are consistent with the aforementioned which revealed that: i) in tissue microarray and clinical statistical analysis, MTSS1 protein was mainly expressed in the plasma and that MTSS1 expression was significantly correlated with tumor differentiation degree (P<0.05), TNM stage (P<0.05) and lymph node metastasis (P<0.001); ii) in Kaplan-Meier survival analysis, MTSS1 expression was positively related to patient survival (P<0.01); and iii) in Cox multivariate survival analysis, MTSS1 expression (P<0.05), tumor tissue differentiation (P<0.05), tumor size (P<0.05), TNM stage (P<0.001) and lymph node metastasis (P<0.01) were independent factors for NSCLC prognosis.

The role of MTSS1 in cell proliferation differed among tumor types and cell lines. MIM overexpression decreased the PC-3 cell proliferation rate in prostate cancer (8), but increased it in head and neck squamous cell carcinoma. In the present study, the proliferation ability of the H1299 cells was not significantly suppressed or promoted by MTSS1 overexpression.

Tumor metastasis is one of the deadliest steps in cancer progression and currently the most difficult to overcome (23,24). Tumor metastasis ability is associated with cell migration and invasion capacity. The wound healing and Transwell results displayed that cell migration and invasion abilities in the LV-MTSS1 group were poorer than those in the other two groups tested. In the clinical statistical analysis, the positive expression rate of the MTSS1 protein in patients with NSCLC and lymph node metastasis was 27% (21/78) vs. 67% (97/145) in patients with NSCLC without lymph node metastasis, showing a statistically significant difference (P<0.001).

The aforementioned findings suggested that MTSS1 inhibited H1299 cell migration and invasion, and its expression was negatively correlated with lymph node metastasis. That is, MTSS1 may be able to indicate NSCLC tumor progression and the presence of lymph node metastasis, which implies that MTSS1 is an antimetastasis agent (10). Such a role may be related to actin, as MTSS1 contains three introns and four exons of 163 bp (2) to 2,903 bp (4) in length over 9.3 kb, and its protein consists of 356 amino acids (25). MTSS1 has a N-terminal IRSp53/MIM homology domain and a WASP-homology domain 2 at the C-terminus, with which MTSS1 can be combined with actin and further influence cell migration and invasion (26-31). MTSS1 expression decline and loss may lead to changes in tyrosine kinase, which changes the binding capacity of MTSS1 and actin. Finally, the cytoskeletal changes lead to primary tumor cells loss and transfer (27,32). The present study showed that the cytoskeleton structural changes that induce apoptosis can be used to treat cancer (33-35).

The mechanism involved in the MTSS1 expression decrease in most tumors may be associated with CpG island DNA methylation or loss of heterozygosity (LOH) (36). So-called CpG island refers to a region of DNA in which C stands for cytosine, G stands for guanine, and p represents the ester bond between them. This DNA contains large proportions of C and G. In the human genome, dinucleotides CpG are not evenly distributed. A large non-methylated CpG diploid is termed a CpG island. Utikal *et al* (36) reported that a CpG island exists in the MTSS1 5'-flanking promoter and pointed out that MIM-induced cell growth inhibition is anchorage-independent, but related to CpG island DNA methylation within the promoter region. They identified and cloned the MIM promoter region and found that the main promoter activity was located at the 5'-flanking sequence CpG island (276 bp). In a gastric cancer study, Yamashita *et al* (37) also confirmed CpG island DNA methylation in the promoter region and found that MTSS1 was located in a genomic region (8q22) with frequent LOH.

LOH means lack of an LOH molecule. A gene on a chromosome when randomly deleted despite existence of the paired chromosome, results in unpredictable mutations or deletions. Chromosome 8 is involved in many human tumors (38,39), while LOH is a very common genetic alteration in tumor cells (40-44). MTSS1 expression was higher in normal gastric mucosa but decreased or absent in gastric cancer tissues, suggesting a correlation with a high frequency of LOH in the 5' promoter sequence (37,45). The same results were also reported about LOH in cancer of the gastro-esophageal junction (46), cervical carcinoma (47) and in other progressed tumors which include transcriptional suppression by DNMT3B (48) or microRNA-mediated effects (49-54).

In summary, MTSS1 can suppress H1299 migration and invasion and be used as a new independent factor for NSCLC prognosis. However the exact role of this adaptor molecule that links intracellular signaling pathways with actin remodeling (10,55,56) remains under debate and may likely depend on cell type, molecular context and disease type (15,16).

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