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Astrocyte elevated gene-1 (AEG-1) promotes osteosarcoma cell invasion through the JNK/c-Jun/MMP-2 pathway

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ABSTRACT

Osteosarcoma is the most common primary malignant bone tumour in children and adolescents and is characterised by high malignant and metastatic potentials. However, the molecular mechanism underlying this invasiveness remains unclear. In this study, we determined that PD98059 and SP600125, the two mitogen-activated protein kinase (MAPK) family inhibitors, decreased the osteosarcoma cell U2OS-AEG-1 migration and invasion that was enhanced by astrocyte elevated gene-1 (AEG-1) in an *in vitro* wound-healing and Matrigel invasion assay independently of cell viability. These findings indicate that AEG-1 promoted osteosarcoma cell invasion is relevant to the MAPK pathways. The up-regulation of AEG-1 increased the levels of phosphor-c-Jun N-terminal kinase (JNK) and phosphor-c-Jun; however, there were no marked changes in the levels of phosphor-extracellular regulated kinase (ERK) 1/2 or phosphor-c-Fos due to the activation of AEG-1 in U2OS. SP600125 (a JNK inhibitor) decreased phosphor-c-Jun and MMP-2 in U2OS-AEG-1, while PD98059 (a ERK1/2 inhibitor) had no influence on the levels of phosphor-c-Jun or MMP-2 in U2OS-AEG-1. Further study revealed that the down-regulation of phosphor-c-Jun not only obviously decreased the MMP-2 protein level and the MMP-2 transcriptional activity that were up-regulated by AEG-1 in Western-blot and luciferase reporter assays, but also inhibited the migration and invasion abilities of the U2OS-AEG-1 cells, which suggests that AEG-1 mediated U2OS invasion at least partially via the JNK/c-Jun/MMP-2 pathway. Consistent with these observations, immunohistochemical (IHC) staining revealed that AEG-1 expression was associated with the protein levels of phosphor-c-Jun and MMP-2 in needle biopsy paraffin-embedded archival human osteosarcoma tissues. Taken together, our findings suggest that AEG-1 plays a crucial role in the aggressiveness of osteosarcoma via the JNK/c-Jun/MMP-2 pathway.

1. Introduction

Osteosarcoma is the most common primary bone tumour in children and adolescents and is characterised by high malignant and metastatic potentials [1]. Up to 20% of patients with osteosarcoma present with clinically detectable metastatic disease at the initial diagnosis, and approximately 40% of patients present with metastases that predominantly develop in the lungs in the advanced stage. Despite rapid advancements of multimodal treatment, patients with metastatic disease and local relapse still have poor outcomes with survival rates of approximately 20% [2–5]. However, the development of the metastatic ability of osteosarcoma and the underlying molecular mechanism are complicated and remain to be elucidated.

Recently, astrocyte elevated gene-1 (AEG-1) has drawn attention. In 2004, "a lung homing domain" was found in a metastatic breast carcinoma and mediated the movement of breast cancer cells to the lung. This domain is part of the protein metadherin (MTDH) [6]. MTDH, also known as AEG-1, was initially identified as an HIV-1- and tumour necrosis factor α (TNF-α)-inducible transcript in primary human foetal astrocytes by a rapid subtraction hybridisation approach [7,8]. Overexpression of AEG-1 is frequently observed in glioma and carcinomas of the breast, colorectum, liver and other malignancies and is correlated with poor clinical outcomes [9–13].

Interestingly, several studies have documented that AEG-1 plays an important role in promoting tumour invasion. Emdad et al. [14] reported that overexpression of AEG-1 significantly enhances the invasion ability of Hela cells and that this enhancement is associated with the activation of the NF-κB pathway. Song et al. [15] demonstrated that the overexpression of AEG-1 is significantly associated with tumour aggressiveness in human

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non-small cell lung cancer and that the function of AEG-1 in the modulation of metastasis is correlated with the activation of the PI3K-Akt and NF-κB signalling pathways. Additionally, the knockdown of AEG-1 by a small interfering RNA inhibits cellular invasive ability of U87 human glioma cells and 9 L rat gliosarcoma cells in which MMP-2 and MMP-9 are involved in AEG-1-mediated invasion [9]. Moreover, Liu et al. [16] reported that AEG-1 up-regulates MMP-9 and induces human glioma invasion. All of these studies suggest an association between AEG-1 and the invasive ability of malignant tumours.

Our previous study also suggested that AEG-1 plays a crucial role in osteosarcoma invasion through MMP-2 [17]. However, the molecular mechanism by which AEG-1 regulates MMP-2 in osteosarcomas is not clear. MMP-2 is a member of the matrix metalloproteinases (MMPs), which play critical roles during tumour invasion and metastasis in cancer tissues including human osteosarcoma [18]. It is well known that activator protein 1 (AP-1), which was one of the first identified transcription factors, acts independently or coordinately to regulate numerous genes that are involved in the regulation of MMPs expression [19]. AP-1 is composed of a variety of combinations of dimerised proteins that include the Jun, Fos, Maf and ATF sub-families. C-Jun is the most potent transcription factor in this group and forms stable heterodimers with c-Fos proteins. The MAPKs regulate a number of transcription factors such as AP-1, which includes c-Jun and c-Fos [20,21]. The MAPK family contains the ERK 1/2 pathway, the JNK pathway, the p38 pathway and the ERK5 pathway, which are activated via reversible phosphorylation and play key roles in the transduction of extracellular signals to cellular responses [22,23]. The ERK1/2 and JNK pathways have been reported to participate in the modulation of the invasion of osteosarcoma cells. The ERK1/2 and JNK pathways might activate c-Fos and c-Jun, respectively, via the induction of phosphorylation in these pathways [24,25]. Interestingly, YOO [13] demonstrated that the activations of the MAPK pathways play important roles in mediating AEG1-induced Matrigel invasion and anchorage-independent growth in hepatocellular carcinomas. Taken together, these studies prompted us to hypothesise that AEG-1 might exert its invasive ability by activating the ERK1/2 and JNK/AP-1/MMPs pathways.

In the present study, we found that AEG-1 promoted osteosarcoma cell invasion through the JNK/c-Jun/MMP-2 pathway. Consistent with this observation, immunohistochemical staining revealed that there were significant correlations between AEG-1 expression and the protein levels of phosphor-c-Jun and MMP-2 in paraffin-embedded human osteosarcoma tissues. Our results suggest that AEG-1 might play a crucial role in osteosarcoma aggressiveness via the JNK/c-Jun/MMP-2 pathway.

2. Material and methods

2.1. Cell culture and reagents

Human osteosarcoma cell lines U2OS and MG63 (obtained from our laboratory's cell bank, Guangzhou, China) were cultured in RPMI1640 media (Gibco, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (Hyclone, Tauranga, New Zealand), 100 μg/μl streptomycin and 100 μg/μl penicillin in a 37 °C incubator containing 5% CO_2. PD98059 and SP600125 were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2. Vector and retroviral infection

The vector pMSCV/AEG-1-overexpressing human AEG-1 was kindly provided by Dr. Meng-Feng Li (Sun Yat-sen University Zhongshan School of Medicine). Retroviral production and infection were performed as previously described [12]. Stable cell lines expressing AEG-1 were selected for 10 days with 0.5 μg/ml puromycin 48 h after infection.

2.3. Immunoblotting analyses

Immunoblotting analyses were conducted as previously described [17]. Anti-AEG-1 rabbit antibody (Invitrogen, Carlsbad, CA, USA), anti-rabbit IgG (Cell Signaling Technology, Beverly, MA, USA), anti-GAPDH rabbit antibody (Cell Signaling Technology, Beverly, MA, USA), anti-MMP-2 mouse antibody, anti-Erk1/2 rabbit antibody, anti-phospho-Erk1/2 rabbit antibody, anti-c-Fos rabbit antibody, anti-phospho-c-Fos rabbit antibody, anti-JNK rabbit antibody, anti-phospho-JNK rabbit antibody, anti-c-Jun rabbit antibody and anti-phospho-c-Jun rabbit antibody (Cell Signaling Technology, Beverly, MA, USA) were used.

2.4. Wound healing assay

Cell migration was assessed by measuring the movement of cells into a scraped cellular area created with a 200 μl pipette tube. After 24 h, the spread of the wound closure was photographed under a microscope. The fraction of cell coverage across the line was measured and taken as the migration rate. All experiments were performed in triplicate.

2.5. Invasion assay

Invasive ability was analysed using 24-well BioCoat cell culture inserts (Costar, Corning, NY, USA) with 8-μm-porosity polyethylene terephthalate membranes coated with Matrigel Basement Membrane Matrix (Trevigen, Gaithersburg, MD, USA) as previously described [17].

2.6. MTT assay

Cell proliferation analyses were conducted using an MTT kit (sterile 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide dye) (Keygen, Nanjing, China) as previously described [17].

2.7. C-Jun knockdown using siRNA transfection

Transient transfections were performed as previously described [17]. The sequence of the c-Jun siRNA was sense 5′ GACCTTATGGCTACAGTAA 3′ (Ribobio, Guangzhou, China).

2.8. Luciferase reporter assay

A total of 1×10^5 cells were seeded per well in 24-well plates and transfected with 1 μg of total DNA (either pGL3-Basic or pGL3-MMP-2 and β-galactosidase) for 48 h using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Luciferase activity was normalised to β-galactosidase activity, and the data are presented as fold activations relative to pGL3-Basic. All experiments were performed in triplicate.

2.9. Human osteosarcoma sample collection, patient information and immunohistochemical staining

Paraffin-embedded needle biopsy specimens that were from primary tumours from 49 osteosarcoma patients, including 10 patients with apparent pulmonary metastases, who underwent

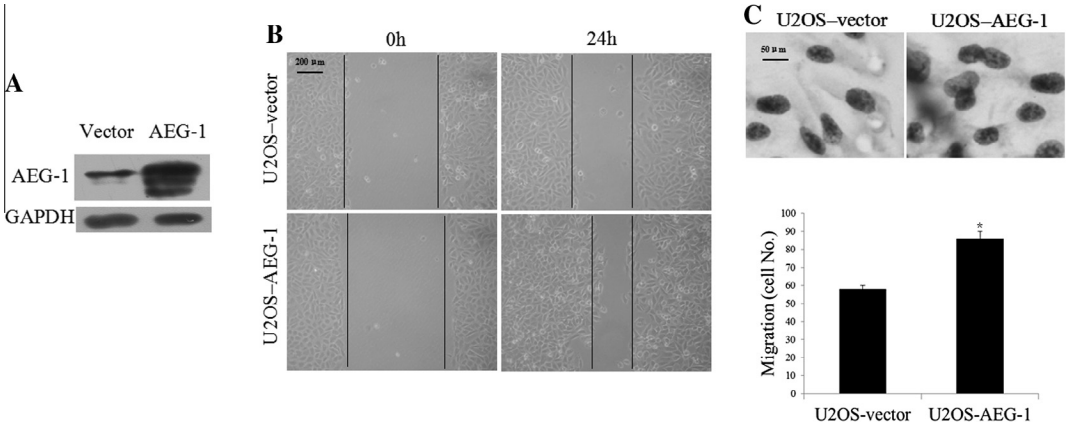

Fig. 1. Upregulation of AEG-1 promotes U2OS invasion. (A) The upregulation of AEG-1 in U2OS cells was analysed by Western blot assay. GAPDH was used as an internal control. (B) The wound-healing assay revealed assay different cell motilities in the indicated cells. (C, upper) AEG-1 regulated the invasive abilities of the U2OS cells in the Matrigel invasion assay. (Lower) Quantification of the invaded cells. The error bars represent the standard deviations calculated from three independent experiments. $^{*}P < 0.05$ compared to the control cells. Original magnifications: ×100 (B), ×400 (C).

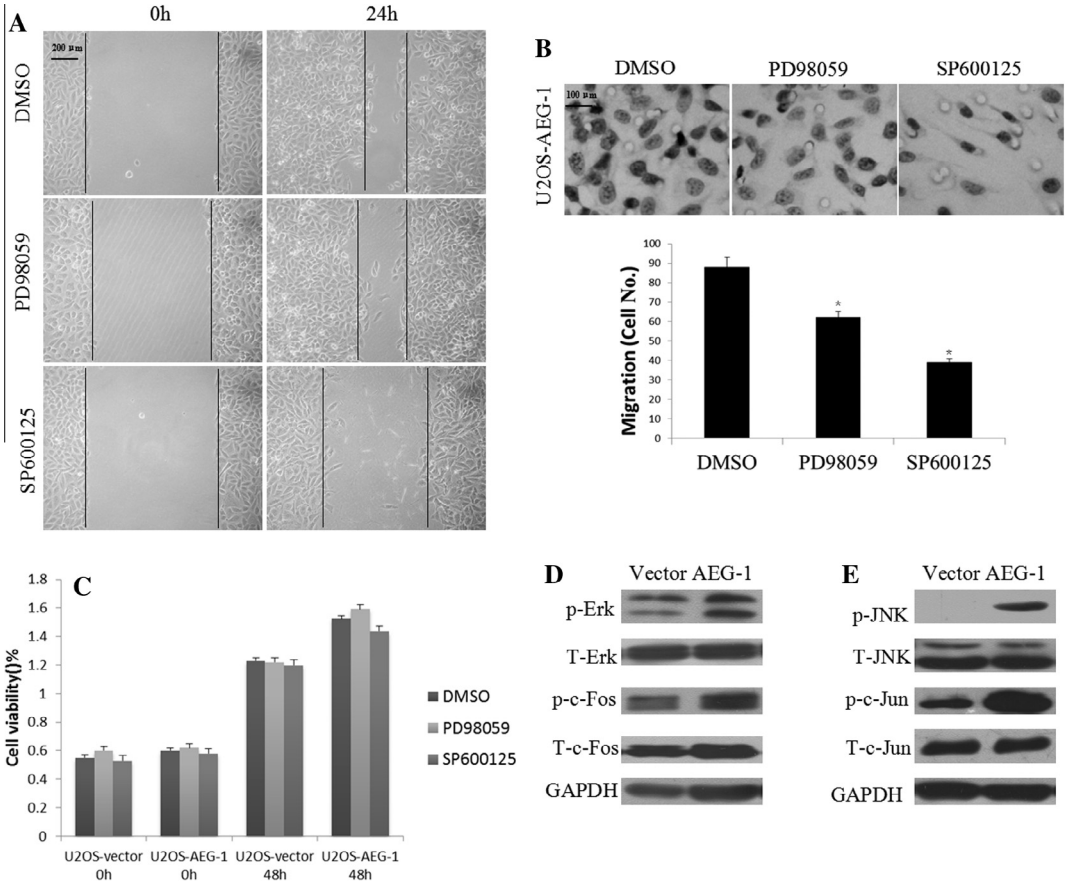

Fig. 2. AEG-1 promoted U2OS-cell invasion is related to the JNK-c-Jun pathway. (A) The wound-healing assays with the U2OS-AEG-1 cells and treatments with PD98059 and SP600125. (B, upper) The Matrigel invasion assay using the U2OS-AEG-1 cells and treatments with PD98059 and SP600125. (Lower) The quantification of the invaded cells. The error bars represent the standard deviations calculated from three independent experiments. $^{*}P < 0.05$ compared to control. (C) Analysis of the viabilities of the U2OS-vector and U2OS-AEG-1 cells upon treatment with PD98059 and SP600125 by MTT assay. The error bars represent the standard deviations calculated from three independent experiments. (D and E) The expressions of the indicated proteins were analysed by Western blot assay in the U2OS-vector and U2OS-AEG-1 cells. GAPDH was used as an internal control. Original magnifications: ×100 (A), ×400 (B).

initial surgical resection between 2011 and 2012 were randomly selected from the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). Prior patient (or guardian) consent and approval from the Institutional Research Ethics Committee were obtained for the use of these clinical materials for research purposes.

Immunohistochemical (IHC) staining was performed using the standard streptavidin–biotin peroxidase method as previously described [17]. Anti-AEG-1 rabbit antibody (Invitrogen, Carlsbad, CA, USA), anti-phosphor-c-Jun rabbit antibody and anti-MMP-2 rabbit antibody (Cell Signaling Technology, Beverly, MA, USA) were used. Digital photomicrographs were obtained at ×400 magnifica-

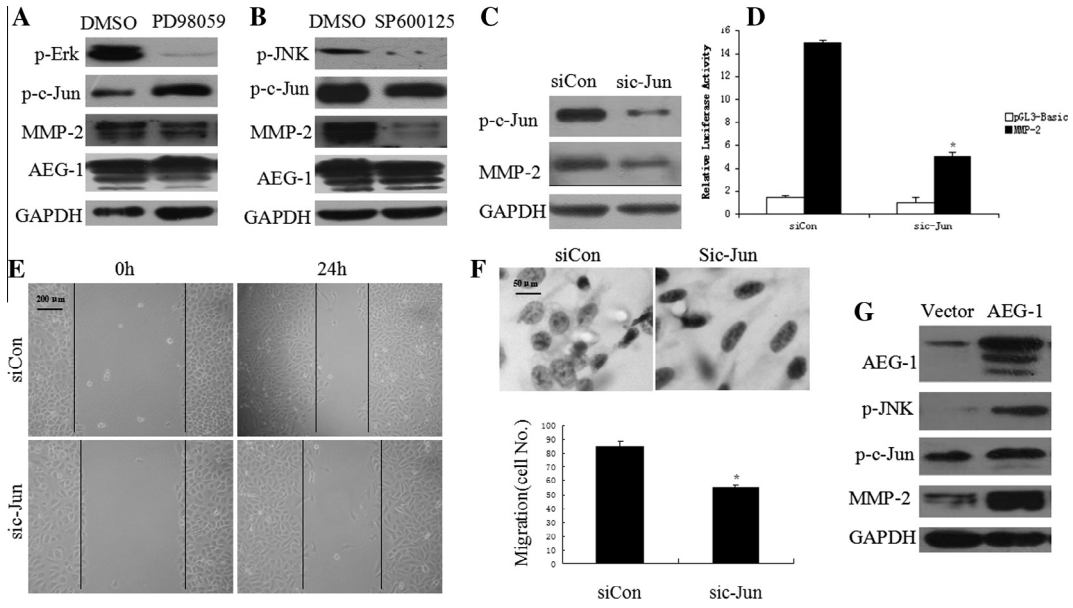

Fig. 3. AEG-1 promotes osteosarcoma cell invasion through the JNK/c-Jun/MMP-2 pathway. (A and B) The expressions of the indicated proteins were analysed by Western blot assay using U2OS-AEG-1 cells that were treated with PD98059 and SP600125. (C) Knockdown of phosphor-c-Jun and the expression of phosphor-c-Jun and MMP-2 in the U2OS-AEG-1 cells were analysed by Western blot assay. (D) Knockdown of phosphor-c-Jun inhibited the transcriptional activity of MMP-2 in the U2OS-AEG-1 cells as assessed by luciferase reporter assay. The error bars represent the standard deviations calculated from three independent experiments. $^{*}P < 0.05$ compared to control. (E) The wound-healing assay using the U2OS-AEG-1 cells following transfection with sic-Jun and siCon. (F, upper) Knockdown of phosphor-c-Jun regulated the invasion abilities of the U2OS-AEG-1 cells in the Matrigel invasion assay. (Lower) The quantification of the invaded cells. The error bars represent the standard deviations calculated from three independent experiments. $^{*}P < 0.05$ compared to control cells. (G) The expressions of the indicated proteins were analysed by Western blot assay in the MG63 cells. GAPDH was used as an internal control for A, B, C and G. Original magnifications: ×100 (E), ×400 (F).

Table 1
The intensity of AEG-1, p-c-Jun and MMP-2 were analysed by IHC in osteosarcoma tissues.

	M0				M1			
	–	+	++	+++	–	+	++	+++
AEG-1	6	10	15	8	0	2	3	5
p-c-Jun	10	14	10	5	1	2	4	3
MMP-2	4	10	12	13	0	0	4	6

Numerical values represent patient's quantity. M0: none metastasis, M1: with pulmonary metastasis.

tion. The scoring of the immunohistochemically stained sections was derived by multiplying the percentage of positive cells by the staining intensity.

2.10. Statistical analyses

Two-tailed Student's *t* tests or ANOVA tests were conducted on the data from the quantitative assays. Spearman correlation analyses were used to assess the associations of the expression of AEG-1 with those of c-Jun and MMP-2, and between the expression of AEG-1 and pulmonary metastasis. All statistical analyses were performed with the SPSS 16.0 statistical software package. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Upregulation of AEG-1 promotes U2OS invasion

In this study, we first increased the expression of AEG-1 in U2OS cells via retroviral infection. As shown in Fig. 1A, AEG-1 protein levels were successfully elevated following transfection with AEG-1 cDNA compared to the controls as assayed by immunoblotting. Next, we detected the effects of AEG-1 on the invasion ability of U2OS cells via the wound-healing and Matrigel invasion assays. We found that the overexpression of AEG-1 markedly increased U2OS migration as assayed with the wound-healing assay and that increasing AEG-1 promoted the U2OS cells' invasion ability as assessed with the Matrigel invasion assay (Fig. 1B and C).

3.2. AEG-1 promoted U2OS invasion is related to the JNK-c-Jun pathway

In this study, the ERK1/2 and JNK pathways were blocked with the specific inhibitors PD98059 (20 μM) and SP600125 (25 μM) in the U2OS-AEG-1 cells to determine whether AEG-1 regulates U2OS invasion through the MAPK pathways. As shown in Fig. 2A and B, both PD98059 and SP600125 inhibited U2OS-AEG-1 cell migration and invasion in the wound-healing and Matrigel invasion assays, and the effects of SP600125 were more obvious. Next, we investigated whether the two inhibitors influenced osteosarcoma cell viability. As shown in Fig. 2C, treatment with PD98059 and SP600125 for 48 h did not significantly inhibit the proliferation of the U2OS-AEG-1 cells, which indicated that the two inhibitors regulated cell invasion independently of cell viability. Finally, we examined the signalling pathways that were activated by AEG-1 using Western blot analysis. As shown in Fig. 2D and E, phosphor-JNK and phosphor-c-Jun were significantly increased in the U2OS-AEG-1 cells compared to the U2OS-vector cells. However, there were no marked changes in the phosphor-ERK1/2 or phosphor-c-Fos that were activated by AEG-1. These results suggest that the JNK-c-Jun pathways might play important roles in mediating AEG-1-induced cell invasion.

3.3. AEG-1 promotes osteosarcoma cell invasion through the JNK/c-Jun/MMP-2 pathway

To determine the relationship between the invasive function of AEG-1 and the phosphorylation levels of JNK and c-Jun, we first treated U2OS-AEG-1 cells with PD98059 (20 μM) and SP600125 (25 μM) for 1 h. Western blot revealed that PD98059 markedly

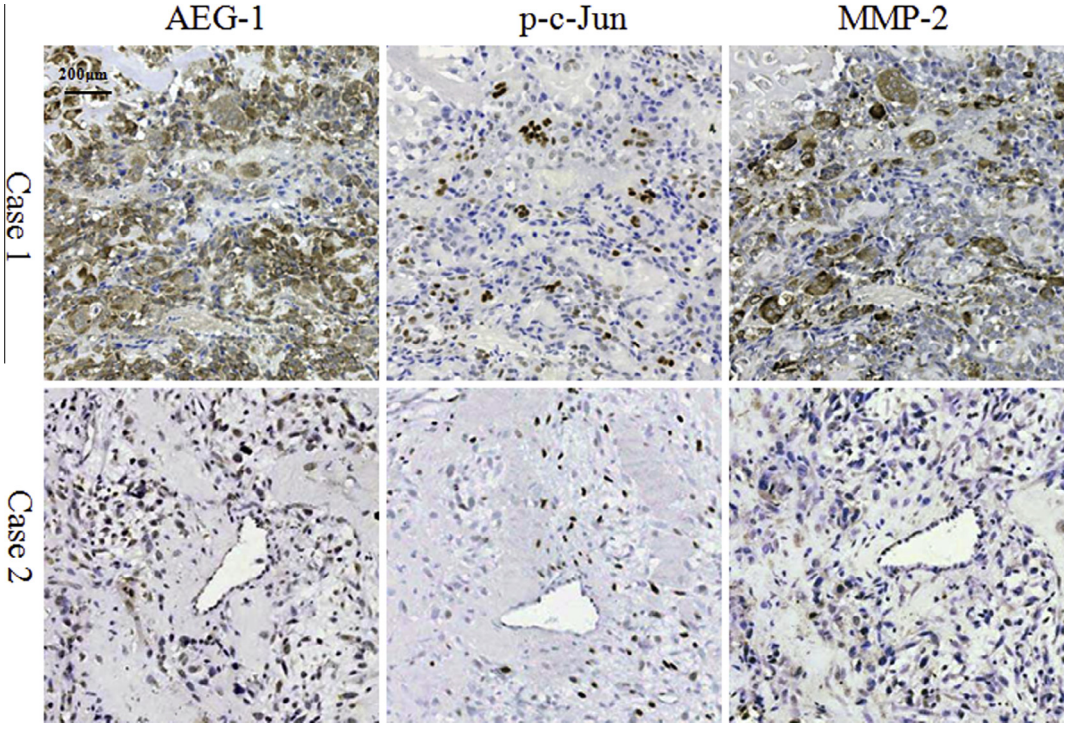

Fig. 4. AEG-1 is associated with phosphor-c-Jun and MMP-2 in osteosarcoma tissues. Representative staining for AEG-1, phosphor-c-Jun and MMP-2 expression in serial sections of formalin-fixed, paraffin-embedded osteosarcoma tissues by IHC. Original magnification: ×200.

inhibited phosphor-ERK1/2 compared to the control, which was treated with DMSO, but PD98059 had no influence on phosphor-c-Jun or MMP-2 (Fig. 3A). However, when phosphor-JNK was decreased by SP600125 in the U2OS-AEG-1 cells, phosphor-c-Jun and MMP-2 were decreased (Fig. 3B). Moreover, there were no marked changes in AEG-1 protein levels when the U2OS-AEG-1 cells were treated with PD98059 and SP600125 (Fig. 3A and B). These results suggest that AEG-1 was located upstream of JNK and might regulate phosphor-c-Jun and MMP-2 through phosphor-JNK in osteosarcoma cells.

Next, we decreased the expression of phosphor-c-Jun in the U2OS-AEG-1 cells with small interfering RNAs. As shown in Fig. 3C, phosphor-c-Jun protein levels were successfully decreased following transfection compared to the controls down to the MMP-2 levels observed by Western blot. Furthermore, the downregulation of phosphor-c-Jun markedly inhibited MMP-2 transcriptional activity in the U2OS-AEG-1 cells compared to the controls as assessed by luciferase reporter assay (Fig. 3D). Finally, we detected the effects of phosphor-c-Jun on the invasion ability of the U2OS-AEG-1 cells via the wound-healing and Matrigel invasion assays. The results revealed that the downregulation of phosphor-c-Jun inhibited the migration and invasion abilities of the U2OS-AEG-1 cells (Fig. 3E and F). These results suggest that AEG-1 mediates U2OS invasion at least partially through phosphor-c-Jun and MMP-2.

Consistently, as shown in Fig. 3G, the upregulation of AEG-1 expression in another osteosarcoma cell line (MG63) increased the expression levels of phospho-JNK, phospho-c-Jun and MMP-2. Together, these results suggest that AEG-1 might regulate osteosarcoma cell invasion through the JNK/c-Jun/MMP-2 pathway.

3.4. AEG-1 is associated with phosphor-c-Jun and MMP-2 in osteosarcoma tissues

To determine the relationships between AEG-1 expression and the expressions of phosphor-c-Jun and MMP-2, we conducted immunohistochemical staining of 49 needle biopsy paraffin-embedded human osteosarcoma specimens. All of these specimens were acquired prior to neoadjuvant chemotherapy, which is different from our previous study. The intensities of AEG-1, phosphor-c-Jun and MMP-2 are shown in Table 1 and are similar to the findings of an earlier study [17]. IHC assay revealed that AEG-1 expression levels were associated with pulmonary metastasis ($r = 0.340$; $P = 0.008$) and that the osteosarcoma samples with high levels of AEG-1 expression exhibited strong phosphor-c-Jun or MMP-2 staining signals, whereas the osteosarcoma specimens with low AEG-1 expression exhibited low or absent phosphor-c-Jun or MMP-2 expressions (Fig. 4). Spearman correlation analyses revealed correlations between the expressions of AEG-1 and phosphor-c-Jun ($r = 0.265$; $P = 0.016$) and between the expressions of AEG-1 and MMP-2 ($r = 0.405$; $P = 0.009$), which were consistent with the *in vitro* observations detailed above.

4. Discussion

Previously, we demonstrated that AEG-1 is overexpressed in osteosarcoma tissues and that the expression of AEG-1 is correlated with the clinical parameters of osteosarcomas, AEG-1 might play a role in the progression of osteosarcoma via MMP-2 [17]. In the present study, we clarified the molecular mechanism by which AEG-1 regulates MMP-2. We found that AEG-1 might promote osteosarcoma cell invasion through the JNK/c-Jun/MMP-2 pathway, and our findings suggested that AEG-1 might be a potential target for anti-osteosarcoma metastasis strategies.

MMPs play important roles in the matrix degradation required for tumour growth, invasion and tumour-induced angiogenesis [26,27], which are regulated by H-ras and the transcription factors AP-1, NF-κB and Akt [28–31]. MAPKs are major signalling pathways that control MMPs [32]. Silibinin suppresses human osteosarcoma MG-63 cell invasion by inhibiting the ERK-dependent c-Jun/AP-1 induction of MMP-2 [33]. Statin-induced HMG-CoA reductase inhibition reduces cell migration and invasion in

osteosarcoma cells, and a molecular mechanism involving JNK-c-Jun-MMP-2 activity is related to these effects [34]. Interestingly, YOO [13] verified that AEG-1 promotes hepatocellular carcinoma progression via the MAPK pathways. Similar to other studies, in the present study, we suggest that AEG-1 might regulate U2OS invasion through the JNK/c-Jun/MMP-2 pathway.

In the present study, we demonstrated that AEG-1 might promote osteosarcoma cell invasion through the MAPK pathway. However, Liu B [35] demonstrated that AEG-1 regulates osteosarcoma cell invasion via endothelin-1/endothelin A receptor signalling in a PI3K-dependent manner. The MAPK pathway and PI3K pathway play important roles in the pathogenesis of osteosarcoma. Bufalin-inhibited migration and invasion in human osteosarcoma U2OS cells is carried out by suppression of the matrix metalloproteinase-2, ERK, and JNK signalling pathways [36]. Knockdown of CXCR7 inhibits proliferation and invasion of osteosarcoma cells through inhibition of the PI3K/Akt and β-arrestin pathways [37]. Experimental data demonstrate that the MAPK pathway and PI3K pathway influence each other both negatively and positively, resulting in dynamic and complex cross-talk [38]. Whether the two pathways activate or inhibit each other regulated by AEG-1 with the invasive abilities of osteosarcoma cells requires further study.

Additionally, in this study, we identified an interesting phenomenon that requires further investigation. Western blot assay revealed that there were only a single large band in the 80 KD area and a single small band in the 75 KD area of AEG-1 in the U2OS and MG63 cells, while there were three bands in the 80 KD, 75 KD and 60 KD areas of the AEG-1 in the U2OS-AEG-1 and MG63-AEG-1 cells. The 75 KD and 60 KD area bands might be isoforms of AEG-1, which would be consistent with other report [39]. We found that the U2OS-AEG-1 cells exhibited a greater invasive ability than did the U2OS cells, and whether the two isoforms of AEG-1 are correlated with the invasive abilities of osteosarcoma cells requires further study.

In summary, our study is the first to show that AEG-1 promoted osteosarcoma invasion through the JNK/c-Jun/MMP-2 pathway *in vitro* and *in vivo*. Understanding the molecular mechanism by which AEG-1 promotes osteosarcoma progression will not only improve our understanding of the mechanisms that underlie the metastasis of osteosarcomas but might also establish AEG-1 as a potential therapeutic target for the treatment of osteosarcoma.

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References

[1] T.A. Damron, W.G. Ward, A. Stewart, Osteosarcoma, chondrosarcoma, and Ewing's sarcoma: National Cancer Data Base Report, Clin. Orthop. Relat. Res. 459 (2007) 40–47.
[2] G. Bacci, M. Rocca, M. Salone, A. Balladelli, S. Ferrari, E. Palmerini, C. Forni, A. Briccoli, High grade osteosarcoma of the extremities with lung metastases at presentation: treatment with neoadjuvant chemotherapy and simultaneous resection of primary and metastatic lesions, J. Surg. Oncol. 98 (2008) 415–420.
[3] S.S. Bielack, D. Carrle, J. Hardes, A. Schuck, M. Paulussen, Bone tumors in adolescents and young adults, Curr. Treat. Options Oncol. 9 (2008) 67–80.
[4] D.P. Hughes, Strategies for the targeted delivery of therapeutics for osteosarcoma, Expert Opin. Drug Deliv. 6 (2009) 1311–1321.
[5] P.J. Messerschmitt, R.M. Garcia, F.W. Abdul-Karim, E.M. Greenfield, P.J. Getty, Osteosarcoma, J. Am. Acad. Orthop. Surg. 17 (2009) 515–527.
[6] D.M. Brown, E. Ruoslahti, Metadherin, a cell surface protein in breast tumors that mediates lung metastasis, Cancer Cell 5 (2004) 365–374.
[7] Z.Z. Su, Y. Chen, D.C. Kang, W. Chao, M. Simm, D.J. Volsky, P.B. Fisher, Customized rapid subtraction hybridization (RaSH) gene microarrays identify overlapping expression changes in human fetal astrocytes resulting from human immunodeficiency virus-1 infection or tumor necrosis factor-alpha treatment, Gene 306 (2003) 67–78.
[8] Z.Z. Su, D.C. Kang, Y. Chen, O. Pekarskaya, W. Chao, D.J. Volsky, P.B. Fisher, Identification of gene products suppressed by human immunodeficiency virus type 1 infection or gp120 exposure of primary human astrocytes by rapid subtraction hybridization, J. Neurovirol. 9 (2003) 372–389.
[9] L. Emdad, D. Sarkar, S.G. Lee, Z.Z. Su, B.K. Yoo, R. Dash, A. Yacoub, C.E. Fuller, K. Shah, P. Dent, J.N. Bruce, P.B. Fisher, Astrocyte elevated gene-1: a novel target for human glioma therapy, Mol. Cancer Ther. 9 (2010) 79–88.
[10] G. Hu, R.A. Chong, Q. Yang, Y. Wei, M.A. Blanco, F. Li, M. Reiss, J.L. Au, B.G. Haffty, Y. Kang, MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer, Cancer Cell 15 (2009) 9–20.
[11] S.J. Huang, B.W. Wu, D.F. Li, W.H. Zhou, P. Deng, K.J. Zhang, Y.J. Li, Knockdown of astrocyte elevated gene-1 inhibits tumor growth and modifies microRNAs expression profiles in human colorectal cancer cells, Biochem. Biophys. Res. Commun. 444 (2014) 338–345.
[12] J. Li, N. Zhang, L.B. Song, W.T. Liao, L.L. Jiang, L.Y. Gong, J. Wu, J. Yuan, H.Z. Zhang, M.S. Zeng, M. Li, Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival, Clin. Cancer Res. 14 (2008) 3319–3326.
[13] B.K. Yoo, L. Emdad, Z.Z. Su, A. Villanueva, D.Y. Chiang, N.D. Mukhopadhyay, A.S. Mills, S. Waxman, R.A. Fisher, J.M. Llovet, P.B. Fisher, D. Sarkar, Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression, J. Clin. Invest. 119 (2009) 465–477.
[14] L. Emdad, D. Sarkar, Z.Z. Su, A. Randolph, H. Boukerche, K. Valerie, P.B. Fisher, Activation of the nuclear factor kappaB pathway by astrocyte elevated gene-1: implications for tumor progression and metastasis, Cancer Res. 66 (2006) 1509–1516.
[15] L. Song, W. Li, H. Zhang, W. Liao, T. Dai, C. Yu, X. Ding, L. Zhang, J. Li, Over-expression of AEG-1 significantly associates with tumour aggressiveness and poor prognosis in human non-small cell lung cancer, J. Pathol. 219 (2009) 317–326.
[16] L. Liu, J. Wu, Z. Ying, B. Chen, A. Han, Y. Liang, L. Song, J. Yuan, J. Li, M. Li, Astrocyte elevated gene-1 upregulates matrix metalloproteinase-9 and induces human glioma invasion, Cancer Res. 70 (2010) 3750–3759.
[17] F. Wang, Z.F. Ke, S.J. Sun, W.F. Chen, S.C. Yang, S.H. Li, X.P. Mao, L.T. Wang, Oncogenic roles of astrocyte elevated gene-1 (AEG-1) in osteosarcoma progression and prognosis, Cancer Biol. Ther. 12 (2011) 539–548.
[18] G. Klein, E. Vellenga, M.W. Fraaije, W.A. Kamps, E.S. de Bont, The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia, Crit. Rev. Oncol. Hematol. 50 (2004) 87–100.
[19] P. Angel, M. Karin, The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation, Biochim. Biophys. Acta 1072 (1991) 129–157.
[20] P.N. Chen, Y.S. Hsieh, H.L. Chiou, S.C. Chu, Silibinin inhibits cell invasion through inactivation of both PI3K-Akt and MAPK signaling pathways, Chem. Biol. Interact. 156 (2005) 141–150.
[21] C. Huang, W.Y. Ma, M.R. Young, N. Colburn, Z. Dong, Shortage of mitogen-activated protein kinase is responsible for resistance to AP-1 transactivation and transformation in mouse JB6 cells, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 156–161.
[22] M. Krishna, H. Narang, The complexity of mitogen-activated protein kinases (MAPKs) made simple, Cell. Mol. Life Sci. 65 (2008) 3525–3544.
[23] X. Wang, C. Tournier, Regulation of cellular functions by the ERK5 signalling pathway, Cell. Signal. 18 (2006) 753–760.
[24] B. Binetruy, T. Smeal, M. Karin, Ha-Ras augments c-Jun activity and stimulates phosphorylation of its activation domain, Nature 351 (1991) 122–127.
[25] T. Deng, M. Karin, C-Fos transcriptional activity stimulated by H-Ras-activated protein kinase distinct from JNK and ERK, Nature 371 (1994) 171–175.
[26] A.F. Chambers, L.M. Matrisian, Changing views of the role of matrix metalloproteinases in metastasis, J. Natl. Cancer Inst. 89 (1997) 1260–1270.
[27] N. Ortega, D. Behonick, D. Stickens, Z. Werb, How proteases regulate bone morphogenesis, Ann. N. Y. Acad. Sci. 995 (2003) 109–116.
[28] S.C. Huang, C.T. Ho, S.Y. Lin-Shiau, J.K. Lin, Carnosol inhibits the invasion of B16/F10 mouse melanoma cells by suppressing metalloproteinase-9 through down-regulating nuclear factor-kappa B and c-Jun, Biochem. Pharmacol. 69 (2005) 221–232.
[29] A. Moon, M.S. Kim, T.G. Kim, S.H. Kim, H.E. Kim, Y.Q. Chen, H.R. Kim, H-ras, but not N-ras, induces an invasive phenotype in human breast epithelial cells: a role for MMP-2 in the H-ras-induced invasive phenotype, Int. J. Cancer 85 (2000) 176–181.
[30] C. Segrelles, S. Ruiz, M. Santos, J. Martinez-Palacio, M.F. Lara, J.M. Paramio, Akt mediates an angiogenic switch in transformed keratinocytes, Carcinogenesis 25 (2004) 1137–1147.
[31] J.H. Woo, J.H. Lim, Y.H. Kim, S.I. Suh, D.S. Min, J.S. Chang, Y.H. Lee, J.W. Park, T.K. Kwon, Resveratrol inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting JNK and PKC delta signal transduction, Oncogene 23 (2004) 1845–1853.
[32] P.M. Reuben, H.S. Cheung, Regulation of matrix metalloproteinase (MMP) gene expression by protein kinases, Front. Biosci. 11 (2006) 1199–1215.
[33] Y.S. Hsieh, S.C. Chu, S.F. Yang, P.N. Chen, Y.C. Liu, K.H. Lu, Silibinin suppresses human osteosarcoma MG-63 cell invasion by inhibiting the ERK-dependent c-Jun/AP-1 induction of MMP-2, Carcinogenesis 28 (2007) 977–987.

[34] O. Fromigue, Z. Hamidouche, P.J. Marie, Blockade of the RhoA-JNK-c-Jun-MMP2 cascade by atorvastatin reduces osteosarcoma cell invasion, J. Biol. Chem. 283 (2008) 30549–30556.
[35] B. Liu, Y.K. Wu, D. Peng, Astrocyte elevated gene-1 regulates osteosarcoma cell invasion and chemoresistance via endothelin-1/endothelin A receptor signaling, Oncol. Lett. 5 (2013) 505–510.
[36] F.S. Chueh, Y.Y. Chen, A.C. Huang, H.C. Ho, C.L. Liao, J.S. Yang, C.L. Kuo, J.G. Chung, Bufalin-inhibited migration and invasion in human osteosarcoma U-2 OS cells is carried out by suppression of the matrix metalloproteinase-2, ERK, and JNK signaling pathways, Environ. Toxicol. 29 (2014) 21–29.
[37] Y. Zhang, C.Q. Yang, Y. Gao, C. Wang, C.L. Zhang, X.H. Zhou, Knockdown of CXCR7 inhibits proliferation and invasion of osteosarcoma cells through inhibition of the PI3K/Akt and β-arrestin pathways, Oncol. Rep. 32 (3) (2014) 965–972.
[38] E. Aksamitiene, A. Kiyatkin, B.N. Kholodenko, Cross-talk between mitogenic Ras/MAPK and survival PI3K/Akt pathways: a fine balance, Biochem. Soc. Trans. 40 (1) (2012) 139–146.
[39] D.E. Britt, D.F. Yang, D.Q. Yang, D. Flanagan, H. Callanan, Y.P. Lim, S.H. Lin, D.C. Hixson, Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells, Exp. Cell Res. 300 (2004) 134–148.