Human Leucine Zipper Protein sLZIP Induces Migration and Invasion of Cervical Cancer Cells via Expression of Matrix Metalloproteinase-9*

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Background: Proteolytic degradation of the extracellular matrix and basement membranes by matrix metalloproteinases (MMPs) is crucial in tumor invasion and metastasis.

Results: sLZIP induces the expression of MMP-9, leading to enhancement of migration and invasion of cervical cancer cells.

Conclusion: A novel regulatory mechanism of MMP-9 expression is characterized.

Significance: sLZIP is a potential target for preventing the invasion and metastasis of cervical cancer.

Extracellular proteolysis mediates tissue homeostasis. In cancer, altered proteolysis leads to abnormal tumor growth, inflammation, tissue invasion, and metastasis. Matrix metalloproteinase-9 (MMP-9) represents one of the most prominent proteases associated with inflammation and tumorigenesis. The recently identified human transcription factor sLZIP is a member of the leucine zipper transcription factor family. Although sLZIP is known to function in ligand-induced transactivation of the glucocorticoid receptor, its exact functions and target genes are not known. In this study, we investigated the role of sLZIP in MMP-9 expression and its involvement in cervical cancer development. Our results show that sLZIP increased the expression of MMP-9 at both the mRNA and protein levels and the proteolytic activity of MMP-9 in HeLa and SiHa cells. sLZIP also increased the transcriptional activity of MMP-9 by binding directly to the cAMP-responsive element of the MMP-9 promoter region. Involvement of sLZIP in MMP-9 expression was further supported by the fact that ME-180 cells expressing sLZIP siRNA were refractory to MMP-9 expression. Results from wound healing and invasion assays showed that sLZIP enhanced both the migration and invasion of cervical cancer cells. The increased migration and invasion of HeLa and SiHa cells that were induced by sLZIP were abrogated by inhibition of the proteolytic activity of MMP-9. These results indicate that sLZIP plays a critical role in MMP-9 expression and is probably involved in invasion and metastasis of cervical cancer.

The interactions of cells with the extracellular matrix (ECM) are critical for the normal development and function of organisms (1, 2). Modulation of cell-matrix interactions occurs through the action of unique proteolytic systems responsible for hydrolysis of a variety of ECM components (1, 3). Proteolytic degradation of ECM and basement membranes is a crucial event in tumor invasion, metastasis, and angiogenesis (4, 5). Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are capable of degrading ECM proteins and basement membranes at physiological pH values and are thus involved in a wide range of normal and pathological conditions, including inflammation, tissue repair, tumor invasion, and metastasis (4, 6–8). Members of the MMP family include collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -7, -10, -11, and -12), and membrane-type MMPs (MT1-MMP to MT6-MMP) (9–11). Among MMPs, gelatinases such as MMP-2 (gelatinase A) and MMP-9 (gelatinase B) can degrade collagenous ECM proteins and collagen IV, a major component of basement membranes (6, 12, 13).

MMP-9 is of particular interest because its basal expression level is normally low in most cells, whereas it is highly expressed in most human cancers in response to diverse growth factors and cytokines (9, 14, 15). In contrast, MMP-2 is expressed constitutively in diverse cell types (10). Furthermore, up-regulation of MMP-9 expression contributes to the development of tumor progression, including angiogenesis (11, 12) and invasion and metastasis (1, 16, 17). High levels of MMP-9 have been related to several inflammatory diseases, such as multiple sclerosis and rheumatoid arthritis (7, 18), in addition to a variety of cancers, such as gliomas and breast and lung cancers (19–21). However, the exact mechanism underlying the up-regulation of MMP-9 in disease states is not clear.

The human leucine zipper protein LZIP is a bZIP transcription factor of the CREB (cAMP-responsive element-binding protein) gene family that contains a basic DNA-binding domain, a putative transmembrane domain, and a leucine zipper domain (22). Ubiquitously expressed human LZIP binds to the canonical cAMP-responsive element (CRE) and regulates cell proliferation (22, 23). It has been reported that human LZIP binds to CC chemokine receptor 1 and is a positive regulator in CC chemokine leukotactin-1-induced cell migration (24). We previously identified a novel isoform of human LZIP and par-
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Materials—DMEM, RPMI 1640 medium, FBS, and Lipofectamine 2000 reagent were purchased from Invitrogen. MMP-2 and -9 inhibitors and phorbol 12-myristate 13-acetate were purchased from Calbiochem. Anti-MMP-9 antibody was purchased from Abcam (Cambridge, United Kingdom). Gelatin was obtained from Difco (Lexington, KY).

Cell Culture and Transient Transfection—HeLa and SiHa cells were grown in DMEM supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. ME-180 cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were plated at a density of 5 × 10^5 cells/well in a 6-well plate. After 24 h, cells were cotransfected with 0.2 μg of the reporter gene plasmid and the experimental plasmid using Lipofectamine 2000 according to the manufacturer’s instructions. After 24 h of transfection, cells were grown in the same medium supplemented with 0.5% FBS for 24 h. Serum-starved cells were used for the assays.

RNA Interference of sLZIP—Scrambled control and human sLZIP-specific siRNAs were purchased from Bioneer Corp. (Daejeon, South Korea). For RNA interference experiments, cells were plated at a density of 5 × 10^5 cells/well in a 6-well plate. After 24 h, cells were transfected with siRNA using Lipofectamine 2000 reagent according to the manufacturer’s instructions.

RNA Extraction and Semiquantitative RT-PCR—Total RNA was extracted from cells with TRIzol (Invitrogen) according to the manufacturer’s protocol. Approximately 2 μg of total RNA was used to prepare cDNA using the SuperScript first-strand cDNA synthesis kit (Bioneer Corp.). The experimental conditions were as follows: 96 °C for 40 s, 60 °C for 40 s, and 72 °C for 40 s for 22 cycles. Primer pairs of sLZIP and MMP-9 are as follows: sense primer 5’-ATGGAGCTGGAATTGGATGC-3’ and antisense primer 5’-CTAGCCTGAGTATCTCTC-3’ for sLZIP and sense primer 5’-TCCCTGGAGACCTGAGAACC-3’ and antisense primer 5’-CGCGAAGTCTTCGAGTAGTT-3’ for MMP-9. GAPDH1 was amplified as an internal control. The PCR products were electrophoresed on a 2% (w/v) agarose gel in 1× Tris acetate/EDTA buffer and stained with ethidium bromide solution. All PCRs were repeated at least three times. The intensity of each band amplified by RT-PCR was analyzed using a Multimage™ light cabinet (version 5.5, Alpha Innotech Corp., San Leandro, CA) and normalized to that of GAPDH mRNA in corresponding samples.

Luciferase Reporter Gene Activity Assay—Luciferase assays were performed using the luciferase assay system (Promega). The transfected cells were washed twice with ice-cold PBS and lysed in the culture dishes with reporter lysis buffer. Luciferase activities were recorded in a 20/20n luminometer (Turner Bio-Systems, Sunnyvale, CA) according to the manufacturer’s instructions. Luciferase activity was normalized to β-galactosidase activity. For β-galactosidase assay, pSV-β-galactosidase was cotransfected with the luciferase reporter gene. Cell extracts were assayed for β-galactosidase activity using the β-galactosidase enzyme assay system (Promega) and analyzed with a DU530 spectrophotometer (Beckman Instruments). The ratio of luciferase to β-galactosidase activity was determined in triplicate samples. All data are presented as the mean ± S.D. of at least three independent experiments.

Gelatin Zymography—The MMP-9 activity was analyzed by gelatin zymography. Briefly, cells were incubated in serum-free DMEM, and the supernatants were collected after incubation for 24 h, clarified by centrifugation, mixed with nonreducing sample buffer, and separated by electrophoresis on a 7.5% SDS-polyacrylamide gel containing 1 mg/ml gelatin. After electrophoresis, gels were renatured by washing twice for 30 min with 2.5% Triton X-100 solution to remove all SDS. The gels were then incubated overnight at 37 °C in 50 mmol/liter Tris-HCl (pH 7.4), 5 mmol/liter CaCl2, and 1 μM ZnCl2. The gels were stained with 0.05% Coomassie Brilliant Blue R-250 and destained in destaining solution (5:1:4 methanol/acetic acid/distilled water).

Electrophoretic Mobility Shift Assay—A double-stranded oligonucleotide containing a consensus binding site for CRE, 5-CTGACCCCTGAGTACGACTTG-3, was 5’-end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. Unincorporated nucleotide was removed by passage over a Bio-Gel P-6 spin column (Bio-Rad). A purified recombinant His-sLZIP protein was incubated with radiolabeled probe for 20 min, and the protein-DNA complexes were separated from free probes by electrophoresis on a 4% native polyacrylamide gel in 0.5× Tris-HCl (pH 7.5), 1 mM MgCl2, 50 mM NaCl, 0.5 mM EDTA, 4% glycerol, 0.5 mM DTT, and 50 mg/ml poly(dI-dC). Dried gels were visualized by autoradiography. For competition experiments, binding reactions were incubated with a 50-fold molar excess of unlabeled CRE-binding oligonucleotide for 20 min before addition of the radiolabeled oligonucleotide. For supershift assays, antibodies were added to the reaction mixture for an additional 30 min.

Chromatin Immunoprecipitation Assay—For ChIP experiments, ~4 × 10^7 cells were used per sample. Cells were washed with PBS and treated with 1% formaldehyde in medium for 10 min at 25 °C, followed by addition of glycine to a final concentration of 0.125 M for 5 min. Cells were then scraped into PBS and centrifuged at 1000 × g for 5 min at 4 °C. ChIP assays were performed by coprecipitating the protein-DNA complexes with anti-HA antibody. Promoter regions −181 to −29 and −426 to −300 of MMP-9 were amplified from the prepared DNA samples using the oligomers.
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**Wound Healing Assay**—After HeLa and SiHa cells were grown to confluence in 6-mm culture plates, an artificial “wound” was created using a P-10 pipette tip to scratch the confluent cell monolayer. Cell migration into the scratched region was recorded using a Nikon microscopy system at 0, 12, 24, and 36 h, and images were obtained at the beginning and at the 36-h time point to compare the cell migration for the closure of the wound.

**In Vitro Invasion Assay**—Matrigel invasion assays were used to assess the effect of sLZIP conditioned medium (CM) on the invasive ability of HeLa and SiHa cells. Transwell inserts (8.0-μm pore size with polycarbonate membrane; BD Biosciences) were coated with 100 μl of Matrigel (Sigma), diluted with serum-free DMEM, and allowed to solidify for 5 h. After harvest, cells were suspended in serum-free DMEM at a concentration of 1 × 10⁶ cells/ml, and 100 μl of the cell suspension immediately placed into the upper compartment of the plate. Subsequently, the lower compartment was filled with sLZIP CM. After a 36-h incubation, the non-invading cells remaining on the upper surface of the membrane were removed by wiping with cotton-tipped swabs. Cells on the lower surface of the membrane were stained with Diff-Quik staining solution (Baxter, Deerfield, IL) according to the manufacturer’s instructions. Five fields of adherent cells were counted randomly in each well, and the results were numerically averaged and counted.

**Generation of Conditioned Medium**—HeLa and SiHa cells at a density of 5 × 10⁵ cells/well were plated in a 6-well culture dish. Cells were transfected with the mock or sLZIP expression plasmids and incubated in serum-free DMEM for 24 h. The supernatants were collected, and cells were removed by centrifugation at 500 × g for 5 min. Cell-free conditioned medium was then either used directly to measure the MMP activity or diluted with fresh co-culture medium and used for further experiments.

**Statistical Analysis**—Data are expressed as the mean ± S.D. Analyses were performed using Student’s t test with SPSS 12.0 software. A p value of <0.05 was considered to be significant.

**RESULTS**

**sLZIP Induces MMP-9 Expression in Cervical Cancer Cells**—MMPs are up-regulated in metastatic cancer cells and are key markers for these cells (26, 27). In an effort to identify new target molecules for the transcription factor sLZIP, we examined the effects of sLZIP on the expression of MMPs in various cancer cell lines derived from breast, prostate, lung, and cervical cancers. Among different types of MMPs, sLZIP significantly increased the mRNA level of MMP-9 in HeLa cervical cancer cells but not in breast and lung cancer cell lines (Fig. 1A). sLZIP slightly increased the mRNA levels of MMP-9, -10, and -13 in DU-145 prostate cancer cells (Fig. 1A). The mRNA expression of MMP-13 was also increased by sLZIP in A549 cells (Fig. 1A). Although sLZIP increased the mRNA levels of MMP-10 and -13 in HeLa cells, we focused on the role of sLZIP in MMP-9 expression in cervical cancer cells because the effect of sLZIP on MMP-9 expression was significant. To determine the expression levels of endogenous MMP-9 and sLZIP in various cervical cancer cells, we performed semiquantitative RT-PCR and real-time PCR. Our results reveal that the mRNA levels of MMP-9 and sLZIP are higher in ME-180 cells compared with HeLa and SiHa cells and that the expression levels of sLZIP and MMP-9 are related (Fig. 1B). We next examined the effect of sLZIP on MMP-9 expression in HeLa and SiHa cells, which exhibit lower expression of sLZIP and MMP-9. The mRNA expression of MMP-9 was increased in a dose-dependent manner in cells transfected with sLZIP compared with cells transfected with a mock vector (Fig. 1C). To further document the role of sLZIP in MMP-9 transcriptional regulation, we depleted sLZIP using siRNA in ME-180 cells expressing higher sLZIP and MMP-9 levels and measured the endogenous mRNA level of MMP-9. With increasing amounts of siRNA for sLZIP (si-sLZIP), the sLZIP mRNA level was decreased to 50% of the control cell level (Fig. 1C). The decrease in the sLZIP level resulted in a 2-fold decrease in the mRNA level of MMP-9 (Fig. 1C).

**sLZIP Enhances MMP-9 Proteolytic Activity in Cervical Cancer Cells**—We next examined the effects of sLZIP on transcriptional activation of the MMP-9 promoter. The results from the luciferase assay showed that sLZIP increased the transcriptional activity of the MMP-9 promoter in a dose-dependent manner in HeLa and SiHa cells (Fig. 2A). We also examined the effect of si-sLZIP on MMP-9 promoter activity. ME-180 cells transfected with si-sLZIP abrogated the positive effect of sLZIP on the transcriptional activity of the MMP-9 promoter (Fig. 2A). Cells containing 50 nmol of si-sLZIP exhibited an 85% decrease in MMP-9 promoter activity compared with cells transfected with a siRNA control (Fig. 2A). These results demonstrate that sLZIP increases MMP-9 promoter activity and regulates MMP-9 expression in cervical cancer cells. We next examined whether sLZIP induces the MMP-9 protein using ELISA. sLZIP-transfected cells showed higher MMP-9 protein production compared with cells transfected with a mock vector (HeLa and SiHa) (Fig. 2B). Phorbol 12-myristate 13-acetate was used as a positive control. However, the protein level of MMP-9 was decreased by si-sLZIP in ME-180 cells (Fig. 2B). These results are consistent with the RT-PCR and promoter activity results. We also examined the effect of sLZIP on the proteolytic activity of MMP-9 using zymographic analysis. Our results showed that sLZIP increased the enzymatic activity of MMP-9 in HeLa and SiHa cells, whereas si-sLZIP decreased the proteolytic activity of MMP-9 in ME-180 cells as shown in a gelatin zymogram (Fig. 2C). These results indicate that sLZIP induces MMP-9 at both the mRNA and protein levels and enhances the proteolytic activity of MMP-9 in cervical cancer cells.

**sLZIP Binds Directly to the CRE Region in the MMP-9 Promoter**—The binding sites of several transcription factors are located in the 5′-flanking region of the MMP-9 gene (11, 16). To determine the regulatory mechanism of sLZIP in the transcriptional activation of MMP-9, multiple constructions containing different lengths of the 5′-flanking region of the MMP-9 gene coupled to the luciferase reporter gene were generated. The results from a luciferase assay showed that sLZIP increased the transcriptional activity of MMP-9 by 3.5-fold (Fig. 3A). The deletion fragment (−223/+13) containing the potential sLZIP-binding CRE site exhibited an ~2.5-fold increase in MMP-9 promoter activity (Fig. 3A). To investigate whether sLZIP binds directly to the promoter of MMP-9, we performed an EMSA using a DNA sequence bearing several motifs of the MMP-9
promoter. The results indicated that sLZIP bound directly to the proximal CRE region of the MMP-9 promoter (Fig. 3B). The ability of sLZIP to bind to the DNA of the proximal CRE in the MMP-9 promoter increased in a dose-dependent manner (Fig. 3C). To determine the specificity of the sLZIP binding activity, 50- and 100-fold excesses of the unlabeled CRE probe were used to compete with the complexes. As shown in Fig. 3C, the sLZIP complex was supershifted by treatment with the antibody against sLZIP, indicating that the sLZIP binding activity for the CRE of the MMP-9 promoter is specific. The sLZIP-CRE complex was detected in the wild-type CRE consensus probe; however, sLZIP did not bind to the mutant CRE probe (Fig. 3C).

We next performed a ChIP assay to investigate sLZIP recruitment to the −181/−29 CRE binding site in the MMP-9 promoter region. Samples of HA-sLZIP-transfected cells that were immunoprecipitated with the anti-HA antibody showed the PCR band, demonstrating that these transcription factors bind to the CRE within the MMP-9 promoter region (Fig. 3D). The PCR band was not detected in the presence of normal rabbit IgG (Fig. 3D). To confirm the specificity of sLZIP binding to the promoter, the same ChIP DNA was amplified using off-target primers. The off-target region (−426/−300) was not amplified, whereas positive results were obtained from input chromatin (Fig. 3D). Our results indicate that sLZIP is recruited to the MMP-9
promoter region, leading to activation of MMP-9 transcription. To further investigate the induction level of the MMP-9 transcript, we performed real-time PCR using samples from the ChIP assay. We found that expression of MMP-9 mRNA in sLZIP-transfected cells was 6-fold higher than the basal level in mock vector-transfected cells (Fig. 3E). These results suggest that sLZIP up-regulates the transcription and activity of MMP-9 by binding directly to the MMP-9 promoter region.

sLZIP Enhances Wound Healing, Migration, and Invasion in Cervical Cancer Cells—It is well known that successful wound healing involves a number of processes, including cell proliferation, cell migration, vascular permeability, and angiogenesis (28, 29). Cell migration is a process that is critical during many stages of cancer cell metastasis. To verify the potential role of sLZIP in regulating cell migration, we performed wound healing assays to investigate the effect of sLZIP on cell motility. Wounds in HeLa, SiHa, and ME-180 cells were allowed to heal for 24 h. The migration distance of HeLa, SiHa, and ME-180 cells was determined compared with the beginning wound state. The wound healing of HeLa cells (Fig. 4A) and SiHa cells (Fig. 4B) transfected with sLZIP was increased in a time-dependent manner compared with control cells. The wound healing of ME-180 cells transfected with si-sLZIP was decreased in a time-dependent manner compared with control cells (Fig. 4C).
All data were quantified from analysis of 10 microscopic fields from three independent experiments and are expressed as the mean relative to the migration distance of control cells. We next examined whether sLZIP is involved in the proliferation of HeLa and SiHa cells using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. As shown in Fig. 4D, sLZIP overexpression did not affect the proliferation of HeLa and SiHa cells, indicating that sLZIP is not involved in proliferation of cervical cancer cells (Fig. 4D). Cell invasion is a complicated process and is necessary for tumor cells to invade through the ECM. Therefore, we performed an invasion assay, and the results were in agreement with the results from the wound healing assay. sLZIP-overexpressing HeLa and SiHa cells exhibited a markedly increased invasive ability compared with mock vector-transfected cells (Fig. 4E). These results indicate that sLZIP regulates the invasive ability of cervical cancer cells.

**sLZIP Conditioned Medium Increases Migration and Invasion of Cervical Cancer Cells**—To further examine the effect of sLZIP on wound healing activity and invasion, we prepared a CM of HeLa cells transfected with sLZIP (Fig. 5A). HeLa cells were cultured in a serum-free medium, and after transfection, cells were extensively washed and transferred to a serum-free environment. The collected supernatants were used in wound healing and invasion assays.
healing assays. We first examined the wound healing ability and invasion of HeLa cells in response to sLZIP CM compared with control CM. The wounds of HeLa cells were allowed to heal for 36 h. The number of cells that migrated was increased by 2.3-fold in sLZIP CM compared with control CM (Fig. 5B). To determine whether sLZIP up-regula-
tion is an event leading to induction of the invasive phenotype in HeLa cells, the adhesion and motility of the cells were investigated. Invasion assays were performed using an invasion chamber coated with Matrigel to determine whether sLZIP CM contributed to the invasive ability of cancer cells. sLZIP CM enhanced the invasive ability of HeLa cells depending on the amount of sLZIP CM (Fig. 5C). The CM mixture containing 30% sLZIP CM and 70% normal medium increased the invasive ability of HeLa cells by ~27-fold compared with the 30% control CM mixture (Fig. 5C). These results indicated that sLZIP CM contained important factors involved in cell migration and invasion. We next examined whether MMP-9 is involved in sLZIP-induced migration of cervical cancer cells. We prepared conditioned media of ME-180 cells transfected with si-sLZIP and MMP-9 siRNA (si-MMP-9), and the migration ability was determined. As shown in Fig. 5D, the cell migration ability of ME-180 cells was decreased in si-sLZIP CM and si-MMP-9 CM compared with control siRNA CM. The inhibitory effect of si-sLZIP CM was similar to that of si-MMP-9 CM, indicating that MMP-9 is involved in sLZIP-induced migration of cervical cancer cells. Our results indicate that sLZIP plays a role in cell migration and invasion by secretion of important factors that are involved in these events.

**MMP-9 Is Involved in sLZIP-induced Cell Mobility**—Because MMP-9 is implicated in the migration of cancer cells, we examined the role of MMP-9 in the cell migration induced by sLZIP in HeLa cells. To examine whether the increased activity of
MMP-9 in sLZIP CM directly contributed to alteration of the wound healing ability, we analyzed the wound healing response in the presence of MMP inhibitors, including MMP inhibitor I (MMP-9 inhibitor), MMP-2 inhibitor, anti-MMP-9 antibody, and EDTA (a pan-inhibitor of MMPs) as a positive control. Cells were incubated with various inhibitors and anti-MMP-9 antibody for 36 h before wound healing assays. The enhanced cell mobility due to sLZIP CM was decreased in the presence of the specific MMP-9 inhibitor and anti-MMP-9 antibody by 2.5-fold (Fig. 6A). We also examined the effect of TIMP1 (tissue inhibitor of metalloproteinases I) on the cell migration that was increased by sLZIP CM. The CM mixture containing sLZIP CM and TIMP1 CM decreased sLZIP CM-induced cell migration by 2-fold (Fig. 6A). The MMP-2 inhibitor slightly suppressed sLZIP CM-induced cell migration (Fig. 6A). Therefore, inhibition of MMP-9 was sufficient to inhibit the wound healing ability that was induced by sLZIP CM. We also investigated whether MMP-9 is a key mediator of the invasive ability of HeLa cells that is induced by sLZIP CM. The results from an invasion assay showed that the MMP-9 inhibitor and anti-MMP-9 antibody decreased the invasive ability of HeLa cells, whereas the MMP-2 inhibitor did not affect the invasion of HeLa cells (Fig. 6B). These findings indicate that sLZIP is involved in migration and invasion of HeLa cells via activation of MMP-9 transcription and secretion of enzymatically active MMP-9.

DISCUSSION

The originally described tumor-associated role of MMP-9 is degradation of the basement membranes (28, 29). Enhanced invasive activity of cancer cells is observed when MMP-9 is ectopically expressed (30, 31). Pathological investigations have also demonstrated that expression of MMP-9 is strongly associated with tumor metastasis (4, 32). In this study, we have provided evidence that sLZIP is a novel regulator of MMP-9 transcription that plays an essential role in tumor invasion and metastasis. The importance of sLZIP in MMP-9 expression is based on several lines of supporting evidence. (a) Overexpression of sLZIP increases both the mRNA and protein levels of MMP-9. (b) Knockdown of sLZIP using siRNA reduces both the expression and proteolytic activity of MMP-9. (c) sLZIP binds directly to the CRE region of the MMP-9 promoter. (d)
sLZIP enhances cell migration in cervical cancer cells. (e) Both the MMP-9 inhibitor and anti-MMP-9 antibody reduce sLZIP-induced cell mobility in HeLa cells.

MMP-9 is known to play an important role in the context of tumorigenesis and metastasis because it degrades collagen IV and weakens the basement membrane (33, 34). Degradation products of the ECM, including fragments of collagen IV, can act as signaling substances that regulate cell motility (35–37). Furthermore, based on its importance in the invasive ability of tumor cells, MMP-9 has a role in tumor angiogenesis and also effects on the signaling cascades leading to tumor progression (7, 32, 38). Therefore, regulation of sLZIP expression in cancer development has been of interest. Although the exact regulatory mechanism of MMP-9 expression in cancer is not clear, MMP-9 expression can be induced by various stimuli, including endothelial growth factor, IL-1α, TNF-α, LPS, and 12-O-tetradecanoylphorbol-13-acetate (7, 39, 40). The promoter region of endothelial growth factor, IL-1β, sLZIP binds directly to the proximal CRE region (−106 to −85) of the MMP-9 promoter as revealed by EMSA and ChIP assays. Overexpression of sLZIP has the potential to increase cell migration and increase the invasive ability of cervical cancer cells, suggesting that sLZIP serves as a metastasis regulator, integrating cell migration and invasion in tumor progression. This is the first study to address the mechanism by which sLZIP promotes tumor migration and invasion.

In general, MMP-9 expression is increased by oncogenic proteins and is elevated in many tumor types, including breast, prostate, and cervical cancers and gliomas, and the level of MMP-9 is correlated with the prognosis of clinical patients (1, 29). Therefore, regulation of sLZIP expression is a potential therapeutic target for blocking the metastasis of tumor cells. Our results also raise the possibility that inhibition of sLZIP suppresses tumor invasion and metastasis.

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