Reduced Expression of Metastasis Suppressor-1 (MTSS1) Accelerates Progression of Human Bladder Uroepithelium Cell Carcinoma

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Abstract. *Background: Metastasis suppressor 1 (MTSS1) is a multi-functional cytoskeletal protein. Recent research showed that MTSS1 is a potential tumor suppressor in many types of cancer cells, including kidney and bladder cancer cells. However, the clinical implication of MTSS1 in human bladder uroepithelium cell carcinoma (BUCC) and its potential in suppressing BUCC tumorigenesis remains undetermined. In the present study, the expression of MTSS1 in human BUCC tissue samples, and correlations between MTSS1 and pathological grade and stage of the tumors were examined in BUCC specimens. The function of MTSS1 in BUCC progression was explored. Materials and Methods: The mRNA and protein expression of MTSS1 were examined in 68 BUCC tissue samples with matching adjacent normal bladder tissues using quantitative real-time PCR and western blot. Furthermore, the bladder cancer cell line 5637 was used to determine the anticancer effect of MTSS1. Results: Lower MTSS1 mRNA expression was seen in BUCC tissues compared with normal bladder tissues. A lower MTSS1 mRNA level was seen in tumors with high clinical stage and with high pathological nuclear grade. Likewise, MTSS1 protein expression in normal bladder tissue was significantly higher than that in BUCC tissue. The protein level of MTSS1 significantly negatively correlated with clinical stage and* pathological nuclear grade of BUCC. Cumulative survival curves indicated that MTSS1 expression was negatively correlated with survival time: patients with a high level of MTSS1 had significantly longer survival time than those with a low level of MTSS1 (p<0.001). Overexpression of MTSS1 reduced BUCC cell proliferation, cell cycle progression and colony formation, but had no influence on BUCC cell apoptosis. Conclusion: These results suggest that overexpression of MTSS1 suppresses BUCC development, providing a novel perspective for BUCC tumorigenesis and a potential therapeutic target for BUCC.*

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Bladder uroepithelium cell carcinoma (BUCC), which accounts for more than 90% of all bladder cancer, is the most common malignancy of the urinary tract and the ninth most common malignancy in the world (1). BUCC is a highly heterogeneous disease, with different histological subtypes and varying prognosis. The overall and 5-year cancer survival rates were significantly different among patients with different pathologic grading, histologic type and AJCC stage of BUCC (2). Although wide use of advanced imaging and surgical techniques have improved the clinical outcome of patients with BUCC, no specific indicator has been found that predicts morbidity and postoperative recurrence of BUCC in patients.

Metastasis suppressor 1 (MTSS1) was originally identified as a metastasis-suppressor protein with 759 amino acids. Its C-terminus is identical to the 356 amino acids encoded by the *MTSS1* gene, which is found on human chromosome 8q24.1 and encodes a 5.3 kb mRNA and a polypeptide predicted to be an actin-binding protein of 356 amino acids with homology to the Wiscott-Aldrich Syndrome protein family (3, 4). MTSS1, also known as missing in metastasis (MIM), MIM-B or basal cell carcinoma-enriched gene 4 (BEG4), was mainly located in the cytoplasm and may be involved in cytoskeletal organization (5). MTSS1 was

originally identified as a protein down-regulated in metastatic bladder carcinoma cell lines (3), and much evidence has indicated that MTSS1 expression may be correlated with different types of cancer (6-10).

We demonstrated that the MTSS1 was a potential tumor suppressor in human bladder cancer and kidney cancer cells in our previous studies (11, 12). Thus, MTSS1 may act as a multifunctional molecule in tumorigenesis and metastasis. However, the role of MTSS1 in the regulation of the cellular behavior of bladder cancer is still very much an open question. In the present study, we investigated the expression of MTSS1 in a cohort of human bladder cancer tissues and its correlation with pathological grade, clinical stage and long-term survival of patients. Furthermore, we examined the biological effect of MTSS1 on migration and invasion in bladder cancer cells *in vitro*.

Materials and Methods

BUCC specimens. A total of 68 (41 males and 27 females) pairs of BUCC and normal bladder tissue samples from newly diagnosed patients who underwent total cystectomy or transurethral resection of bladder cancer from May 2014 to December 2015 at Peking University Cancer Hospital were snap-frozen in liquid nitrogen immediately after surgery. The average age of patients was 52.7±10.4 (range=26-78) years. In the cohort, 52 (76.5%) and 16 (23.5%) cases were staged as non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC), respectively. Pathological grades of the cohort were low grade in 48 cases (70.6%) and high grade in 20 (29.4%). The present study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of Peking University Cancer Hospital, and written informed consent was obtained from each patient.

Cell cultures. Bladder cancer cell line 5637 was obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured routinely in RPMI-1640, supplemented with 5% fetal bovine serum, 100 U/ml penicillin and 50 μg/ml streptomycin (all Gibco, Carlsbad, CA, USA). All cell lines were grown in a humidified incubator at 37°C and 5% CO_2.

Expression vectors and lentiviral infection. Lentiviral transfected with *MTSS1* expression plasmid (NM_014751) (GV287-RBM5) and plasmids containing scrambled control sequence (GV287) were constructed by Genechem Company (Genechem, Shanghai, China). Bladder cancer 5637 cells were infected with *MTSS1* overexpression lentiviral vectors and negative control lentiviral vectors according to the manufacturer's instruction, with 1 μg DNA for 4×10^5 5637 cells in a 24-well plate. Five hours after the transfection, the cell culture medium was replaced with 10% fetal bovine serum.

Bromodeoxyuridine labeling. For the bromodeoxyuridine (BrdU) labeling, cells were plated into 96-well culture plates at a 2×10^4 cells per well in 100 μl cell culture medium and maintained at 37°C. The cell growth rate was determined using a BrdU cell proliferation ELISA kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions after 1 and 4 days. Each experiment was performed in triplicate and repeated three times independently.

Cell-cycle analysis. For cell-cycle analysis, cells were seeded on 6-well plates at 80% confluence and incubated at 37°C for 24 h. The cells were harvested, washed with ice-cold phosphate buffered saline and resuspended in ice-cold 70% ethanol for 30 min. The cells were then treated with 10 mg/ml RNase at 37°C for 30 min. The cells were stained with 10 mg/ml propidium iodide (Sigma, St. Louis, MO, USA) for 30 min. The DNA content was measured by flow cytometry.

Colony formation in soft agarose gel. For the cell colony formation assay, cells were seeded into 6-well plates at 800 cells per well and cultured at 37°C for 14 days. The medium was replaced every 3-4 days. Cells were washed twice with phosphate-buffered saline, fixed with paraformaldehyde, stained with Giemsa (Sigma) for 20 minutes, and washed with ddH_2O three times. The plates were photographed with a microscope. Each experiment was performed in triplicate and repeated three times independently.

Cell apoptosis detection. For cell apoptosis detection, cells were seeded on 6-well plates at 80% confluence and incubated at 37°C for 24 h. The cells were harvested, washed with ice-cold phosphate-buffered saline and resuspended in staining buffer. Flow cytometry was performed to analyze apoptosis. An annexin V-allophycocyanin (APC) stain assay (eBioscience, Vienna, Austria) was performed using the manufacturer's protocol, and the apoptotic cells were analyzed by flow cytometry.

RNA extraction and quantitative real-time polymerase chain reaction. Total RNA was extracted from tissues or cells using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). FastQuant RT Kit (With gDNase kit and SuperReal PreMix Plus (SYBR Green) (TIANGEN BIOTECH, Beijing, P.R. China) were used. The PCR primers used were as follows: *MTSS1* forward: 5'-ACTGGGAATCTGACCACTAT-3'; *MTSS1* reverse: 5'-GACCT GACTGCTAAGGGAC-3'; glyceraldehyde 3-phosphate dehydro-genase (*GAPDH*) forward: 5'-TGACTTCAACAGCGACACCCA-3', *GAPDH* reverse: 5'-CACCCTGTTGCTGTAGCCAAA-3'. PCR cycling conditions were as follows: an Initial denaturation period at 95°C for 15 min, followed by a two-step PCR program consisting of denaturation at 95°C for 10 s and annealing/extension at 60°C for 32 s for 40 cycles. All samples were normalized against the internal control (*GAPDH*) and analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis. Total protein was extracted from the cells or tissue using lysis buffer containing protease inhibitors. Thirty micrograms of protein from each sample were loaded on 10% sodium dodecyl sulfonate polyacrylamide gel electrophoresis. The gels were electrophoresed, and then transferred onto polyvinylidene difluoride membrane (GE Healthcare, Buckinghamshire, UK), and blocked with 10% non-fat milk in Tween/Tris-buffered salt solution [20 mM Tris–Cl, pH 7.5, 0.15 M NaCl and 0.05% Tween-20] for 1 h. Following incubation with the antibody to MTSS1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4°C overnight, the membrane was washed and incubated with IRDye® 800CW Goat anti-rabbit IgG secondary antibody at room temperature for 2 h followed by detection using a LiCor Odyssey gel imaging scanner (LI-COR Biosciences, Lincoln, NE, USA). To verify equal loading of protein, the blots were re-probed with a primary monoclonal antibody against GAPDH (Proteintech Group, Inc., Chicago, IL, USA).
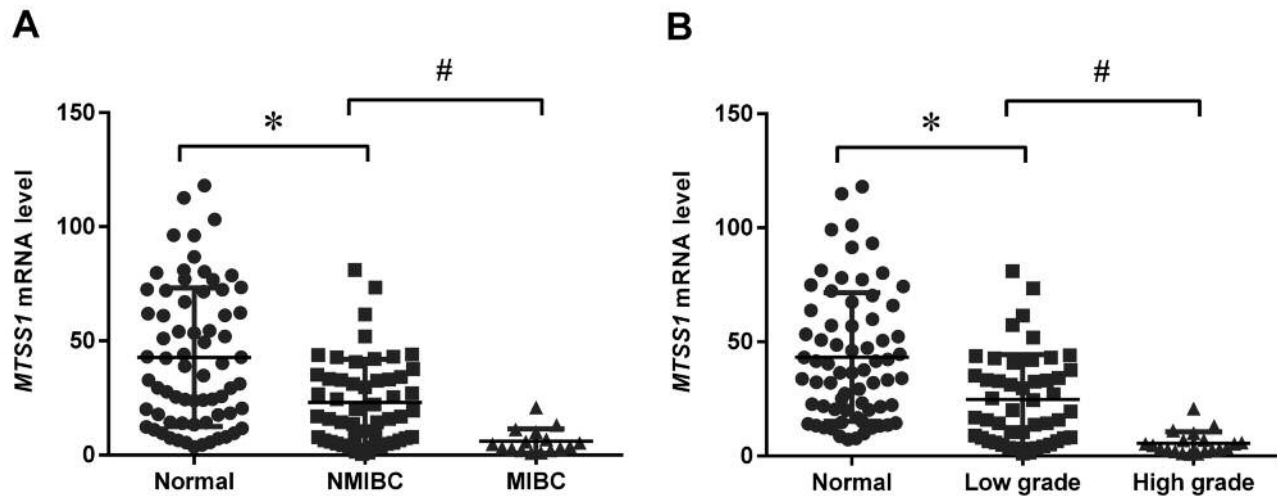
Figure 1. *Lower expression of metastasis suppressor 1 (MTSS1) mRNA in patients with bladder uroepithelium cell carcinoma (BUCC). The relative expression of MTSS1 mRNA in BUCC tissues was significantly lower than that of normal bladder tissues. Furthermore, the mRNA level of MTSS1 was higher in non-muscle-invasive bladder cancer (NMIBC) than muscle-invasive bladder cancer (MIBC), *p<0.05 vs. Normal, #p<0.05 vs. NMIBC (A). Furthermore, the mRNA level of MTSS1 was higher in low pathological grade cancer than high pathological grade cancer tissues, *p<0.05 vs. Normal, #p<0.05 vs. low grade. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) acted as an internal loading control. Each sample was detected by triplicates.*

Statistical analysis. Quantitative analysis of western blot was performed using the GelDoc-2000 Imaging System (UVi Company, Cambridge, UK). For protein expression level, the protein ratio (band density of study protein/band density of GAPDH) was considered as 100% in normal bladder tissues, and that of the other tissues was expressed as a percentage of that of the normal bladder tissues. Comparison between groups was performed using one-way analysis of variance followed by all pairwise multiple comparison procedures using Bonferroni test and Student *t*-test or nonparametric test. In addition, Spearman correlation was used for comparison and estimation of correlations between MTSS1 protein expression levels and clinicopathological parameters. Survival curves were constructed using the Kaplan–Meier method. All data are presented as the mean±standard deviation, and a value of *p*<0.05 was regarded as significant.

Results

Differential expression level of MTSS1 mRNA in BUCC and normal bladder tissues. The clinical and pathological characteristics of patients are shown in Table I. In order to compare the mRNA levels of *MTSS1* in human BUCC and normal bladder tissues, total RNA was isolated from bladder tissues of 68 patients with BUCC and normal bladder tissues The transcript level of *MTSS1* was examined using qRT-PCR. The mRNA level of *MTSS1* in BUCC tissues was significantly lower than that of normal bladder tissues. Furthermore, the mRNA level of *MTSS1* was higher in NMIBC than MIBC, and lower *MTSS1* mRNA levels were seen in BUCC with high pathological grade (*p*<0.05) (Figure 1).

Table I. *Baseline clinicopathological characteristics of patients.*

Characteristic	Value
Gender	
Male	41 (60.3%)
Female	27 (39.7%)
Age, years	52.7±10.4 (26-78)
Clinical stage	
NMIBC	52 (76.5%)
MIBC	16 (23.5%)
Pathological grade	
Low	48 (70.6%)
High	20 (29.4%)
Follow-up, years	2.84 (0.9-3)

MIBC: Muscle-invasive bladder cancer; NMIBC: non-muscle-invasive bladder cancer. Data are the mean±SD, median (range) or number (%).

Expression profile of MTSS1 protein in BUCC and normal bladder tissues. To further determine MTSS1 expression status in BUCC tissues, the protein level of MTSS1 in BUCC and normal bladder tissues were determined. As shown in Figure 2, decreased MTSS1 expression was seen in BUCC tissues compared with normal bladder tissues, and negativity correlated with clinical stage of BUCC (MIBC *vs.* NMIBC, *p*<0.05). The protein level of MTSS1 was also significantly negatively correlated with pathological grade of BUCC (high grade *vs.* low grade, *p*<0.05).
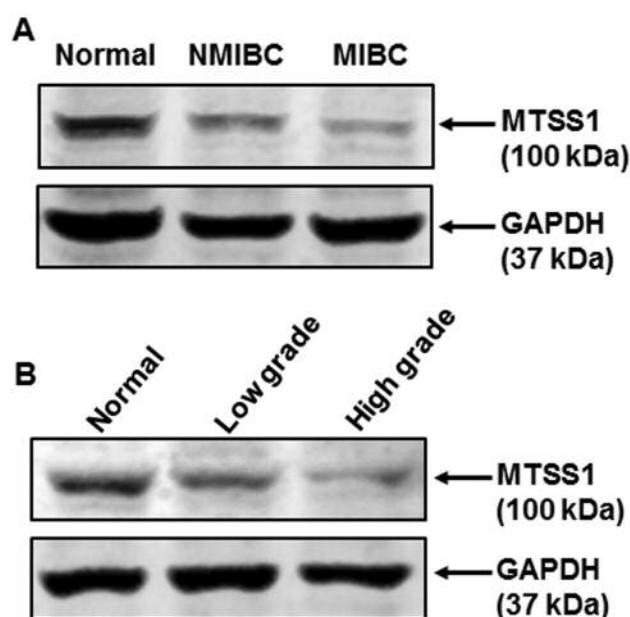
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Figure 2. *The expression of metastasis suppressor 1 (MTSS1) protein decreased in cancer tissues of high pathological grade. A: Typical results of western blot showed that MTSS1 protein level decreased in non-muscle-invasive bladder cancer (NMIBC) tissues, and the protein level was significantly lower in muscle-invasive bladder cancer (MIBC) tissues than that in NMIBC tissues. B: The MTSS1 protein level was also lower in cancer tissues with low pathological grade, and the protein level was significantly lower in cancer tissues with high pathological grade.*

Figure 3. *Low metastasis suppressor 1 (MTSS1) expression predicted poor survival of patients with bladder uroepithelium cell carcinoma (BUCC). Patients with muscle-invasive bladder cancer (MIBC) with low MTSS1 expression had significantly shorter survival than those with NMIBC with high MTSS1 expression. B: Patients with high pathological grade cancer with low MTSS1 expression also had significantly poor survival than those with low pathological grade cancer with high MTSS1 expression.*

Decrease of MTSS1 expression was a predictor of poor survival of patients with BUCC. Kaplan–Meier survival analysis was used to determine the overall survival of patients according to MTSS1 expression. Regardless of cancer classification of MIBC and NMIBC, patients with low MTSS1 expression had significantly shorter survival than those with high MTSS1 expression (Figure 3A). Furthermore, regardless of cancer grade, patients with low MTSS1 expression also had significantly shorter survival than those with high MTSS1 expression (Figure 3B). These findings suggest that a decrease of MTSS1 expression in bladder cancer predicts poor survival.

Overexpression of MTSS1 reduced bladder cancer cell proliferation, cell cycle progression and colony formation in vitro. To explore the role of *MTSS1* in bladder cancer cell proliferation, the 5637 cells were infected with *MTSS1* overexpression lentiviral vectors and control lentiviral vectors. As shown in Figure 4, both the mRNA and protein expression levels were significantly up-regulated in 5637 cells after
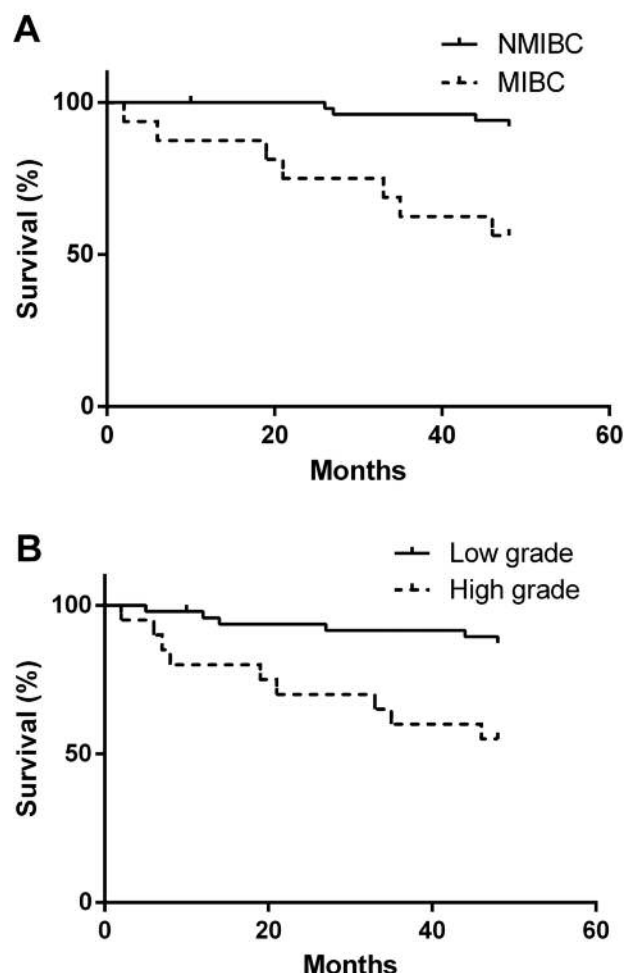
infection with the lentiviral vectors for *MTSS1* overexpression. The BrdU proliferation assay was conducted to confirm the effect of *MTSS1* overexpression on bladder cancer cells proliferation. As shown in Figure 5A, overexpression of *MTSS1* reduced cancer cell proliferation significantly. Cell cycle analysis and colony formation analysis were used to examine if *MTSS1* overexpression influences bladder cancer cell growth. Figure 5B demonstrates that there was a significant increase in the rate of G_2/M phase cells when *MTSS1* was overexpressed, but there was no significant influence on cells in S and G_1 phase. Moreover, 5637 cells
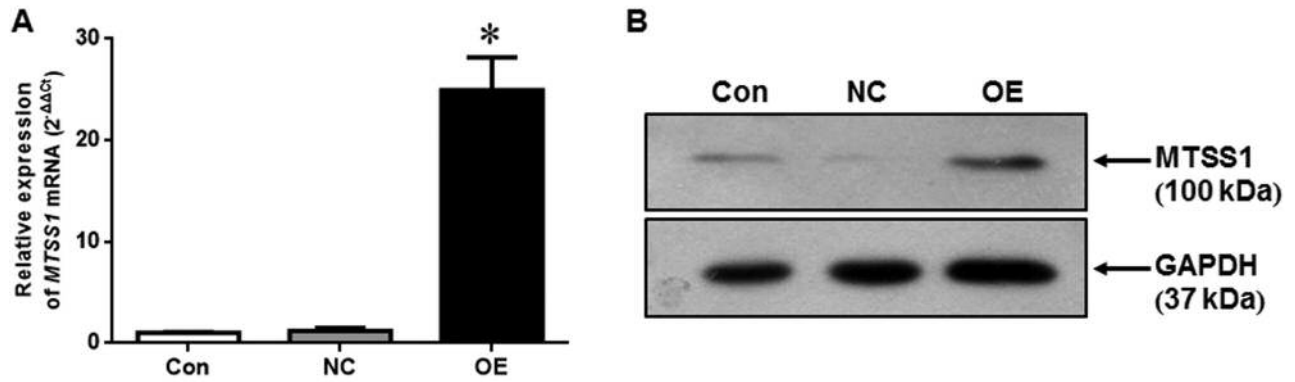
Figure 4. *Overexpression of metastasis suppressor 1 (MTSS1) in 5637 cells. The relative expression of MTSS1 mRNA significantly increased in 5637 cells after infection with MTSS1 overexpression lentiviral vectors. Con: 5637 cells without any treatment, NC: 5637 cells infected with negative control lentiviral vectors, and OE: 5637 cells infected with MTSS1 overexpression lentiviral vectors. B. Western blot analysis shows that MTSS1 protein level was significantly increased in the OE cells compared to NC cells. n=3 per group, *p<0.05 vs. NC group.*
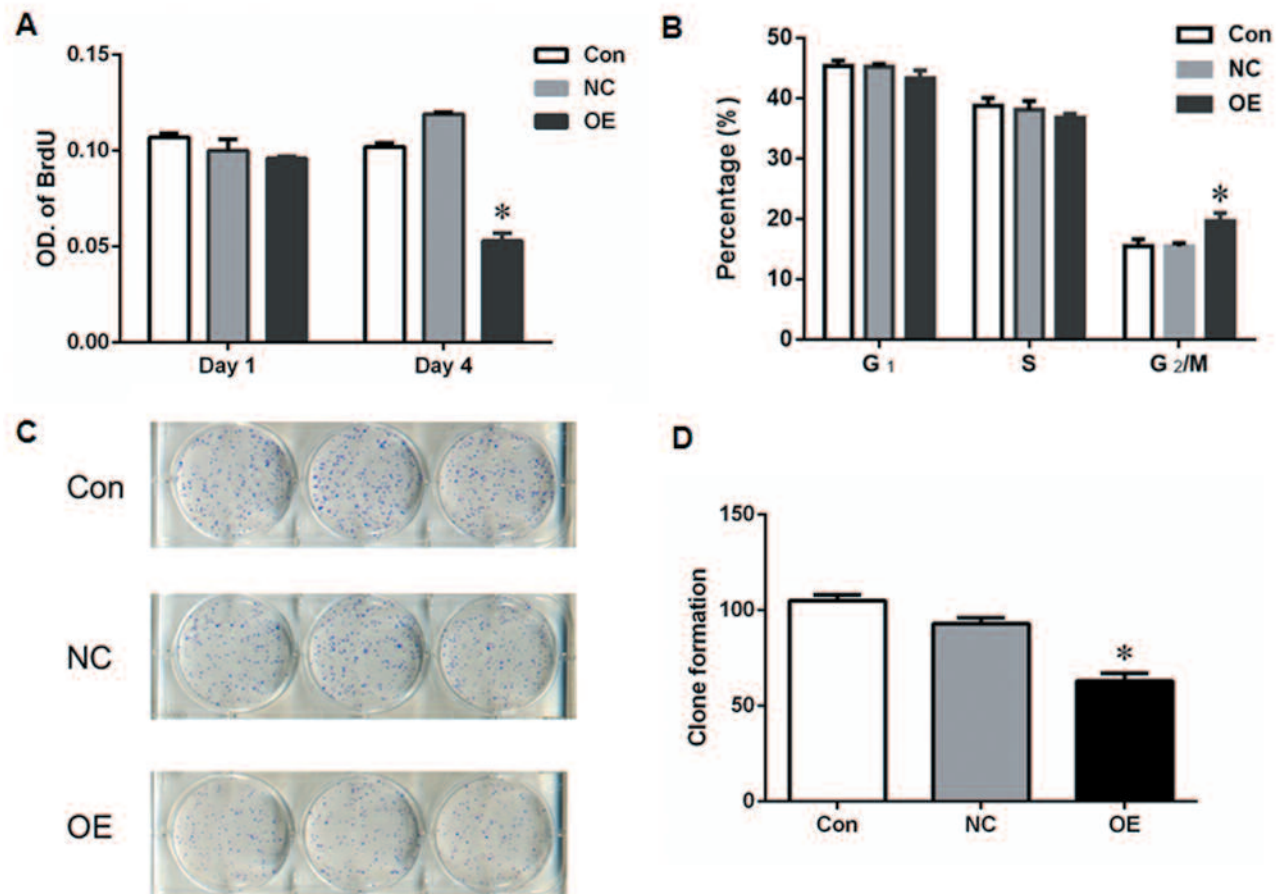

Figure 5. *Up-regulation of metastasis suppressor 1 (MTSS1) reduces cell proliferation. A: Quantitative analysis of bromodeoxyuridine (BrdU)-positive cell number was reduced in 5637 cells transfected with MTSS1 overexpression lentiviral vectors (OE) compared with the negative control lentiviral vector (NC) group. B: The quantitative analysis demonstrated that overexpression of MTSS1 significantly reduced the number of cells in the G_2/M phase. C: OE cells formed fewer and smaller colonies per plate than the NC cells after 14 days seeded. n=3 per group, *p<0.05 vs. NC group. Con: 5637 cells without any treatment.*
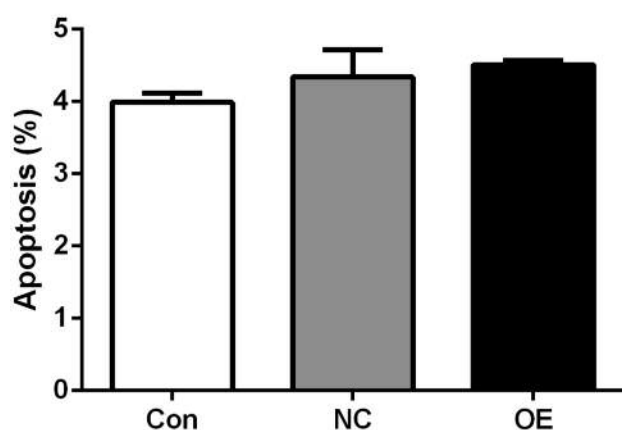
Figure 6. *Overexpression of metastasis suppressor 1 (MTSS1) had no effect on cell apoptosis. Quantification of apoptotic cell number indicated that cells transfected with MTSS1 overexpression lentiviral vectors (OE) had no effect on 5637 cell apoptosis when compared with the negative control lentiviral vector (NC) group, n=3 per group. Con: 5637 cells without any treatment.*

showed significantly less colony formation when *MTSS1* was overexpressed (Figure 5C and D).

Up-regulation of MTSS1 had no influence on apoptosis of bladder cancer cells. To further elucidate the role of MTSS1 in bladder cancer cell apoptosis, 5637 cells infected with *MTSS1* overexpression lentiviral vectors were subjected to annexin V-APC staining and flow cytometric analysis. Figure 6 demonstrates that *MTSS1* overexpression in 5637 cells had no significant effects on the cellular apoptosis rate.

Discussion

BUCC is one of the most common types of cancer, and an estimated 74,000 new cases are diagnosed per year. Since the molecular heterogeneity of BUCC is considered as one of the most important factors responsible for large variation in the response to clinical treatment, molecular diagnosis becomes more and more important in patients suffering BUCC (13). In our previous study, we confirmed reduced expression of MTSS1 in human bladder cancer tissues using immunohistochemical analysis and also found a decrease in the mRNA expression in bladder cancer cell lines (12). In this study, we further confirmed decreased expression of MTSS1 in bladder cancer tissues and cell lines, especially in MIBC and high-grade BUCC. We demonstrated that low MTSS1 expression was a predictor of poor patient survival, and overexpression of MTSS1 reduced BUCC cell proliferation, cell cycle progression and colony formation, but had no influence on BUCC cell apoptosis.

The role of MTSS1 in cancer diagnosis and progression has been elucidated. There are multiple published studies indicating cancer-suppressive functions of MTSS1 in a variety of cancer models. Kayser *et al.* found that the expression of MTSS1 significantly increased in non-small-cell lung carcinomas compared with normal lung, and MTSS1 expression was inversely correlated with cancer stage and histological grading. In addition, patients with low MTSS1 expression had a significantly worse outcome (14). Zeleniak *et al.* demonstrated that primary pancreatic ductal adenocarcinoma displayed higher MTSS1 expression levels than did metastatic pancreatic ductal adenocarcinoma (15). Another report also showed that loss of MTSS1 expression was associated with lymph node metastasis and poor differentiation of hilar cholangiocarcinoma (16), and high expression of MTSS1 suppressed the invasive, migratory, growth and adhesive properties of breast cancer cell lines (17). Similarly, data from our previous study demonstrated MTSS1 expression to be reduced in human kidney cancer cells, and to be are inversely correlated with the growth, invasion, adhesion and migration of kidney cancer cells (11).

However, there are conflicting data on whether MTSS1 expression is an indicator of good or poor prognosis in patients with advanced cancer. In hepatitis B-related hepatocellular carcinoma, elevated MTSS1 expression was found to be associated with cancer metastasis and poor prognosis (18). Up-regulation of MTSS1 expression in colorectal cancer tissues was also significantly correlated with poor differentiation, tissue invasion, high preoperative carcinoembryonic antigen level, presence of lymph node metastasis and high TNM stage (19).

Interestingly, our present data showed an increase in the proliferative potential of BUCC cells with decreased MTSS1 expression, but intrinsically overexpression of MTSS1 did not result in any difference in apoptosis. This suggested that MTSS1 mainly functions as a specific proliferation suppressor protein in BUCC, regulating cell proliferation and cancer growth. The mechanisms for down-regulation of MTSS1 in BUCC are not fully understood. Actin polymerization plays a very important role in cell polarization, cytokinesis and cell adhesions, which is ultimately implicated in a variety of stages of cancer progression. Multiple studies revealed that MTSS1 may act as a cytoskeletal scaffold protein to regulate cytoskeletal dynamics through interaction with other proteins. It can regulate cell motility by modulating different actin-related protein (ARP)2/3 activators (20). Overexpression of MTSS1 increased the formation of lamellipodia, membrane ruffles, and filopodia-like structures (21). In addition, MTSS1 was reported to behave as a sonic hedgehog-responsive gene, which association with the Gli complex during both development and tumorigenesis (22). Methylation is also known to be involved in the down-regulation of MTSS1. Yamashita *et al.* found when gastric cancer cells were treated

with the demethylating agent 5-aza-2'-deoxycytidine, MTSS1 expression level increased significantly (23).

In summary, our current study demonstrated that MTSS1 plays an important role in BUCC tumorigenesis and development. These findings enrich our understanding of the molecular function of MTSS1 underlying BUCC progression, and is useful for understanding the role of MTSS1 in the proliferation of BUCC cells. MTSS1 therefore could be a promising diagnostic biomarker and therapeutic target for BUCC. Further studies are necessary to determine MTSS1-related signaling pathways in BUCC progression.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgements

References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin *65*: 5-29, 2015.

2 Feng H, Zhang W, Li J and Lu X: Different patterns in the prognostic value of age for bladder cancer-specific survival depending on tumor stages. Am J Cancer Res *5*: 2090-2097, 2015.

3 Lee YG, Macoska JA, Korenchuk S and Pienta KJ: MIM, a potential metastasis suppressor gene in bladder cancer. Neoplasia *4*: 291-294, 2002.

4 Woodings JA, Sharp SJ and Machesky LM: MIM-B, a putative metastasis suppressor protein, binds to actin and to protein tyrosine phosphatase delta. Biochem J *371*: 463-471, 2003.

5 Gonzalez-Quevedo R, Shoffer M, Horng L and Oro AE: Receptor tyrosine phosphatase-dependent cytoskeletal remodeling by the hedgehog-responsive gene MIM/BEG4. J Cell Biol *168*: 453-463, 2005.

6 Liu R, Martin TA, Jordan NJ, Ruge F, Ye L and Jiang WG: Metastasis suppressor 1 expression in human ovarian cancer: The impact on cellular migration and metastasis. Int J Oncol *47*: 1429-1439, 2015.

7 Ma S, Guan XY, Lee TK and Chan KW: Clinicopathological significance of missing in metastasis B expression in hepatocellular carcinoma. Hum Pathol *38*: 1201-1206, 2007.

8 Schemionek M, Herrmann O, Reher MM, Chatain N, Schubert C, Costa IG, Hanzelmann S, Gusmao EG, Kintsler S, Braunschweig T, Hamilton A, Helgason GV, Copland M, Schwab A, Muller-Tidow C, Li S, Holyoake TL, Brummendorf TH and Koschmieder S: MTSS1 is a critical epigenetically regulated tumor suppressor in CML. Leukemia *30*: 823-832, 2016.

9 Shi W, Hasimu G, Wang Y, Li N, Chen M and Zhang H: MTSS1 is an independent prognostic biomarker for survival in intrahepatic cholangiocarcinoma patients. Am J Transl Res *7*: 1974-1983, 2015.

10 Zhou L, Li J, Shao QQ, Guo JC, Liang ZY, Zhou WX, Zhang TP, You L and Zhao YP: Expression and significance of MTSS1 in pancreatic cancer. Pathol Oncol Res *22*: 7-14, 2016.

11 Du P, Ye L, Li H, Yang Y and Jiang WG: The tumour-suppressive role of metastasis suppressor-1, MTSS1, in human kidney cancer, a possible connection with the SHH pathway. J Exp Ther Oncol *10*: 91-99, 2012.

12 Du P, Ye L, Ruge F, Yang Y and Jiang WG: Metastasis suppressor-1, MTSS1, acts as a putative tumour suppressor in human bladder cancer. Anticancer Res *31*: 3205-3212, 2011.

13 Mohammed AA, El-Tanni H, El-Khatib HM, Mirza AA, Mirza AA and Alturaifi TH: Urinary bladder cancer: biomarkers and target therapy, new era for more attention. Oncol Rev *10*: 320, 2016.

14 Kayser G, Csanadi A, Kakanou S, Prasse A, Kassem A, Stickeler E, Passlick B and Zur Hausen A: Down-regulation of *MTSS1* expression is an independent prognosticator in squamous cell carcinoma of the lung. Br J Cancer *112*: 866-873, 2015.

15 Zeleniak AE, Huang W, Brinkman MK, Fishel ML and Hill R: Loss of MTSS1 results in increased metastatic potential in pancreatic cancer. Oncotarget *8*: 16473-16478, 2017.

16 Wang F, Liu Y and Zhang H: Loss of MTSS1 expression is an independent prognostic factor for hilar cholangiocarcinoma. Pathol Oncol Res *19*: 815-820, 2013.

18 Huang XY, Huang ZL, Xu B, Chen Z, Re TJ, Zheng Q, Tang ZY and Huang XY: Elevated MTSS1 expression associated with metastasis and poor prognosis of residual hepatitis B-related hepatocellular carcinoma. J Exp Clin Cancer Res *35*: 85, 2016.

17 Parr C and Jiang WG: Metastasis suppressor 1 (MTSS1) demonstrates prognostic value and anti-metastatic properties in breast cancer. Eur J Cancer *45*: 1673-1683, 2009.

19 Wang D, Xu MR, Wang T, Li T and Zhu J: MTSS1 overexpression correlates with poor prognosis in colorectal cancer. J Gastrointest Surg *15*: 1205-1212, 2011.

20 Lin J, Liu J, Wang Y, Zhu J, Zhou K, Smith N and Zhan X: Differential regulation of cortactin and N-WASP-mediated actin polymerization by missing in metastasis (MIM) protein. Oncogene *24*: 2059-2066, 2005.

21 Xie F, Ye L, Ta M, Zhang L and Jiang WG: MTSS1: a multifunctional protein and its role in cancer invasion and metastasis. Front Biosci *3*: 621-631, 2011.

22 Callahan CA, Ofstad T, Horng L, Wang JK, Zhen HH, Coulombe PA and Oro AE: MIM/BEG4, a Sonic hedgehog-responsive gene that potentiates Gli-dependent transcription. Genes Dev *18*: 2724-2729, 2004.

23 Yamashita S, Tsujino Y, Moriguchi K, Tatematsu M and Ushijima T: Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-aza-2'-deoxycytidine treatment and oligonucleotide microarray. Cancer Sci *97*: 64-71, 2006.