Metastasis Suppressor-1, MTSS1, Acts as a Putative Tumour Suppressor in Human Bladder Cancer

PENG DU[1,2], LIN YE[1], FIONA RUGE[1], YONG YANG[2] and WEN G. JIANG[1]

[1]*Metastasis & Angiogenesis Research Group, Cardiff University School of Medicine, Cardiff, U.K.;*
[2]*Department of Urology, Chaoyang Hospital, Capital Medical University, Beijing, P.R. China*

Abstract. *Background: Metastasis suppressor 1 (MTSS1) is proposed to function as a cytoskeletal-associated protein, which may play a role in the aggressiveness of cancer cells. Recent studies have demonstrated a clinical significance of MTSS1 in certain types of cancer, but its role in bladder cancer remains unknown. We investigated the expression of MTSS1 in normal and malignant human bladder tissues and its molecular interaction within bladder cancer cells. Materials and Methods: The expression of MTSS1 in human bladder tissues and bladder cancer cell lines was assessed at both the mRNA and protein levels using reverse transcription-PCR (RT-PCR) and immunohistochemistry, respectively. Full-length MTSS1 cDNA was amplified from normal mammary tissues. The effect of MTSS1 overexpression on cellular functions was examined in bladder cancer cells using a variety of in vitro assays. Results: Transitional cells of bladder tissues stained positively for MTSS1. However, cancer cells from tumour tissues did not stain for MTSS1. Similarly, in bladder cancer cell lines, MTSS1 was almost absent from T24 and RT112 cells, and the EJ138 cell line expressed a very low level of MTSS1. Overexpression of MTSS1 reduced the growth and adhesion of bladder cell lines in vitro. However, overexpression of MTSS1 had no bearing on the invasion and migration of bladder cell lines in vitro. Conclusion: MTSS1 is expressed at low levels or is absent from human bladder cancer cells. MTSS1 levels are inversely correlated with the growth and adhesion of bladder cancer cells in vitro. MTSS1 appears to be a potential tumour suppressor in human bladder cancer.*

Correspondence to: Professor Wen G. Jiang, Metastasis & Angiogenesis Research Group, Cardiff University School of Medicine, Cardiff, CF14 4XN, U.K. Tel: +44 2920742895, Fax: +44 2920742896, e-mail: jiangw@cf.ac.uk

Key Words: Metastasis suppressor-1, MTSS1, cellular migration, invasion, bladder cancer, metastasis.

Tumour metastasis is the most significant contributor to the mortality of patients with cancer. Metastasis of cancer cells proceeds *via* a long series of sequential, interrelated steps, modulated largely by activators and suppressors of metastasis. The accumulation of many genetic alterations has been implicated in tumourigenesis and metastasis of bladder cancer. These include, the altered expression and function of oncogenes such as RAS, ERBB2 and epidermal growth factor (EGF) receptor, cell cycle genes *p15* and *p16*, DNA-repair genes, and tumour-suppressor genes *Rb* and *p53* (1). Loss of heterozygosity of chromosome 9p and 9q is a crucial event in the transition of normal urothelium to papillary transitional cell carcinoma, while *p53* is primarily involved in the development of carcinoma *in situ* (1). Metastasis suppressor genes are defined, by their ability to inhibit metastasis at any step of the metastatic cascade. To date, some metastasis suppressor genes have been identified, such as nonmetastatic gene 23 (NM23), Kangai 1 (KAI1), *KISS1*, mitogen-activated protein kinase kinase 4 (MKK4), breast cancer metastasis suppressor 1 (BRMS1), Rho GDP dissociation inhibitor 2 (RhoGDI2), cofactor required for Sp1 transcriptional activation subunit 3 (CRSP3) and Vitamin D3 up-regulated protein 1 (VDUP1) (2-4). These metastasis suppressor genes inhibit metastasis of cancer cells, *in vivo*, without blocking tumourigenicity.

Metastasis suppressor-1 (*MTSS1*) is a novel potential metastasis suppressor gene, which is also known as missing-in-metastasis (*MIM*), MIM-B, basal cell carcinoma-enriched gene 4 (*BEG4*) or *KIAA042*. *MTSS1* was first identified as a potential metastasis suppressor gene missing in metastatic bladder carcinoma cell lines (5). This gene 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 (5). The MTSS1 protein, a much longer 759 amino acid protein, whose C-terminus is identical to the 356 amino acids encoded by the MTSS1 gene (6) and the mouse homologue of MIM (mMIM) (7), is cytoplasmic in location. Subsequently, MTSS1 was investigated in some types of cancer. In prostate cancer and breast cancer, expression of MTSS1 has been shown to be

Table I. *Sequences of the primers used in this study.*

Primer	Sense (5'-3')	Antisense (5'-3')
MTSS1 screening	TCAAGAACAGATGGAAGAATGG	TGCGGTAGCGGTAATGTG
MTSS1 expression	ATGGAGGCTGTGATTGAG	CTAAGAAAAGCGAGGGG
GAPDH	ATGATATCGCCGCGCTCGTC	GCTCGGTGAGGATCTTCA

reduced, whereas up-regulation of MTSS1 expression has also been observed in hepatocellular carcinoma (8) and breast cancer (9). Recent research showed that down-regulation of *MTSS1* expression was observed both in oesophageal tumour tissues and oesophageal cancer (ESCC) cell lines (10). Patients with high levels of *MTSS1* transcripts had a favourable prognosis in comparison with those who had reduced or lack of expression. Sublines from ESCC cells were created and further demonstrated that *MTSS1* expression in ESCC cells significantly influenced the aggressiveness of the oesophageal cancer cells, by reducing their cellular migration and *in vitro* invasiveness (10). These studies suggest that *MTSS1* plays contrasting roles in different malignancies.

Although MTSS1 has been implicated in the disease progression of certain tumours, its role in bladder cancer remains unknown. In the present study we first examined the expression of *MTSS1* in normal and malignant human bladder tissues, then the effect of *MTSS1* on growth, adhesion, migration and invasion of bladder cancer cells.

Materials and Methods

Materials, cell lines and tissue samples. Human bladder cancer cell lines RT112, EJ138 and T24 (ECACC, European Collection of Animal Cell Culture, Salisbury, UK) were routinely maintained in DMEM-F12 medium supplemented with 10% foetal bovine serum and antibiotics. Polyclonal rabbit anti-MTSS1 and monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Other reagents or kits were obtained from Sigma-Aldrich (Sigma-Aldrich, Poole, UK). Nineteen bladder samples were collected from patients with bladder cancer, immediately after surgery at the University Hospital of Wales, including 12 bladder tumour tissues and 9 normal background bladder tissues. These tissues were collected immediately after radical cystectomy and transurethral resection of bladder tumour. All protocols were reviewed and approved by the Ethical Committee and all patients gave written informed consent.

Immunohistochemical staining procedure for bladder tissues. Frozen sections (8-10 μm thickness) were fixed using a mixture of acetone and methanol (50:50). The sections were then placed in Optimax wash buffer for 5-10 min to rehydrate and incubated for 20 min in a 0.6% bovine serum albumin (BSA) blocking solution and probed with the primary antibody. Following extensive washing, sections were incubated for 30 min with a biotinylated secondary antibody (Multilink swine anti-goat/mouse/rabbit immuno-globulin; Dako Inc. Carpinteria, CA, USA). Following washing, an avidin-biotin complex (Vector Laboratories, Peterborough, UK) was applied to the sections followed by extensive washing. Diaminobenzidine chromogen (Vector Laboratories) was then added to the sections, which were incubated in the dark for 5 min. Sections were then counterstained in Gill's haematoxylin and dehydrated in ascending grades of methanol before clearing in xylene and mounting under a coverslip.

Construction of MTSS1 expression vectors and transfection. This was carried out as we previously reported (9). The first strand cDNA was synthesized from RNA isolated from normal human mammary tissues using a DuraScript™ RT-PCR kit. PCR was then used to amplify the coding sequence of human MTSS1 using the Extensor Hi-Fidelity PCR master mix (ABgene Ltd., Epsom, UK). The sequences of primers are shown in Table I. The verified MTSS1 insert was cloned into a mammalian expression plasmid vector (pEF/His TOPO TA plasmid vector, Invitrogen, Inc., Paisley, UK). The recombinant plasmid vectors were transformed into chemically competent TOP10 *Escherichia coli* (Invitrogen, Inc., Paisley, UK), and the colonies were then analyzed. Colonies carrying correct recombinant plasmids were amplified and plasmids extracted. Purified *MTSS1* transgenes and control plasmid vectors were then transfected into RT112 and EJ138 cells individually using an Easjet Plus electroporator (EquiBio Ltd, Kent, UK), both RT112 and EJ138 were derived from bladder carcinoma. After up to 3 weeks of selection with blasticidin, the transfectants were verified for their expression of *MTSS1* and successful clones were used in subsequent studies.

RNA isolation and reverse transcription PCR. RNA was isolated using Total RNA Isolation Reagent (ABgene Ltd). Reverse transcription was performed using the DuraScript™ RT-PCR kit, followed by PCR using a REDTaq™ ReadyMix PCR reaction mix (primer sequences are shown in Table I). Cycling conditions were 94˚C for 5 min, followed by 30 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 40 s. This was followed by a final 10 min extension period at 72˚C. The products were visualized on 1.5% agarose gel stained with ethidium bromide.

Immunoprecipitation and Western blot analysis. The protein concentrations in cell lysates were determined using the DC Protein Assay kit (BIO-RAD, Hemel Hempstead, UK) and an ELx800 spectrophotometer (BIO-TEK™ , Winooski, VT, US). Equal amount of proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and blotted onto nitrocellulose sheets. Proteins were then respectively probed with anti-MTSS1 antibody and peroxidase-conjugated secondary antibody, with stringent washings between each step. Protein bands were visualized using the Supersignal™ West Dura system (Pierce Biotechnology, Inc., Rockford, IL, USA), and photographed using an UVITech imager (UVITech, Inc., Cambridge, UK).

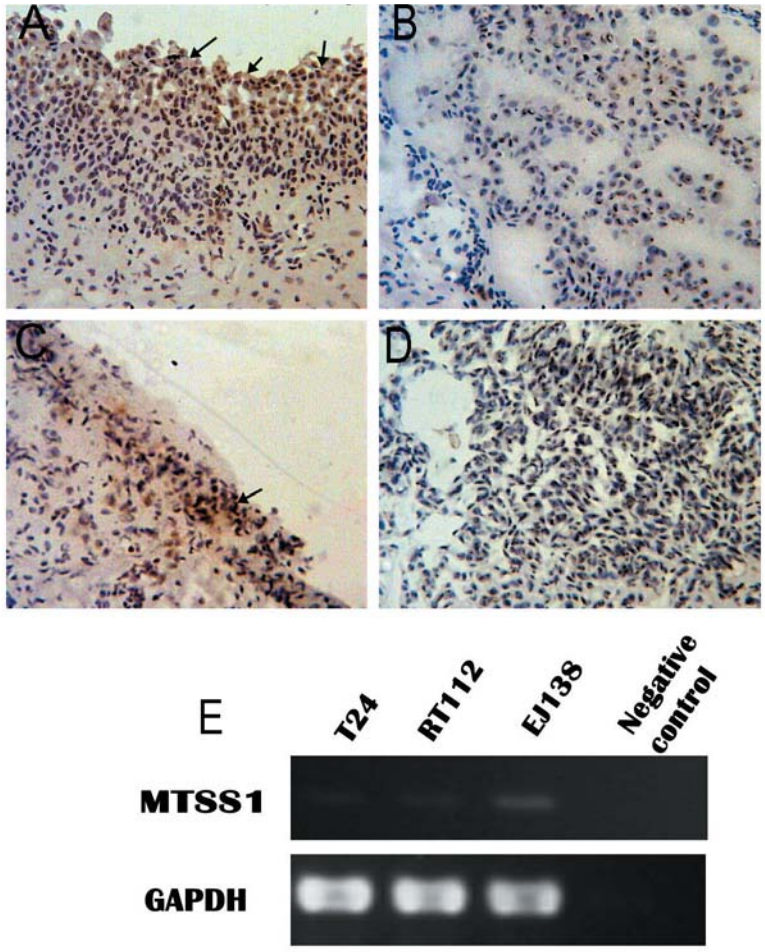

Figure 1. *Expression of MTSS1 in bladder cancer. Immunohistochemical staining of MTSS1 in human bladder tissues. A and C: Background normal bladder tissues; B and D: bladder cancer tissues. The staining for MTSS1 was seen in the cytoplasmic region of the transitional cells (indicated by arrows) and was absent from the cancer cells of tumour tissues. E: The mRNA expression of MTSS1 was examined in bladder cancer cell lines using RT-PCR. Expression of MTSS1 was almost absent from T24 and RT112 cells, and the EJ138 cell line expressed a very low level of MTSS1.*

In vitro cell growth assay. Cell growth was assessed using a method previously reported by our laboratories (11, 12). Briefly, the cells were plated into a 96-well plate (3,000 cells/well). Cell growth was assessed after a period of incubation (up to 5 days). Crystal violet was used to stain cells. Following washing, stained crystal was extracted with 10% acetic acid and absorbance was determined at a wavelength of 540 nm using a spectrophotometer (Elx800).

In vitro invasion assay. This was performed according to a standard procedure (13-15). Transwell inserts with 8 μm pore size were coated with 50 μg Matrigel (BD Matrigel™ Basement Membrane Matrix; Oxford, UK) and air dried. Matrigel was rehydrated before use. A total of 30,000 cells were added to each insert, and the same number of cells were loaded into another well as control. After 96 h cells that had migrated through the matrix to the other side of the insert were fixed in 4% formalin, then stained with 0.5% (w/v) crystal violet. The stained crystal violet was extracted with 10% acetic acid and the absorbance was determined at a wavelength of 540 nm.

In vitro cell matrix adhesion assay. Cell matrix adhesion was assessed according to a previously described method (13-15). A total of 30,000 cells was added to each well of a 96-well plate, previously coated with Matrigel (5 μg/well). After 40 min of incubation, non adherent cells were washed off using balanced saline solution buffer. The remaining adherent cells were then fixed and stained with crystal violet. The number of adherent cells in random fields were observed and counted under a microscope.

Electric cell-substrate impedance sensing (ECIS) based attachment and migration assay. An ECIS Z-Theta instrument and 96W1E arrays (Applied Biophysics, Inc., NY, USA) were used in the study, according to a method recently reported (16). Briefly, the same number of test cells (60,000 per well) was added to each well of the ECIS arrays. Impedance and resistance of the cell layer was immediately recorded for a period of up to 20 h. When confluence was reached, the monolayer in each well was electrically wounded at 1,400 μA and 6,000Hz for 30 s to create a 250 μm wound per well. Impedance and resistance of the wounded cells as they migrated in

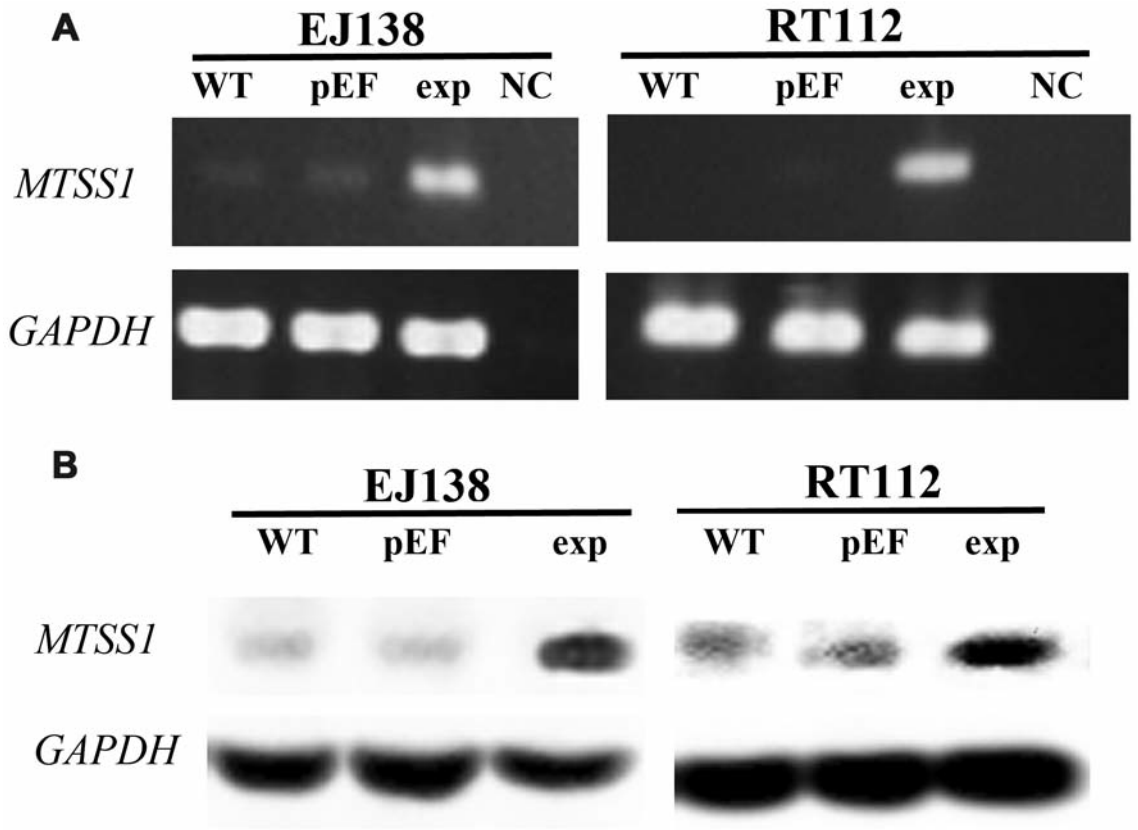

Figure 2. *Overexpression of MTSS1 in bladder cancer cells. A: RT-PCR showed increased MTSS1 transcripts in the RT112 and EJ138 cells for transfected MTSS1 over-expression, respectively. GAPDH was examined as a housekeeping gene. NC, Negative control. B: Changes of MTSS1 protein by MTSS1 overexpression in the corresponding bladder cancer cells were verified using Western blot analysis. GAPDH was used as the housekeeping control.*

the wound was then recorded for a period of up to 20 h. Data were analysed using the ECIS software, supplied by the manufacturer.

Statistical analysis. All statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Non-normally distributed data were assessed using the Mann-Whitney test, while the two sample *t*-test was used for normally distributed data. Differences were considered to be statistically significant at $p<0.05$.

Results

Expression of MTSS1 in human bladder tissues and cell lines. In order to estimate the protein levels of MTSS1 in human bladder tissues, we conducted immunohistochemical analysis using malignant bladder tissues. As shown in Figure 1, Normal bladder transitional cells stained positively for MTSS1 (A and C). The staining was largely confined to the cytoplasmic region of the cells. Staining for MTSS1 was absent from cancer cells of tumour tissues (B and D). The mRNA expression of *MTSS1* was also examined in three bladder cancer cell lines using RT-PCR. In bladder cancer cell lines, *MTSS1* was expressed at trace levels in T24 and RT112 cells, and the EJ138 cell line expressed low levels of MTSS1 (Figure 1E).

Overexpression of MTSS1 in bladder cancer cells. To investigate the impact of *MTSS1* on functions of bladder cancer cells, the constructed *MTSS1* expression vectors were utilised to overexpress *MTSS1* in bladder cancer cells. After the selection using blasticidin, the expression of MTSS1 in the transfected cells was verified using both RT-PCR and Western blot (Figure 2). Increased expression of both mRNA (Figure 2A) and protein (Figure 2B) of MTSS1 was seen in $RT112^{exp}$, in comparison with the controls, $RT112^{WT}$ and $RT112^{pEF}$. Similarly, overexpression of MTSS1 was confirmed in $EJ138^{exp}$ cells, in comparison with $EJ138^{WT}$ and $EJ138^{pEF}$ control cells.

MTSS1 is associated with the growth rate and in vitro invasiveness of bladder cancer cells. RT112 and EJ138 cells with forced overexpression of *MTSS1* displayed a slower growth rate compared with the controls (Figure 3). The

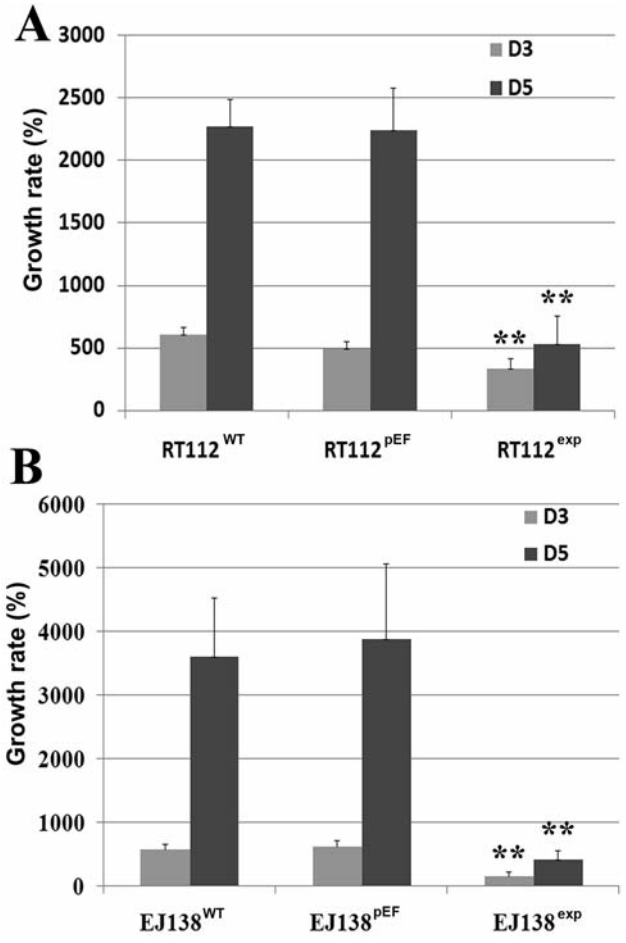

Figure 3. *Effects of MTSS1 on in vitro growth of bladder cancer cells. A: The cell growth rate on third (D3) and fifth day (D5), normalized by data of the first day of RT112^{exp} cells was significantly reduced in comparison to the two controls, RT112^{WT} and RT112^{pEF}. B: the cell growth rate of EJ138^{exp} cells was significantly reduced in comparison to the two controls, EJ138^{WT} and EJ138p^{EF}, p<0.01. Representative data from three independent experiments are shown.*

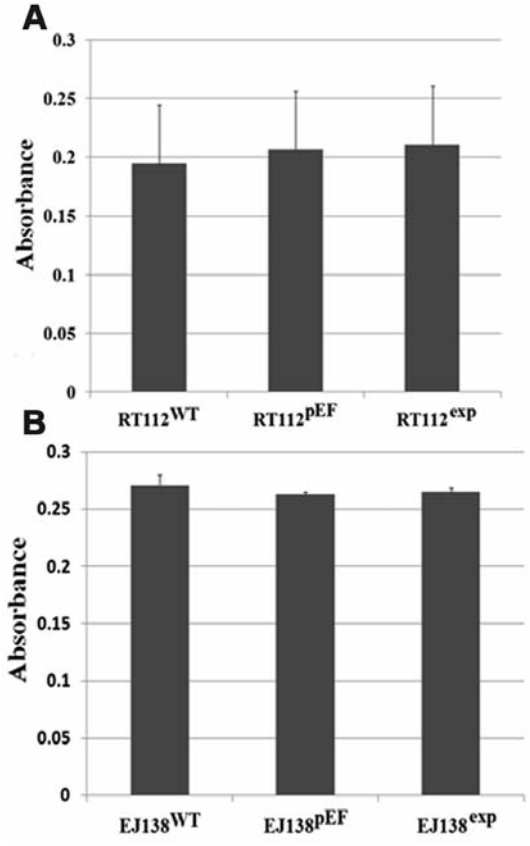

Figure 4. *Effects of MTSS1 overexpression on the invasion of bladder cancer cells. A: RT112 cells; B: EJ138 cells. There were no significant differences among the three groups. Representative data from three independent experiments are shown.*

invasiveness was then examined in the genetically modified cells. Interestingly, the overexpression of *MTSS1* had no bearing on the invasion of bladder cell lines *in vitro* (Figure 4).

Effect of MTSS1 overexpression on cell-matrix adhesion in bladder cancer cells. We first examined the effect of MTSS1 on the cell-matrix adhesion of bladder cancer cell lines. Overexpression of *MTSS1* exhibited a significant inhibitory effect on cell-matrix adhesion of the cells ($p<0.01$ *vs*. both controls) (Figure 5A). Similarly, compared with EJ138^{WT} and EJ138^{pEF}, the number of adherent cells for EJ139^{exp} was significantly reduced ($p<0.01$ *vs*. both controls) (Figure 5B).

The ECIS system was used to further investigate the effect of enhanced expression of *MTSS1* on RT112 and EJ138 cell adhesion. The attachment capacity was markedly reduced in RT112^{exp} cells compared with RT112^{WT} and RT112^{pEF} cells (Figure 5C and D). Similarly, the attachment capacity was markedly reduced in EJ138^{exp} cells compared with EJ138^{WT} and EJ138^{pEF} cells (Figure 5E and F).

Effect of MTSS1 on migration of bladder cancer cells. The effect of MTSS1 on RT112 and EJ138 cellular motility was assessed using the ECIS system. There was no significant difference in the migration capacity between RT112^{exp} cells and the control RT112^{WT} and RT112^{pEF} cells (Figure 6A and B), and also between EJ138^{exp} cells and the respective EJ1382^{WT} and EJ138^{pEF} control cells (Figure 6C and D).

Discussion

Bladder cancer is the fourth most common cancer in men and the second most common malignancy affecting the genitourinary system (17), accounting for 90%-95% of

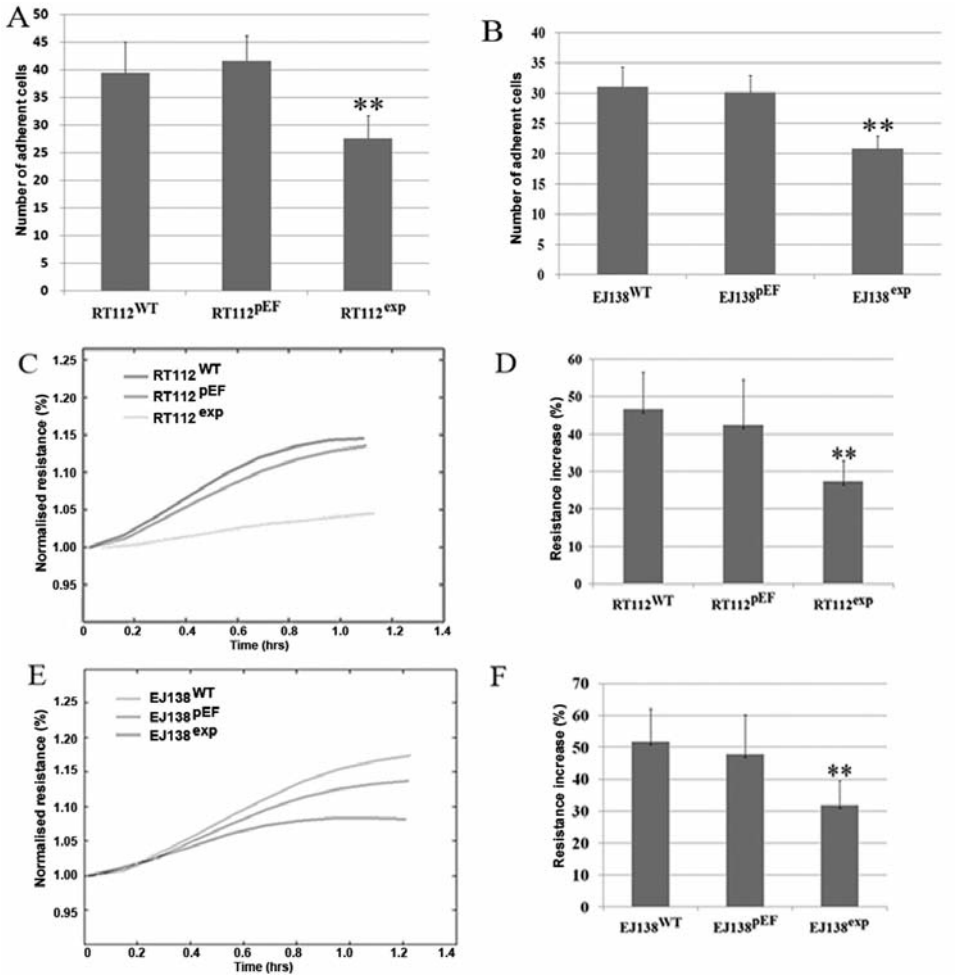

Figure 5. *Effects of MTSS1 on adhesion of bladder cancer cells in vitro. A: Overexpression of MTSS1 reduced the number of adherent RT112^{exp} cells. **p<0.01 versus RT112^{WT} and RT112^{pEF} cells. B: Overexpression of MTSS1 reduced the number of adherent EJ138^{exp} cells. **p<0.01 versus EJ138^{WT} and EJ138p^{EF} cells. C and D: RT112^{exp} cells which overexpressed MTSS1 showed markedly reduced attachment using an ECIS model. E and F: EJ138^{exp} cells which overexpressed MTSS1 showed markedly reduced attachment. ECIS RbA modelling of cell attachment indicated a significant reduction of attachment in MTSS1 transfected cells. **p<0.01 versus wild-type and empty plasmid control cells. All experiments were repeated 3 times.*

urothelial carcinomas (18), and is also called transitional cell carcinomas (TCCs) (19). It is a heterogeneous disease with considerable variations in its natural history. For example, the five-year survival rate is ~98% for patients with a monofocal, well-differentiated and small papillary tumour, whereas this could be 0% if patients have invasive bladder cancer extending throughout the bladder wall and with gross nodal metastases. It is also characterized as a carcinoma with multifocality and a high recurrence rate (20). At initial diagnosis, 75% of patients present with non-muscle-invasive bladder cancer (NMIBC), the remaining 25% present with muscle-invasive bladder cancer (MIBC). The main problems of NMIBC are recurrence and progression, while MIBC is frequently associated with metastatic disease and is the major cause of mortality. In NMIBC, approximately 50%-80% of patients will experience disease recurrence after transurethral resection and intravesical therapy (21). Despite the improvements in both early detection and treatment, there remains a significant challenge to manage this disease.

The role of *MTSS1*, in tumour progression remains unclear and in some ways controversial; whether or not *MTSS1* serves as a metastasis suppressor has not been clearly defined to date. Several lines of evidence have indicated that the expression of *MTSS1* can be up-regulated and up-regulation of MTSS1 expression has been observed in hepatocellular carcinoma, and higher levels of *MTSS1* expression were found to be associated with early-stage disease of hepatocellular carcinoma (8). However, more

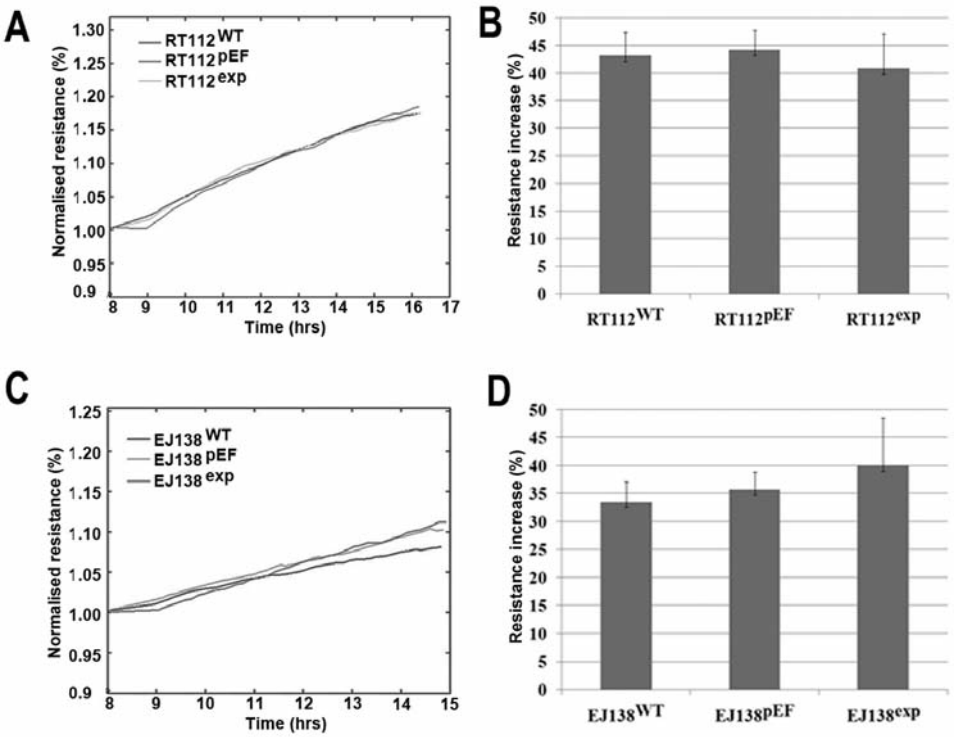

Figure 6. *Effect of MTSS1 on migration of bladder cancer cells as analysed by ECIS (wounding assays). Cell monolayers were first wounded at 6 V for 30 s. The impedance changes during the migration process are shown. Migrating capacity was not significantly different in RT112^{exp} cells compared with RT112^{WT} and RT112^{pEF} cells (A), not in EJ138^{exp} cells compared with EJ1382^{WT} and EJ138p^{EF} cells (C). ECIS RbA modelling of cell attachment indicated no significant reduction of attachment in MTSS1-transfected RT112 cells (B), not of MTSS1-transfected EJ138 cells (D). All experiments were repeated 3 times.*

studies have indicated a down-regulation of MTSS1 in solid tumours (9, 10, 22). Recent research showed that down-regulation of *MTSS1* expression was observed both in oesophageal tumour tissues and ESCC cell lines (9). To our best knowledge, the current study is the first report to examine the staining pattern of *MTSS1* in human bladder tissues and to test the impact on growth, adhesion, migration and invasion of bladder cancer cells by genetically manipulating the expression of MTSS1.

Similar to human breast cancer and oesophageal cancer (9, 10), MTSS1 expression was seen at a lower level or absent from tumour cells of bladder tissues. Similarly, in bladder cancer cell lines, MTSS1 expression was almost absent from T24 and RT112 cells, and the EJ138 cell line expressed a very low level of MTSS1. Moreover, the overexpression of *MTSS1* reduced the growth and adhesion of bladder cell lines *in vitro*. This is in contrast to that observed with hepatocellular cancer cells, in which cancer cells had higher staining than normal cells (8). This would indicate that in some human tumours, MTSS1 is expressed at lower levels, although the opposite may also operate in other selected tumours.

In the present study, we employed methods to genetically alter the expression of *MTSS1* in bladder cancer cells, namely the overexpression approach. Forced overexpression of MTSS1 in two bladder cancer cell lines, RT112 and EJ138, resulted in a reduction of adhesion, but not invasion and migration. This indicates that *MTSS1* plays a key role in the control of the aggressiveness of bladder cancer cells. The current study also demonstrated that overexpression of *MTSS1* resulted in reduced cell growth *in vitro*. This growth-regulatory role of MTSS1 has been tentatively indicated in other tumour cells, including breast cancer cells and oesophageal cancer cells (10, 22). In oesophageal cancer cells, overexpression of MTSS1 was associated with inhibitory effects on cell growth (10). This evidence strongly suggests that MTSS1 is a potential tumour suppressor in these tumours.

In conclusion, the current study shows reduced expression of *MTSS1* in bladder cancer cells. *MTSS1* overexpression can suppress the aggressiveness of bladder cancer cells through inhibiting cell growth, and adhesion. The study, thus, suggests that MTSS1 may be putative tumour suppressor in bladder cancer.

Acknowledgements

Dr P. Du is a recipient of Cardiff University China Medical Scholarship. The Authors wish to thank the Albert Hung Foundation and Cancer Research Wales for supporting the study.

References

1 Brandau S and Bohle A: Bladder cancer. I. Molecular and genetic basis of carcinogenesis. Eur Urol *39*: 491-497, 2001.
2 Malik FA, Sanders AJ, Jones AD, Mansel RE and Jiang WG: Transcriptional and translational modulation of KAI1 expression in ductal carcinoma of the breast and the prognostic significance. Int J Mol Med *23*: 273-278, 2009.
3 Malik FA, Sanders AJ, Kayani MA and Jiang WG: Effect of expressional alteration of KAI1 on breast cancer cell growth, adhesion, migration and invasion. Cancer Genomics Proteomics *6*: 205-213, 2009.
4 Steeg PS: Metastasis suppressors alter the signal transduction of cancer cells. Nat Rev Cancer *3*: 55-63, 2003.
5 Lee YG, Macoska JA, Korenchuk S and Pienta KJ: *MIM*, a potential metastasis suppressor gene in bladder cancer. Neoplasia *4*: 291-294, 2002.
6 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.
7 Mattila PK, Salminen M, Yamashiro T and Lappalainen P: Mouse MIM, a tissue-specific regulator of cytoskeletal dynamics, interacts with ATP-actin monomers through its C-terminal WH2 domain. J Biol Chem *278*: 8452-8459, 2003.
8 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.
9 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.
10 Xie F, Ye L, Chen J, Wu N, Zhang Z, Yang Y, Zhang L and Jiang WG: The impact of metastasis suppressor-1, MTSS1, on oesophageal squamous cell carcinoma and its clinical significance. J Transl Med *9*: 95-104, 2011.
11 Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mansel RE and Mason MD: The potential lymphangiogenic effects of hepatocyte growth factor/scatter factor *in vitro* and *in vivo*. Int J Mol Med *16*: 723-728, 2005.
12 Jiang WG, Hiscox S, Hallett MB, Scott C, Horrobin DF and Puntis MC: Inhibition of hepatocyte growth factor-induced motility and *in vitro* invasion of human colon cancer cells by gamma-linolenic acid. Br J Cancer *71*: 744-752, 1995.
13 Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD and Mansel RE: Expression of membrane type-1 matrix metalloproteinase, MT1-MMP in human breast cancer and its impact on invasiveness of breast cancer cells. Int J Mol Med *17*: 583-590, 2006.
14 Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD, Mokbel K and Mansel RE: Targeting matrilysin and its impact on tumor growth *in vivo*: the potential implications in breast cancer therapy. Clin Cancer Res *11*: 6012-6019, 2005.
15 Jiang WG, Hiscox S, Singhrao SK, Nakamura T, Puntis MC and Hallett MB: Inhibition of HGF/SF-induced membrane ruffling and cell motility by transient elevation of cytosolic free Ca^{2+}. Exp Cell Res *220*: 424-433, 1995.
16 Jiang WG, Martin TA, Lewis-Russell JM, Douglas-Jones A, Ye L and Mansel RE: Eplin-alpha expression in human breast cancer, the impact on cellular migration and clinical outcome. Mol Cancer *7*: 71-80, 2008.
17 Siegel R, Ward E, Brawley O and Jemal A: Cancer statistics, 2011. CA Cancer J Clin *61*: 212-236, 2011.
18 Ploeg M, Aben KK and Kiemeney LA: The present and future burden of urinary bladder cancer in the world. World J Urol *27*: 289-293, 2009.
19 Epstein JI, Amin MB, Reuter VR and Mostofi FK: The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. Am J Surg Pathol *22*: 1435-1448, 1998.
20 Oyasu R: World Health Organization and International Society of Urological Pathology classification and two-number grading system of bladder tumors. Cancer *88*: 1509-1512, 2000.
21 Youssef RF and Lotan Y: Predictors of outcome of non-muscle-invasive and muscle-invasive bladder cancer. Scientific World J *11*: 369-381, 2011.
22 Utikal1 J GA, Muller-Molinet I, Oerther S, Kzhyshkowska J, Arens N, Grobholz R, Kannookadan S and Goerdt S: The expression of metastasis suppressor MIM/MTSS1 is regulated by DNA methylation. Int J Cancer *119*: 2287-2293, 2006.

Received August 2, 2011
Revised September 5, 2011
Accepted September 6, 2011