Biochemical and Biophysical Research Communications 425 (2012) 462–467

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Overexpression of myeloid zinc finger 1 suppresses matrix metalloproteinase-2 expression and reduces invasiveness of SiHa human cervical cancer cells

Su-Ju Tsai [a,b], Jin-Ming Hwang [c], Shu-Ching Hsieh [d], Tsung-Ho Ying [e,f,*,1], Yi-Hsien Hsieh [g,h,i,*,1]

[a] Department of Physical Medicine and Rehabilitation, Chung Shan Medical University Hospital, Taichung, Taiwan
[b] Department of Physical Medicine and Rehabilitation, College of Medicine, Chung Shan Medical University, Taichung, Taiwan
[c] Department of Applied Chemistry, School of Medicine, College of Medicine, Chung Shan Medical University, Taichung, Taiwan
[d] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[e] Department of Obstetrics and Gynecology, School of Medicine, College of Medicine, Chung Shan Medical University, Taichung, Taiwan
[f] Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan
[g] Institute of Biochemistry and Biotechnology, College of Medicine, Chung Shan Medical University, Taichung, Taiwan
[h] Department of Biochemistry, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[i] Department of Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 21 July 2012
Available online 27 July 2012

Keywords:
MZF1
MMP-2
Migration
Invasion
Cervical cancer

ABSTRACT

Myeloid zinc finger 1 (MZF1) gene belongs to the Kruppel family of zinc finger transcription factors. MZF1 has been suggested to play an important role in the tumorigenesis, invasion, and apoptosis of various tumor cells. However, the role of MZF1 in human cervical cancer remains unclear. To investigate the molecular mechanisms of MZF1 and its functional role in human cervical cancer cell migration and invasion, we experimented on stable SiHa cells overexpressing MZF1. We found that MZF1 overexpression inhibits the migratory and invasive abilities of SiHa cervical cancer cells. In addition, the overexpression of MZF1 significantly reduces MMP-2 protein and mRNA levels. Luciferase and ChIP assays suggested that MZF1 directly binds to MMP-2 gene regulatory sequences in vivo and suppresses MMP-2 promoter activity in vitro. This study shows that MZF-1 represses MMP-2 transcription and suggests that this repression may be linked to inhibition of human cervical cancer cell migration and metastasis.

1. Introduction

Cervical cancer is the second most frequently diagnosed malignancy and one of the major causes of death in women worldwide. Human papillomavirus (HPV) infection is the most significant risk factor in the etiology of cervical cancer [1]. Genetic changes and the resulting genomic instability have long been known as important in the mechanism underlying cervical cancer progression. Cytogenetic studies have shown non-random chromosomal changes in cervical cancer cells [2,3], and several molecular genetic studies have found frequent loss-of-heterozygosity sites, suggesting tumor suppressor gene involvement in the development of cervical cancer [4,5].

The metastatic process involves multiple events involving cell migration, invasion, adhesion, and degradation of the extracellular matrix (ECM). The proteolytic degradation of ECM components is an event central to this process. Specifically, the ability to pierce the basement membrane is related to an increased potential for metastasis. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that can degrade various ECM components [6]. Two members of the MMP family, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B), which show substrate specificity toward type IV collagen, play a pivotal role in ECM degradation. The expression of MMP-2 and MMP-9 are strongly linked to tumor migration, invasion, and metastasis in various types of human cancer [7,8]. In cervical cancer, the expression of MMP-2 and MMP-9 is involved in invasion and metastasis [9,10]. Additionally, MMP-2 increases with the increasing grade of CIN, suggesting that the overexpression of MMP-2 may be associated with a potential risk for invasion and metastasis [11]. These observations suggest that MMP-2 has a crucial effect on human cervical cancer cells.

The myeloid zinc finger 1 (MZF1) gene, a member of the Kruppel family of C_2H_2 zinc finger transcription factors [12], was originally identified in the peripheral blood leukocytes of a chronic myelogenous leukemia (CML) patient as an mRNA transcript that encodes a 485 amino acid protein [13]. MZF1 is a bi-functional transcription factor that can act both as transcription repressor and transcription activator, and it is involved in cell differentiation,

* Corresponding authors. Address: Department of Biochemistry, School of Medicine, Chung Shan Medical University, Chung Shan Medical University Hospital, No. 110, Section 1, Chien-Kuo N. Road, Taichung, Taiwan. Fax: +886 4 23248195.
E-mail addresses: ying.steve@gmail.com (T.-H. Ying), hyhsien@csmu.edu.tw (Y.-H. Hsieh).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.125

migration, and proliferation in hematopoietic and non-hematopoietic cells [14,15]. The overexpression of MZF1 leads to the transactivation of Axl promoter activity and induces the migratory, invasive, and in vivo metastatic potential of solid tumor cells [16]. In addition, reducing the expression of MZF1 inhibits PKCα expression and cell migration and invasion in SK-Hep-1 cells [17]; the suppression of MZF1 also inhibits tumor cell growth in human hepatocellular carcinoma xenografts in nude mice [18]. However, little is known about the molecular mechanisms by which MZF1 regulates the expression of MMP-2 in highly invasive cervical cancer cells. In this study, we demonstrate the expression of MZF1 in cervical cancer cell lines, examine the overexpression of MZF1, and evaluate the overexpressed effect of stable transfection on the migration and invasiveness of cervical cancer cells. Furthermore, we determine the effect of MZF1 overexpression on the expression of MMP-2 transcription levels that play crucial roles in the migration and invasion of cervical cancer cells. This study hopes to provide insight into the mechanism by which MZF1 suppresses the migration ability and invasiveness of human cervical cancer cells.

2. Methods and materials

2.1. Reagents

Antibodies to MZF1, MMP-2, MMP-9, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-labeled anti-mouse and anti-rabbit secondary antibodies were obtained from Promega (Madison, WI). The MMP-2 promoter constructs cloned into the pGL3-Basic luciferase vector (Promega) and β-galactosidase plasmids were donated by Dr. JL Ko of the Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan.

2.2. Construction of plasmids and stable transfection

The pcDNA3.0 expression vector was obtained from Invitrogen. The full-length MZF1 gene (GenBank Accession No. M58297, 1091–2548) was obtained from SK-Hep-1 cells by RT-PCR assay using the primer pairs 5′-TATAAGCTTATGAATGAATGGTC CCCTTGTGTAT-3′ (nucleotides of 234–253) and 5′-TATCTCGAGCTACTC GGCGCT GTGGACGCGCTGG-3′ (nucleotides of 1520–1502). The PCR products were isolated and subcloned into the pcDNA3.0 expression vector and verified by automated DNA sequencing. For stable transfection, SiHa cells were plated at 2×105 cells per 100 mm dish. At 70% confluency, cultures were transfected with pcDNA3.0 (Neo) or pcDNA-MZF1 (MZF1) using Jet-PEI reagent according to manufacturer's instructions. SiHa cells overexpressing Neo or MZF1 cells were passaged at 1:10 split ratio 48 h after transfection, selected in medium containing G418 (800 μg/mL) for approximately 28 days, and tested for the expression of stable transfection by Western blot analysis.

2.3. Cell culture

SiHa cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). They were grown in DMEM medium supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified environment (5% CO_2 at 37 °C).

2.4. RNA isolation and reverse transcriptase-PCR

Using TRIOL reagent, total cellular RNA was isolated from SiHa cells overexpressing Neo or MZF1. RT-PCR was performed as described previously [17]. The following primers were used: MZF1 forward, 5′-AGGTCCAGGTAGTGTAAGCCCT-3′; MZF1 reverse, 5′-ACTCTCCGATGCTCTTCCAG-3′; β-actin forward, 5′-GCACTCTTC-CAGCCTTCCTTCC-3′; and β-actin reverse, 5′-TCACCTTCACCGTTC-CAGTTTTT-3′. The PCR products were analyzed by 1.5% agarose gel electrophoresis and direct visualization after EtBr staining.

2.5. Western blot analysis

Equal amounts of protein extracts (30 μg) were subjected to immunoblot following methods described previously [19]. Antibodies to MZF1, MMP-2, MMP-9, and β-actin were used. The blots were incubated with HRP-conjugated anti-mouse or anti-rabbit antibody at room temperature for 2 h. Signals were detected via enhanced chemiluminescence using Super-Signal West Dura Extended Duration Substrate (Pierce) or Immobilon Western HRP Substrate (Millipore).

2.6. Cell viability assay

SiHa cells overexpressing Neo or MZF1 cell viability was detected by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay as described in the manufacture's manual. Briefly, SiHa cells overexpressing Neo or MZF1 were cultured in 24-well culture plates (1×10^4cells per well). Cell proliferation was determined at 24 and 48 h after incubation by adding MTT (5 mg/ml) and incubating the cells further for 4 h. After shaking for 20 min, the absorbance of solubilized formazan crystals was read at 570 nm (A570) was detected using a spectrophotometer microplate reader (BIORAD Laboratory, Hercules, CA).

2.7. Cell cycle analysis

Cell proliferation was determined by flow cytometry assay [19]. SiHa cells overexpressing Neo or MZF1 were fixed in 70% ethanol at −20 °C overnight. The fixed cells were washed with PBS and incubated in PBS containing 100 mg/ml RNase A, 0.1% Triton X-100 and 0.5 mg/ml propidium iodide at room temperature for 20 min. Cell-cycle analysis was performed on the FACSCalibur flow cytometer and the Cellquest software (Becton Dickinson, San Jose, CA).

2.8. Gelatin zymography

A conditioned medium from an equal number of cells was prepared as described above and separated by 10% acrylamide gels containing 0.1% gelatin. The gels were washed in 2.5% Triton X-100 for 15 min twice and incubated in a buffer solution [50 mM Tris–HCl (pH 8.8) containing 5 mM $CaCl_2$ and 0.02% (w/v) NaN_3] for 16 h. After reaction, the gels were stained for 1 h with staining solution (0.1% Coomassie Brilliant Blue, 30% methanol, and 10% acetic acid) and destained in the same solution but without Coomassie Brilliant Blue. The gelatinolytic activity of MMP-2 and MMP-9 was visualized as a clear band against a dark background of stained gelatin.

2.9. Migration assay

Migration assay was performed using the 48-well Boyden chamber (Neuro Probe, Gaithersburg, MD) plated with 8 μm pore size polycarbonate membrane filters (Neuro Probe). The lower compartment was filled with DMEM containing 20% FBS. SiHa cells were placed in the upper part of the Boyden chamber and incubated for 12 h. After incubation, the cells were fixed with methanol and stained with 0.05% Giemsa for 1 h. Cells on the upper surface of the filter were removed with a cotton swab. Subsequently, the filters were rinsed in distilled water until no additional stains

leached. The cells were then air dried for 30 min. Migratory phenotypes were determined by counting the cells that migrated to the lower side of the filter with microscopy at 200× magnification. Fourth fields were counted for each filter, and each sample was assayed in triplicate.

2.10. Invasion assay

Invasion assay was performed using a 48-well Boyden chamber with polycarbonate filters. The upper side was precoated with 10 μg/mL Matrigel (Collaborative Biomedical Products, Bedford, MA). SiHa cells were placed in the upper part of the Boyden chamber and incubated at 37 °C for 24 h. The experimental procedures were the same as in the migration assay.

2.11. Luciferase assay

SiHa cells overexpressing Neo or MZF1 were grown in a 24-well dish and transiently cotransfected with 0.5 μg pGL3 or pGL3-MMP-2 and 0.25 μg cytomegalovirus (CMV)-β-galactosidase plasmids using Jet-PEI reagent. Cells were then lysed, and luciferase activity was measured using a luminometer (Luminoscan Ascent, Thermo Electron Co.). Luciferase activity was normalized to β-galactosidase activity in cell lysates using an assay system (Promega, Madison, WI) and expressed as an average of three independent experiments.

2.12. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation assay was performed as previously described [17]. Chromatin was sonicated and immunoprecipitated with 2 μg rabbit polyclonal MZF1 antibody (Santa Cruz Biotechnology) or rabbit immunoglobulin G antibody as the negative control. The cross-links were reversed by overnight incubation at 65 °C in the presence of 8 M NaCl, followed by the addition of proteinase K (10 mg/mL) for 1 h at 45 °C and RNAase (10 mg/mL) for 30 min at 37 °C. After extraction and precipitation, DNA was dissolved in 30 μL of ddH2O. Primers (forward 5′-AGGCAAGTGGGGTGACGAGGTCG-3′ and reverse 5′- TACTCGCCCTCCTCCACTTTTCT-3′) were used for the amplification of the specific section of the 150 bp MMP-2 promoter region containing the predicted MZF1 binding site. The PCR products were analyzed by 2% agarose gels and visualized by ethidium bromide staining.

2.13. Statistical analysis

All experiments were performed in triplicate. Results are expressed as means ± SD and representative of at least three independently performed experiments. Statistical differences in values were analyzed by Student's t-test for unpaired data and one-way ANOVA with threshold for significance set at $P < 0.05$.

3. Results

3.1. Inhibition of cervical cancer cell migration and invasion ability by the overexpression of MZF1

The molecular mechanism by which MZF1 inhibits cell migration and invasion was determined through the transfection of SiHa cervical cancer cells with Neo (pcDNA) or MZF1 expression vectors (pcDNA-MZF1) and the selection of a stable cell line expressing a high level of MZF1 protein (Fig. 1A) and mRNA (Fig. 1B). Proliferation is important in tumor invasion. To determine the effect of MZF1 on the viability of SiHa cells, MTT assay was performed. The overexpression of MZF1 did not affect cell viability within 24 and 48 h (Fig. 2A). To determine whether overexpression of MZF1 affected cell proliferation, flow cytometric analysis was used to quantify proliferation of SiHa cells overexpressing Neo or MZF1. Our data show that no effect on SiHa cells overexpressing Neo or MZF1 cell proliferation (Fig. 2B). Cell migration and invasion is indispensable in cancer metastasis, so migration assay was performed to determine the role of MZF1 in this process. MZF1 reduced the migration ability of SiHa cells as proven by a 72% decrease in cell movement (Fig. 2C). In addition, the number of cells overexpressing MZF1 that moved through the membrane of the Matrigel chamber was about 82% less than that of Neo cells (Fig. 2D). The results indicate that MZF1 reduces the cell invasiveness of SiHa cervical cancer cells.

3.2. Down-regulation of MMP-2 by MZF1

The expression of MMP-2 and MMP-9 is strongly associated with cervical cancer cell invasion [20]. In this study, the role of MMP-2 and MMP-9 in MZF1-regulated cell migration and invasion was examined. Western blot analysis was performed to determine the effects of MZF1 on MMP-2 and MMP-9 protein expression levels. Overexpressing-MZF1 cells showed a decrease in MMP-2 protein levels but not in MMP-9 protein levels (Fig. 3A). RT-PCR assay confirmed the down-regulation of MMP-2 in overexpressing-MZF1 cells (Fig. 3B). Furthermore, the activity of MMP-2 and MMP-9 was confirmed through gelatin zymography assays. Only the levels of active MMP-2 decreased in the culture medium of overexpressing-MZF1 cells, and no differences in MMP-9 activity were observed in overexpressing-MZF1 cells (Fig. 3C). These findings suggest that MZF1 reduces the expression and enzymatic activity of MMP-2 without affecting MMP-9 activity.

3.3. Suppression of MMP-2 promoter activity by MZF1

The regulation of MMP-2 transcription activity by MZF1 transcription factors in cells overexpressing MZF1 was tested. The promoter sequence of MMP-2 genes was analyzed using the TRANSFAC 4.0 database to identify the putative MZF1 binding sequence. Only one putative MZF1 binding site was identified within the MMP-2 promoter. The MZF1 binding site for the MMP-2 promoter was located within the proximal promoter region upstream of the transcriptional start site (Fig. 4A). MZF1 and a luciferase reporter of MMP-2, which reflects maximum MMP-2 promoter activity [21], were used to determine the ability of MZF1 to regulate MMP-2 promoter activity. Cells were transiently cotransfected

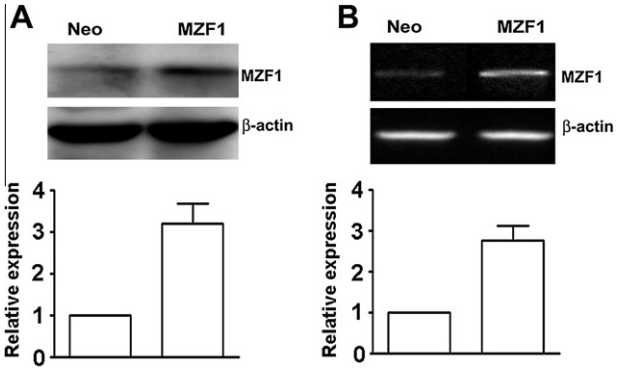

Fig. 1. SiHa cells were transfected with Neo (pcDNA3) or MZF1 expression vectors to establish stable transfectants. (A) Expression of MZF1 level was determined by Western blot analysis. (B) Total mRNA was isolated from Neo or overexpressing-MZF1 cells, and the expression of MZF1 was investigated by RT-PCR with β-actin as internal control. Results from three independent assays are expressed as means ± SD.

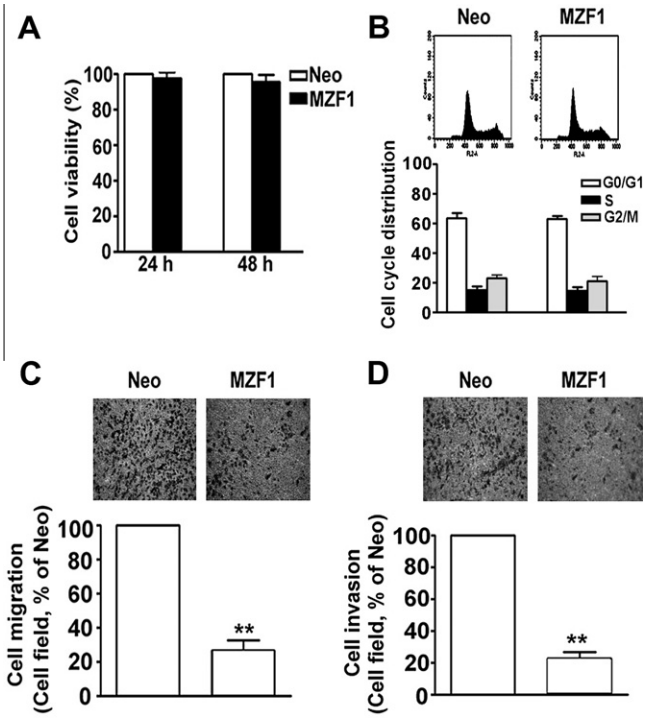

Fig. 2. Overexpression of MZF1 decreases cell migration and invasion ability. (A) The effect of Neo or MZF1 overexpression on cell proliferation after 24 and 48 h was determined by MTT assay. Absorbance values obtained from Neo cells were taken as 100%. (B) Flow cytometry of Neo or overexpressing-MZF1 cells assessed for their distribution in the cell cycle. Values are means ± S.E. of the percentage of cells in the G0/G1 (White bars), S (Black bars) and G2/M (Gray bars) phases of the cell cycle from three independent experiments. Neo or overexpressing-MZF1 cells were plated on Boyden chambers without Matrigel for migration assay (C) or with Matrigel (D) for invasion assay, and then collected after 8 h for migration assay or 16 h for invasion assay. Cells from the top and migrated/invaded cells from the bottom of the chambers were counted. Migrating and invading cells were imaged under a phase contrast microscope. Neo cells were used as control. Each bar represents the mean ± S.E calculated from three independent experiments. ** $P < 0.01$.

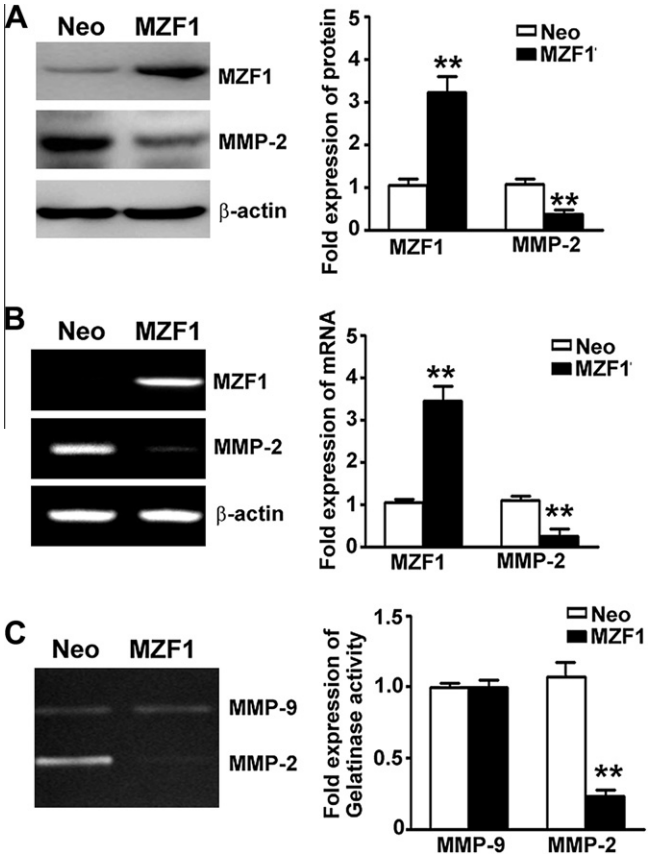

Fig. 3. Down-regulation of MMP-2 expression and secretion by MZF1. (A) Total lysate was extracted from Neo or overexpressing-MZF1 cells, and the protein expression of MZF1, MMP-2, and MMP-9 was measured by Western blot analysis. (B) Semi-quantitative RT-PCR was performed to test the mRNA levels of MZF1, MMP-2, and MMP-9 in Neo or overexpressing-MZF1 cells. (C) Cell-conditioned medium was collected and analyzed for the expression of MMP-2 and MMP-9 by gelatin zymography assays with β-actin as internal control. Results from three independent assays are expressed as means ± S.E. ** $P < 0.01$.

with a reporter gene that included the pGL3 or pGL3-MMP-2 promoter and β-galactosidase. Cells overexpressing MZF1 showed a greater decrease in MMP-2 promoter activity compared with Neo cells (Fig. 4B). These findings indicate that MZF1 suppresses MMP-2 promoter activity.

3.4. Transcriptional repression of MMP-2 by MZF1

To confirm whether MZF1 binds directly to the promoter region of MMP-2, chromatin immunoprecipitation (ChIP) experiments were performed using specific MZF1 antibodies followed by the human MMP-2 promoter. Immunoprecipitated chromatin DNA fragments from Neo or overexpressing-MZF1 cells were analyzed by PCR of the MZF1 region of the MMP-2 promoter (Fig. 4C). In overexpressing-MZF1 cells, the PCR amplification of the MZF1 binding region of the MMP-2 promoter increased, but no changes were observed in Neo overexpressing cells. These findings indicate that MZF1 may directly bind to the promoter region of MMP-2 and repress MMP-2 transcription activity.

4. Discussion

Although MZF1 has been implicated in cell migration and invasion [16], its underlying molecular mechanisms in human cervical cancer progression and invasion have not yet been clearly elucidated. Therefore, characterizing the biological, functional, and molecular mechanisms of MZF1 on cervical cancer is of strategic importance. In this study, relatively low endogenous MZF1 levels were used to establish stable MZF1 overexpression in cervical cancer cells. We observed that MZF1 inhibited the expression and secretion of MMP-2 by directly binding to the MMP-2 promoter region and suppressing MMP-2 transcription activity. These results explain the role of MZF1 as a tumor suppressor in cervical cancer cell metastasis.

MZF-l, a putative transcription factor belonging to the C2H2 zinc finger gene family, contains 13 zinc fingers arranged in two distinct sets: the first set contains zinc fingers 1–4 domains, and the second set in the carboxyl terminus contains zinc fingers 5–13 domains. Both zinc fingers can bind to DNA, and DNA consensus binding sites have been identified for each of these DNA-binding domains [22]. Some studies have found that the overexpression of MZF1 inhibits apoptosis and promotes oncogenesis [14,23], and leads to the transactivation of Axl promoter activity and gene expression, resulting in the induced migratory, invasive, and metastatic potential of solid cancer cells [16]. In addition, the knockout expression of MZF1 inhibits HCC cell growth in vitro and tumor formation in human hepatocellular carcinoma xenografts in vivo [17,18]. Recent study shows that, MZF1 activation have demonstrated to signaling networks of cathepsins B on ErbB2 induced invasive phenotype in breast cancer cells. These authors revealed that the ErbB2-induced cathepsins B expression and invasive ability were critical for ErbB2-induced activation of MZF1, leading to

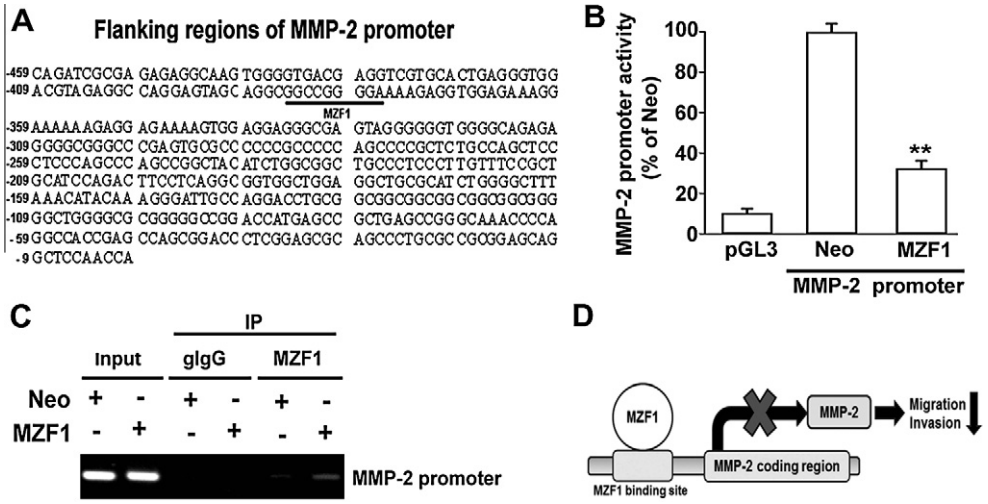

Fig. 4. (A) MZF1 directly binds to the nucleotide sequence of the MMP-2 promoter region. The binding consensus sites of transcription factors are labeled. (B) pGL3-basic or pGL3-MMP-2 promoter plasmid was cotransfected with a β-galactosidase expression vector in Neo or overexpressing-MZF1 cells. After 16 h of transfection, MMP-2 promoter activities in cell lysates were determined by luciferase reporter assay. Luciferase activity, determined in triplicates, was normalized to β-galactosidase activity. The values presented are means ± S.E from three independent experiments. $^{**}P < 0.01$. (C) Chromatin immunoprecipitation using anti-MZF1 antibodies was performed on chromatin extracted from cells overexpressing Neo or MZF1, and specific MMP-2 promoter regions were amplified by PCR. Input samples were used as positive control. (D) Proposed mechanism by which MZF1 inhibits cell migration and invasion in cervical cancer.

MZF1 binding to an ErbB2-responsive enhancer element in the first intron of cathepsins B, induces cathepsins B activation and promotes invasion of breast cancer cells. These functioning downstream of ErbB2, such as PAK4, Cdc42bpβ, PKCα and ERK2 signaling networks [24]. Taken together, all convenient evidence suggests that MZF1 might function as a potential oncogene contributing to the development and progression of human cancers. In contrast, other studies demonstrated that nude mice lacking MZF1 show increased tumorigenesis and that MZF1 may act as a tumor suppressor in tumorigenesis [25]. MZF1, therefore, has bifunctional properties depending on the cellular environment.

Cancer cell metastasis is a complex process involving a number of stages, including invasiveness, intravasation, extravasation, and growth in a secondary organ. Tumor invasion across the ECM is thought to be one of the critical stages. The ability of tumor cells to degrade ECM components is correlated with the presence of cells with metastatic potential [26]. Several proteolytic enzymes, such as MMP-2 and MMP-9, are thought to be involved directly in the migration, invasion and metastasis of tumor cells [27,28]. The elevated expression of MMP-2 has been observed in various solid tumors, including cervical cancer, and is associated with the prognosis factor [29,30]. With Western blot analysis and RT-PCR assay, we also assessed the expression levels of MMP-2 in cells overexpressing MZF1. Endogenous MMP-2 protein mRNA expression was markedly down-regulated in cells overexpressing MZF1. Furthermore, with gelatin zymography, we noted a decreased secretion of MMP-2 in cells overexpressing MZF1. MMP-9 is another factor playing an important role in cell invasiveness in cervical cancer cells [20]. Surprisingly, in our study, MMP-9 expression levels remained unchanged in Neo or overexpressing-MZF1 cells as seen through Western blot analysis, RT-PCR, and gelatin zymography, suggesting that protein and MMP-9 secretion are not involved in MZF1-dependent invasion in cervical cancer cells. This finding suggests that the down-regulation of MMP-2 is involved in the MZF1-mediated reduction in cell migration and invasion potential.

Our RT-PCR experiment showed a decreased expression of MMP-2 mRNA in cells overexpressing MZF1, confirming that the transcription factor MZF1 may function as a transcription repressor of MMP-2 expression in human cervical cancer cells (Fig. 3B). Evidence indicates that MMP-2 activity is regulated by several mechanisms, including gene expression, at the transcription level. The transcriptional up-regulation of MMP-2 involves the activation of several well-known factors, including p53, AP-1, Ets-1, C/EBP, CREB1, PEA3, YB-1, Sp1, and AP-2 [31]. Promoter region analysis indicated that the upstream region between −475 and +7 of the MMP-2 gene, which we characterized previously as the site of maximum MMP-2 promoter activity [21], contains the only MZF1 binding motifs. Interestingly, the transcription factor MZF1 has not been earlier reported to regulate MMP-2 promoter activity. We used luciferase activity and ChIP assays to confirm that MZF1 can bind to one region in the MMP-2 promoter area (nucleotides −690 to −483 bp) because MZF1 itself contains the DNA-binding domain, suggesting that the functional trans-element is MMP-2. Therefore, as a transcription repressor, MZF1 regulates human MMP-2 promoter activity in human cervical cancer cells.

In summary, we have demonstrated for the first time that the overexpression of MZF1 inhibits MMP-2 promoter activity and gene expression, resulting in the suppressed migration and invasion of human cervical cancer cells (Fig. 4D). Further work is required to better understand the signaling pathways by which MZF1 regulates MMP-2 gene expression in cervical cancer cells, and the screening of cervical cancer patients is clearly warranted.

Acknowledgments

This work was supported by Grants from Chung Shan Medical University Hospital, Taichung, Taiwan (CSH-2010-C-018). Freeze centrifugation was performed in the Instrument Center of Chung Shan Medical University, which is supported by the National Science Council, Ministry of Education, and Chung Shan Medical University.

References

[1] C.M. Martin, K. Astbury, J.J. O'Leary, Molecular profiling of cervical neoplasia, Expert Rev. Mol. Diagn. 6 (2006) 217–229.
[2] C.P. Harris, X.Y. Lu, G. Narayan, et al., Comprehensive molecular cytogenetic characterization of cervical cancer cell lines, Genes Chromosom. Cancer 36 (2003) 233–241.
[3] P. Sova, Q. Feng, G. Geiss, et al., Discovery of novel methylation biomarkers in cervical carcinoma by global demethylation and microarray analysis, Cancer Epidemiol. Biomarkers Prev. 15 (2006) 114–123.

[4] Q. Feng, A. Balasubramanian, S.E. Hawes, et al., Detection of hypermethylated genes in women with and without cervical neoplasia, J. Natl Cancer Inst. 97 (2005) 273–282.
[5] H.C. Lai, Y.W. Lin, T.H. Huang, et al., Identification of novel DNA methylation markers in cervical cancer, Int. J. Cancer 123 (2008) 161–167.
[6] M. Egeblad, Z. Werb, New functions for the matrix metalloproteinases in cancer progression, Nat. Rev. Cancer 2 (2002) 161–174.
[7] O.R. Mook, W.M. Frederiks, C.J. Van Noorden, The role of gelatinases in colorectal cancer progression and metastasis, Biochim. Biophys. Acta 1705 (2004) 69–89.
[8] W.G. Stetler-Stevenson, A.E. Yu, Proteases in invasion: matrix metalloproteinases, Semin. Cancer Biol. 11 (2001) 143–152.
[9] T. Fernandes, L.A. de Angelo-Andrade, S.S. Morais, et al., Stromal cells play a role in cervical cancer progression mediated by MMP-2 protein, Eur. J. Gynaecol. Oncol. 29 (2008) 341–344.
[10] A. Schropfer, U. Kammerer, M. Kapp, et al., Expression pattern of matrix metalloproteinases in human gynecological cancer cell lines, BMC Cancer 10 (2010) 553.
[11] M.A. Gaiotto, J. Focchi, J.L. Ribalta, et al., Comparative study of MMP-2 (matrix metalloproteinase 2) immune expression in normal uterine cervix, intraepithelial neoplasias, and squamous cells cervical carcinoma, Am. J. Obstet. Gynecol. 190 (2004) 1278–1282.
[12] R. Hromas, S.J. Collins, D. Hickstein, et al., A retinoic acid-responsive human zinc finger gene, MZF-1, preferentially expressed in myeloid cells, J. Biol. Chem. 266 (1991) 14183–14187.
[13] P. Hui, X. Guo, P.G. Bradford, Isolation and functional characterization of the human gene encoding the myeloid zinc finger protein MZF-1, Biochemistry 34 (1995) 16493–16502.
[14] R. Hromas, J. Morris, K. Cornetta, et al., Aberrant expression of the myeloid zinc finger gene, MZF-1, is oncogenic, Cancer Res. 55 (1995) 3610–3614.
[15] D. Perrotti, P. Melotti, T. Skorski, et al., Overexpression of the zinc finger protein MZF1 inhibits hematopoietic development from embryonic stem cells: correlation with negative regulation of CD34 and c-myb promoter activity, Mol. Cell. Biol. 15 (1995) 6075–6087.
[16] G. Mudduluru, P. Vajkoczy, H. Allgayer, Myeloid zinc finger 1 induces migration, invasion, and in vivo metastasis through Axl gene expression in solid cancer, Mol. Cancer Res. 8 (2010) 159–169.
[17] Y.H. Hsieh, T.T. Wu, J.H. Tsai, et al., PKCalpha expression regulated by Elk-1 and MZF-1 in human HCC cells, Biochem. Biophys. Res. Commun. 339 (2006) 217–225.
[18] Y.H. Hsieh, T.T. Wu, C.Y. Huang, et al., Suppression of tumorigenicity of human hepatocellular carcinoma cells by antisense oligonucleotide MZF-1, Chin. J. Physiol. 50 (2007) 9–15.
[19] T.H. Ying, S.F. Yang, S.J. Tsai, et al., Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8-/caspase-3-dependent pathway, Arch. Toxicol. 86 (2012) 263–273.
[20] M. Libra, A. Scalisi, N. Vella, et al., Uterine cervical carcinoma: role of matrix metalloproteinases (review), Int. J. Oncol. 34 (2009) 897–903.
[21] M.L. Lin, Y.C. Lu, J.G. Chung, et al., Down-regulation of MMP-2 through the p38 MAPK-NF-kappaB-dependent pathway by aloe-emodin leads to inhibition of nasopharyngeal carcinoma cell invasion, Mol. Carcinog. 49 (2010) 783–797.
[22] J.F. Morris, R. Hromas, F.J. Rauscher 3rd, Characterization of the DNA-binding properties of the myeloid zinc finger protein MZF1: two independent DNA-binding domains recognize two DNA consensus sequences with a common G-rich core, Mol. Cell. Biol. 14 (1994) 1786–1795.
[23] R. Hromas, S. Boswell, R.N. Shen, et al., Forced over-expression of the myeloid zinc finger gene MZF-1 inhibits apoptosis and promotes oncogenesis in interleukin-3-dependent FDCP.1 cells, Leukemia 10 (1996) 1049–1050.
[24] B. Rafn, C.F. Nielsen, S.H. Andersen, et al., ErbB2-driven breast cancer cell invasion depends on a complex signaling network activating myeloid zinc finger-1-dependent cathepsin B expression, Mol. Cell 45 (2012) 764–776.
[25] M. Gaboli, P.A. Kotsi, C. Gurrieri, et al., Mzf1 controls cell proliferation and tumorigenesis, Genes Dev. 15 (2001) 1625–1630.
[26] U. Cavallaro, G. Christofori, Cell adhesion in tumor invasion and metastasis: loss of the glue is not enough, Biochim. Biophys. Acta 1552 (2001) 39–45.
[27] A.F. Chambers, A.C. Groom, I.C. MacDonald, Dissemination and growth of cancer cells in metastatic sites, Nat. Rev. Cancer 2 (2002) 563–572.
[28] P. Mignatti, D.B. Rifkin, Biology and biochemistry of proteinases in tumor invasion, Physiol. Rev. 73 (1993) 161–195.
[29] M. Rauvala, K. Aglund, U. Puistola, et al., Matrix metalloproteinases-2 and -9 in cervical cancer: different roles in tumor progression, Int. J. Gynecol. Cancer 16 (2006) 1297–1302.
[30] Y.T. Tee, C.P. Han, J.L. Ko, et al., Evaluation of matrix metalloproteinase 2 expression in cervical carcinogenesis using tissue array and integrated optical density for immunoreactivity, Reprod. Sci. 14 (2007) 719–726.
[31] H. Qin, Y. Sun, E.N. Benveniste, The transcription factors Sp1, Sp3, and AP-2 are required for constitutive matrix metalloproteinase-2 gene expression in astroglioma cells, J. Biol. Chem. 274 (1999) 29130–29137.