Metastasis-associated gene 1 promotes invasion and migration potential of laryngeal squamous cell carcinoma cells

HAILI ZHANG\textsuperscript{1,2}, DONG YANG\textsuperscript{2}, HAIJUAN WANG\textsuperscript{2}, SHUXIN WEN\textsuperscript{1}, JIAN LIU\textsuperscript{2}, QINGCHUN LUAN\textsuperscript{2}, YIXUAN HUANG\textsuperscript{2}, BINQUAN WANG\textsuperscript{1}, CHEN LIN\textsuperscript{2} and HAILI QIAN\textsuperscript{2}

\textsuperscript{1}Department of Otolaryngology Head and Neck Surgery, The First Hospital, Shanxi Medical University, Taiyuan, Shanxi 030001; \textsuperscript{2}State Key Laboratory of Molecular Oncology, Cancer Institute/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Chao Yang, Beijing 100021, P.R. China

Received April 28, 2013; Accepted November 1, 2013

DOI: 10.3892/ol.2013.1729

Abstract. Overexpression of the metastasis-associated gene 1 (MTA1) has previously been found to be associated with progression of various cancer types to the metastasis stage. The function of MTA1 in laryngeal squamous cell carcinoma (LSCC) remains unclear. To explore the significance of MTA1 in the invasion and migration processes in LSCC, gene transfection and RNA interference (RNAi) were performed to study the biological function of MTA1 in the LSCC cell line, HEP-2. Results showed that MTA1 promoted the invasion, adhesion and migration behavior of LSCC cells. RNAi against MTA1 significantly decreased the malignant phenotypes of cancer cells. MTA1 may be important in the process of LSCC invasion and metastasis.

Introduction

Laryngeal squamous cell carcinoma (LSCC) represents the second most common malignant neoplasm of the respiratory tract after lung cancer (1). LSCC has a strong propensity to metastasize to regional lymph nodes, which decreases the cure and survival rates (2,3). Multiple steps and factors are involved in the process of malignant cancer progression (4,5). This process is under the control of many metastasis-associated genes. Among these, metastasis-associated gene 1 (MTA1) is a subunit of the nucleosome remodeling and deacetylase (NURD) complex, which is involved in chromatin remodeling and histone deacetylation in gene expression regulation (7). MTA1 functions as a transcriptional coregulator, regulating the downstream target genes that encode effector proteins controlling cancerous processes (8). MTA1 overexpression is positively correlated with in vitro migration and invasion ability in KYSE150 and B16/F10 melanoma cell lines, and inhibition of MTA1 protein expression results in growth inhibition of cancer cell lines (9,10). Sasai et al (11,12) reported that MTA1 mRNA was overexpressed in thymoma and advanced lung cancer. It has also been reported that MTA1 may be involved in initiating carcinogenesis (13,14). Miyatani et al (15) compared the expression of MTA1 in normal esophageal epithelium, normal gastric epithelium and gastro-esophageal junction cancer, and found that MTA1 levels were significantly higher in cancer samples than in their normal counterparts. Moreover, in tonsil cancer, MTA1 is positively correlated with lymphatic metastasis (16). It has also been indicated that MTA1 is correlated with tumor angiogenesis and poor outcome in patients with early-stage non-small cell lung cancer (NSCLC) (17). This line of evidence indicates that MTA1 may become a new marker for predicting cancer metastasis, or even cancer outcome.

Concerning the molecular mechanism of MTA1 in cancer cell metastasis, MTA1 has been reported to be involved in cancer development in several ways. MTA1-interacting coactivator has been identified as a molecule that interacts with MTA1 to regulate estrogen receptor-\(\alpha\) transactivation (18). Yoo et al (19) reported that MTA1 stabilizes hypoxia-inducible factor-1\(\alpha\) protein by recruiting histone deacetylase 1, and is correlated with angiogenesis in cancer development (20). Since MTA1 is a histone deacetylase (HDAC)-interacting protein that modulates the epigenetic status of its target genes, it is expected to widely influence the expression pattern of the cancer-related gene spectrum. Ghanta et al (21) revealed, using a profiling assay, that MTA1 regulation was partially under the control of p53. When p53 is functional, MTA1 mainly focuses on inflammatory and antimicrobial responses; when p53 is absent, MTA1 predominantly targets genes in cancer signaling. MTA1 is correlated with cigarette smoking in NSCLC, indicating its importance in the smoking-related progression...
of this type of cancer (22). MTA1 has also been reported to regulate the anoikis of human prostate cancer cells (23), which reveals a new subfield of MTA1 mechanisms.

MTA1 is a corepressor responsible for estrogen receptor repression at the transcriptional level (24). A naturally occurring MTA1 variant, MTA1s, can sequester estrogen receptor-α in the cytoplasm (25). Estrogen receptor involvement is the first insight into the p53-independent function of MTA1 in the DNA damage response involving the p21/WAF1-proliferating cell nuclear antigen pathway (26). MTA1 is required for the ATR-mediated DNA damage checkpoint function (27). UV radiation stabilizes MTA1 and increases MTA1 binding to ATR. Other molecules found to be associated with MTA1 expression include RECK (28), HDAC1 (15) and MMP-9 (29). Silencing MTA1 by RNA interference (RNAi) reverses the malignant phenotypes, including adhesion, migration and invasiveness of cervical cancer cells (SiHa) via altered expression of p53 and the E-cadherin/β-catenin complex (30).

No systematic biological studies have been performed on LSCC to date. This study aimed to determine the biological role of MTA1 in LSCC using gain-of-function and RNAi techniques.

Materials and methods

Cell lines. The human LSCC cell line HEp-2 and the human keratinocyte HaCaT cell line (State Key Laboratory of Molecular Oncology, Beijing, China) were cultured in RPMI-1640 and DMEM medium (Gibco-BRL, Grand Island, NY, USA), respectively, supplemented with 10% (v/v) fetal calf serum (FCS) (HyClone Laboratories, Inc., Logan, UT, USA), 2 mM L-glutamine and antibiotics (penicillin-streptomycin) at 37°C in a humidified atmosphere of 5% CO₂ at 37°C. The keratinocyte HaCaT cell line is an immortalized normal epithelial cell line.

Reagents. Lipofectamine 2000 was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). siRNA sequence was chemically synthesized by Jikai Co. (Shanghai, China). The MTA1 primary antibody was from Santa Cruz Biotechnology, Inc. (sc-9446; Santa Cruz, CA, USA) and the horseradish peroxidase-conjugated secondary antibody was from Zhongshan Biotech, Co., Ltd (Zhongshan, China). The ECL detection system was purchased from Amersham Biosciences (Piscataway, NJ, USA). The Boyden chamber system and polycarbonate membrane (8 µm pore size) were obtained from Neuro Probe, Inc. (Canada). Matrigel was purchased from BD Biosciences (San José, CA, USA).

siRNA and plasmid transfection. The 21-nt siRNA sequence was chemically synthesized (Jikai Co.). The target sequences of the siRNA for the MTA1 gene (MTA1-siRNA) were as follows: Sense, 5'-GAACACUCU ACGACAUCCUdTdT-3' and antisense, 5'-GGAGAU GUCGUAGAUGUUUdTdT-3' (9). The MTA1-siRNA was dissolved in sterilized and RNase-free water and annealed. The final concentration was 20 µM. Lipofectamine 2000 (20 µl/ml, Invitrogen Life Technologies) was used to transfect the HEp-2 cell line according to the manufacturer’s instructions. A sequence non-specific to any known gene was used as a negative control (Jikai Co.).

The pcDNA3-MTA1 plasmid was provided by Dr Mahoney (Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, USA), and the transfection was performed according to the manufacturer's instructions. The cells transfected with pcDNA3-MTA1 were selected by G418 prior to use.

Western blotting analysis. Cells were grown to 80% confluence and rinsed twice with 1X PBS prior to harvesting. Total cell protein was extracted using PBS buffer containing aprotonin (2 µg/ml), PMSF (100 µg/ml), leupeptin (2 µg/ml) and 1% Nonidet P-40. Protein concentration was determined using the Gene Quant Pro-91738 protein assay system (Bio-Rad Laboratories, Inc, Hercules, CA, USA). Samples were briefly electrophoresed in 10% SDS-PAGE and transferred to nitrocellulose membranes using a semidy transfer system. Nonspecific binding was blocked for 2 h in 5% fat-free milk in PBS buffer, pH 7.6. Blots were first incubated with MTA1 primary antibody (1:200) (Santa Cruz Biotechnology, Inc.) for 2 h at 37°C, and then with corresponding horseradish peroxidase-conjugated secondary antibody (1:2000, Zhongshan Biotech) for 1 h at room temperature. Signals were visualized using the ECL detection system according to the manufacturer's instructions (Amersham Biosciences). The detection was repeated three times.

Migration and invasion assay. Migration assays were performed using a Boyden chamber system (Neuro Probe, Inc., Gaithersburg, MD USA) with a fibronectin precoated (0.5 mg/ml) polycarbonate membrane (8 µm pore size) (AP48; Neuro Probe, Inc.) as described previously, with minor modifications (9). The invasion chamber was identical to the migration chamber, but with the 250 µg/ml Matrigel (BD Biosciences) precoated polycarbonate membrane. For both assays, the bottom chambers were filled with medium containing 10% FCS, RPMI-1640 and 2% BSA as chemoattractant, and medium containing serum-free RPMI-1640, and 0.2% BSA was added into the top chambers. Treated or control cells (2x10⁴) were added to the top chambers, followed by a 10-h incubation at 37°C and 5% CO₂. Three independent experiments were performed for each set. The cells migrated through and adhered to the bottom of the membrane were then fixed and stained with Giemsa dye. The cells that migrated to the lower side of the membrane were mounted under a microscope and averaged. This experiment was repeated three times.

Adhesion assay. Adhesion assay was performed by the MTT assay. The same numbers of MTA1-siRNA-, control-siRNA- and pcDNA3-MTA1-transfected HEp-2 cells (1x10⁴) were plated into the Matrigel precoated (50 µg/ml) 96-well plate in triplicate. The groups of cells were washed for 30, 60 and 90 min, respectively, to remove the non-adherent cells. After washing, the adherent cells were measured with MTT assay at 490 nm wavelength. The OD values reflect the proportion of cells that adhered to the Matrigel coated 96-well plate. This experiment was repeated in triplicate.

Wound healing assay. To perform the wound healing assay, pcDNA3-MTA1-, MTA1-siRNA- and control-siRNA-trans-
fected HEP-2 cells were implanted into the Matrigel (50 \( \mu \)g/ml)-coated 35-mm culture dishes, as described by Qian et al (9). When the cells grew to 80% confluence, a sterilized tip was used to draw a line with the same width on the bottom of the dishes. Images were captured at 8, 16 and 24 h after the wounding. Data shown in the text are representative of three independent repeats.

**RT-PCR analysis.** Total RNA was isolated from HEP-2 and HaCaT cells, and RT-PCR was performed according to the manufacturer's instructions to detect gene expression (K0011 RT-PCR kit, Vigorous Biotechnology, Beijing, China). The primers used for amplification were as follows: MTA1 (forward primer: 5’-CCCGGCGCTGCCAGCCGTGATTAC-3’, reverse primer: 5’-CACCGGCTTCCAGCGGCTTTGCGTAC-3’); \( \beta \)-actin (forward primer: 5’-ACCACAGTCCATGCCCATCAC-3’, reverse primer: 5’-TCCACACCTGTTGCGTGA-3’). The cycling conditions were as follows: Initial denaturation at 94°C for 5 min, followed by 28 cycles at 94°C for 30 sec, 55°C for 40 sec and 72°C for 30 sec.

**Statistical analyses.** The data were analyzed by ANOVA. The statistical analysis was performed using SPSS 11.0 software (SPSS, Inc, Chicago, IL, USA), and \( P<0.05 \) was considered to indicate a statistically significant difference.

**Results**

**MTA1 mRNA expression in HEP-2 and HaCaT cell lines.** Expression of MTA1 mRNA in HEP-2 and HaCaT cell lines was evaluated by RT-PCR. The level of MTA1 mRNA was significantly higher in the LSCC cell line, HEP-2, than in the immortalized keratinocyte HaCaT cell line (Fig. 1). This result indicates that LSCC cells have higher levels of MTA1 expression than normal cell lines, as indicated by the active role of MTA1 in LSCC. Thus, for further experiments in this study, the HEP-2 cell line was selected to study MTA1 biological functions.

**Confirmation of MTA1 expression by RT-PCR and western blot analysis in the gain-of-function and loss-of-function studies.** We performed gene transfection and RNA interference to study MTA1 functions. RT-PCR and western blot analysis confirmed the changes in MTA1 levels after pcDNA3-MTA1 and \( p \)Silencer3.1-MTA1-siRNA transfection. We found that the expression of MTA1 was either suppressed or increased at the mRNA and protein level after treatment. The mRNA levels corresponding to pcDNA3-MTA1, control-siRNA and MTA1-siRNA, presented by density value, were 2.78±0.046, 1.04±0.119 and 0.32±0.046, respectively. The protein levels were 2.69±0.267, 1.07±0.112 and 0.36±0.069, respectively. Compared with the control-siRNA-transfected cells, MTA1-siRNA significantly decreased the expression of MTA1 (\( P<0.05 \)), while pcDNA3-MTA1 significantly enhanced MTA1 expression (\( P<0.05 \)) (Figs. 2 and 3).

**In vitro migration and invasion ability of HEP-2 cells following transfection of pcDNA3-MTA1 and \( p \)Silencer3.1-MTA1-siRNA.** The in vitro migration and invasion ability of HEP-2 cells transfected with pcDNA3-MTA1 and MTA1-siRNA were studied using the Boyden chamber model as described in Materials and methods. In the migration assay, responding to pcDNA3-MTA1, control-siRNA and MTA1-siRNA, the number of cells that migrated to the lower side of the membrane was 549.2±21.51, 352±25.03 and 120.8±17.28, respectively. The number of cells that migrated to the lower side of the membrane in the invasion assay was 423.6±14.15, 301.2±25.4 and 115.4±15.52, respectively. Compared with the control-siRNA-transfected cells, MTA1-siRNA significantly decreased the migration and invasion ability of HEP-2 cells (\( P<0.05 \)), while pcDNA3-MTA1 significantly enhanced the migration and invasion ability of HEP-2 cells (\( P<0.05 \)) (Figs. 4 and 5).

**Adhesion assay of pcDNA3-MTA1- and MTA1-siRNA-treated HEP-2 cell line.** The adhesion of cancer cells to the extracellular matrix and cell surface molecules is a key step during metastasis. The adhesion assay was 423.6±14.15, 301.2±25.4 and 115.4±15.52, respectively. Compared with the control-siRNA-transfected cells, MTA1-siRNA decreased MTA1 mRNA levels, while MTA1-siRNA decreased MTA1 mRNA levels.
ZHANG et al: MTA1 PROMOTES INVASION AND MIGRATION POTENTIAL OF LSCC CELLS

We evaluated the effects of the MTA1 gene on the adhesion ability of cancer cells. The results showed that, at the early stage of adhesion, pcDNA3-MTA1 transfection promoted the adhesion of HEP-2 cells to the Matrigel matrix, while silencing of MTA1 by RNAi inhibited the adhesion process. The adhesion rates of HEP-2 cells transfected with pcDNA3-MTA1, control-siRNA and MTA1-siRNA at 30 min post-cell seeding were 41.8±7.16, 35.6±6.08 and 26.6±2.97% (P<0.05, MTA1-siRNA vs. control and pcDNA3-MTA1 groups), respectively; 63±10.44, 46±8.34 and 38.6±7.86% (P<0.05, MTA1-siRNA vs. control and pcDNA3-MTA1 groups) at 60 min post-cell seeding, respectively; and 71.2±6.83, 63.6±7.56 and 45±6.08% (P<0.05, MTA1-siRNA vs. control and pcDNA3-MTA1 groups) at 90 min post-cell seeding, respectively (Fig. 6). These results indicate that MTA1 may exert its effects on metastasis by regulating the adhesion molecules on the cell surface.
Wound healing assay. For the wound healing assay, equal numbers of transfected HEp-2 cells were reseeded into 35-mm-diameter culture wells. The wound healing ability of cells reflects their movement on the surface to which they are anchored for growth. At 8, 16 and 24 h after wounding, the healing ability of MTA1-siRNA-transfected cells was significantly poorer than that of the pcDNA3-MTA1- and control-siRNA-transfected cells (Fig. 7).

Discussion

Cancer metastasis is the most common factor that causes cancer patient mortality and is a complex process involving a wide range of biological behaviors. It is urgent that the mechanisms underlying cancer metastasis be explored. Carcinogenesis involves several metastasis-associated molecules, many of which are altered during the process. Recent studies have revealed that MTA1 is important in regulating cancer behaviors. MTA1 expression has been associated with cancer malignancy in various cancer types, and has been found to increase the metastatic and invasive potential of carcinoma cells (12,31-33). LSCC is a type of cancer with a high potential for metastasis and invasion. To investigate whether MTA1 is responsible, at least partially, for the metastatic potential of LSCC, MTA1 gene function in the LSCC HEp-2 cell line was biologically studied using gene expression and RNAi techniques.

To confirm the correlation between MTA1 and cancerous potential in LSCC cells, the MTA1 level in HEp-2 and HaCaT cells was detected. It was observed that MTA1 was expressed at relatively low levels in normal human keratinocyte HaCaT cell lines, while its expression was upregulated in HEp-2 cell lines. This result provided evidence supporting the function of MTA1 in the development of human LSCC.

For further confirmation, gene transfection and RNA interference were performed to study MTA1 functions in LSCC. RT-PCR and western blot analysis showed that the expression of MTA1 was either suppressed or increased at the mRNA and protein level after pcDNA3-MTA1 and pSilencer3.1-MTA1-siRNA transfection. To confirm the association between MTA1 expression and the metastatic potential of cancer cells, migration, invasion, adhesion and wound healing assays were performed after manipulating MTA1 expression. The results showed that overexpression of MTA1 promoted the metastasis potential of HEp-2 cells, while MTA1 silencing by RNAi reversed the malignant phenotypes. The in vivo metastasis ability of HEp-2 was inhibited by MTA1 silencing. In the cellular biological studies, a causal correlation between MTA1 expression and the in vitro migration and invasion ability of LSCC cancer cells was identified.

Silencing of MTA1 also impairs the angiogenesis of prostate cancer, partially eliminating the circumstance of cancer growth (34). These studies support the idea that MTA1 may be a potential target for cancer therapy. In the current study, silencing of MTA1 reversed the cancerous behaviors of LSCC, extending the therapeutic value of MTA1 in cancer treatment. MTA1-silencing was observed to result in a prominent loss of function in cancer cells, while overexpression of MTA1 only increased cancerous behaviors to a certain extent. This may indicate that MTA1 is critical in the life activity of cancer cells.

Molecular pathways were not the focus of the current study. Therefore, our next aim is to identify the mechanisms of MTA1 responsible for the change in biological phenotypes and in the structure-function relationship of MTA1.

In brief, the results of the present study demonstrate that the expression of MTA1 promotes the migration and invasion ability of HEp-2 cells, indicating its importance in the progression of LSCC. Suppressing HDAC activity is currently the target of chemotherapy (35-37). MTA1, as an HDAC component of NURD complexes, may be a potential powerful target for LSCC biotherapy or chemotherapy.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (81071773) and the Major State Basic Research Development Program (2009CB521807). The authors are greatly appreciative of their support.

References

1. Cattaruzza MS, Maisonneuve P and Boyle P: Epidemiology of laryngeal cancer. Eur Arch Otorhinolaryngol 228: 293-305, 1996.
2. Fortin O, Koch W, Trotti A and Sidransky D: Head and neck cancer. N Engl J Med 345: 1890-1900, 2001.
3. Greenlee RT, Hill-Harmon MB, Murray T and Thun M: Cancer statistics. CA Cancer J Clin 51: 15-36, 2001.
4. Song I, Cancer metastasis and metastasis suppressors. Korean J Gastroenterol 43: 1-7, 2004.
5. Stracke ML and Liotta LA: Multi-step cascade of tumor cell metastasis. In Vivo 6: 309-316, 1992.
6. Toh Y, Pencil SD and Nicolson GL: A novel candidate metastasis-associated gene, mta1, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses. J Biol Chem 269: 22958-22963, 1994.
7. Xue Y, Wong J, Moreno GT, Young MK, Côté J and Wang W: NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell 2: 851-861, 1998.
8. Gururaj AE, Singh RR, Rayala SK, Holcm C, den Hollander P, Zhang H, Balasenthil S, Talukder AH, Landberg G and Kumar R: MTA1, a transcriptional activator of breast cancer amplified sequence 3. Proc Natl Acad Sci USA 103: 6670-6675, 2006.
9. Qian H, Lu N, Xue L, Liang X, Zhang X, Fu M, Xie Y, Zhan Q, Liu Z and Lin C: Reduced MTA1 expression by RNAi inhibits in vitro invasion and migration of epidermal squamous cell carcinoma cell line. Clin Exp Metastasis 22: 653-662, 2005.
10. Qian H, Yu J, Li Y, Wang H, Song C, Zhang X, Liang X, Fu M and Lin C: RNA interference of metastasis-associated gene 1 inhibits metastasis of B16F10 melanoma cells in a C57BL/6 mouse model. Biol Cell 99: 573-581, 2007.
11. Sasaki H, Yukue H, Kobayashi Y, Nakashima Y, Kaji M, Fukui I, Kiriyama M, Yamakawa Y and Fuji Y: Expression of the MTA1 mRNA in thymoma patients. Cancer Lett 174: 159-163, 2001.
12. Sasaki H, Moriyama S, Nakashima Y, Kobayashi Y, Yukue H, Kaji M, Fukui I, Kiriyama M, Yamakawa Y and Fuji Y: Expression of the MTA1 mRNA in advanced lung cancer. Lung Cancer 35: 149-154, 2002.
13. Zhang H, Stephens LC and Kumar R: Metastasis tumor antigen family proteins during breast cancer progression and metastasis in a reliable mouse model for human breast cancer. Clin Cancer Res 12: 1479-1486, 2006.
14. Bagheri-Yarmand R, Talukder AH, Wang RA, Vadlamudi RK and Kumar R: Metastasis-associated protein 1 deregulation causes inappropriate mammary gland development and tumorigenesis. Development 131: 3469-3479, 2004.
15. Miyatani T, Kurita N, Mikami C, Kashihara H, Higashijima J, Yoshikawa K, Nishimura M, Sato H, Iwata T and Shimada M: Malignant potential of Barrett's esophagus: special reference to HDAC-1 and MTA1 expression. Hepatogastroenterology 58: 472-476, 2011.
16. Park JO, Jung CK, Sun DJ, Joo YH and Kim MS: Relationships between metastasis-associated protein (MTA) 1 and lymphatic metastasis in tonsil cancer. Eur Arch Otorhinolaryngol 268: 1329-1334, 2011.

17. Zhu X, Guo Y, Li X, Ding Y and Chen L: Metastasis-associated protein 1 nuclear expression is associated with tumor progression and clinical outcome in patients with non-small cell lung cancer. J Thorac Oncol 5: 1159-1166, 2010.

18. Mishra SK, Mazumdar A, Vadlamudi RK, Li F, Wang RA, Yu W, Jordan VC, Santen RJ and Kumar R: MiCoA, a novel metastasis-associated protein 1 (MTA1) interacting protein coactivator, regulates estrogen receptor-alpha transactivation functions. J Biol Chem 278: 19209-19219, 2003.

19. Yoo YG, Kong G and Lee MO: Metastasis-associated protein 1 enhances stability of hypoxia-inducible factor-1alpha protein by recruiting histone deacetylase 1. EMBO J 25: 1231-1241, 2006.

20. Li SH, Tian H, Yue WM, Li L, Li WJ, Chen ZT, Hu WS, Zhu YC and Qi L: Overexpression of metastasis-associated protein 1 is significantly correlated with tumor angiogenesis and poor survival in patients with early-stage non-small cell lung cancer. Ann Surg Oncol 18: 2048-2056, 2011.

21. Ghanta KS, Li DQ, Eswaran J and Kumar R: Gene profiling of MTA1 identifies novel gene targets and functions. PLoS One 6: e17135, 2011.

22. Xu L, Mao XY, Fan CF and Zheng HC: MTA1 expression correlates significantly with cigarette smoke in non-small cell lung cancer. Virchows Arch 459: 415-422, 2011.

23. Cui FL, Gong DD, Zhou YJ, Zhu L and Fan Y: Regulatory effect of MTA1 on the anoikis of human prostate cancer cells. Zhonghua Nan Ke Xue 17: 427-430, 2011 (In Chinese).

24. Mazumdar A, Wang RA, Mishra SK, Adam L, Bagheri-Yarmand R, Mandal M, Vadlamudi RK and Kumar R: Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. Nat Cell Biol 3: 30-37, 2001.

25. Kumar R, Wang RA, Mazumdar A, Talukder AH, Mandal M, Yang Z, Bagheri-Yarmand R, Sahin A, Hörtbogyi G, Adam L et al: A naturally occurring MTA1 variant sequesters oestrogen receptor-alpha in the cytoplasm. Nature 418: 654-657, 2002.

26. Li DQ, Pakala SB, Reddy SD, Ohshiro K, Peng SH, Lian Y, Fu SW and Kumar R: Revelation of p53-independent function of MTA1 in DNA damage response via modulation of the p21 WAF1-proliferating cell nuclear antigen pathway. J Biol Chem 285: 10044-10052, 2010.

27. Li DQ, Ohshiro K, Khan MN and Kumar R: Requirement of MTA1 in ATR-mediated DNA damage checkpoint function. J Biol Chem 285: 19802-19812, 2010.

28. Deng Y, Zhou D and Zeng L: Expression and significance of MTA1 and RECK gene in nasopharyngeal carcinoma. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 25: 534-538, 2011 (In Chinese).

29. Jiang Q, Zhang H and Zhang P: ShRNA-mediated gene silencing of MTA1 influenced on protein expression of ER alpha, MMP-9, CyclinD1 and invasiveness, proliferation in breast cancer cell lines MDA-MB-231 and MCF-7 in vitro. J Exp Clin Cancer Res 30: 60, 2011.

30. Mao XY, Wang H, Fan L and Chen G: Silencing MTA1 by RNAi reverses adhesion, migration and invasiveness of cervical cancer cells (SiHa) via altered expression of p53, and E-cadherin/β-catenin complex. J Huazhong Univ Sci Technol Med Sci 31: 1-9, 2011.

31. Holter MD, Kuefer R, Varambally S, Li H, Ma J, Shapiro GI, Gschwend JE, Hautmann RE, Sanda MG, Giehl K, et al: The role of metastasis-associated protein 1 in prostate cancer progression. Cancer Res 64: 825-829, 2004.

32. Toh Y, Ogita T, Endo K, Adachi E, Kusumoto H, Haraguchi M, Okamura T and Nicolson GL: Expression of the metastasis-associated MTA1 protein and its relationship to deacetylation of the histone H4 in esophageal squamous cell carcinomas. Int J Cancer 110: 362-367, 2004.

33. Kumar R, Wang RA and Bagheri-Yarmand R: Emerging roles of MTA family members in human cancers. Semin Oncol 30 (5 Suppl 16): 30-37, 2003.

34. Kai L, Wang J, Ivanovic M, Chung YT, Laskin WB, Schulze-Hoepfner F, Mirochnik Y, Satcher RL Jr and Levenson AS: Targeting prostate cancer angiogenesis through metastasis-associated protein 1 (MTA1). Prostate 71: 268-280, 2011.

35. Denlinger CE, Keller MD, Mayo MW, Broad RM and Jones DR: Combined proteasome and histone deacetylase inhibition in non-small cell lung cancer. J Thorac Cardiovasc Surg 127: 1078-1080, 2004.

36. Warrell RP Jr, He LZ, Richon V, Calleja E and Pandolfi PP: Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. J Natl Cancer Inst 90: 1621-1625, 1998.

37. Phillips T, Collins T and Davies J: American Association for Cancer Research - 96th Annual Meeting. Targeting the cell cycle and HDAC inhibitors. J Drugs 8: 450-453, 2005.