Biochemical and Biophysical Research Communications 464 (2015) 635–641

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

XCR1 promotes cell growth and migration and is correlated with bone metastasis in non-small cell lung cancer

Ting Wang [1], Shuai Han[1], Zhipeng Wu [1], Zhitao Han, Wangjun Yan, Tielong Liu, Haifeng Wei, Dianwen Song, Wang Zhou[***], Xinghai Yang[**], Jianru Xiao[*]

Department of Bone Tumor Surgery, Changzheng Hospital, Second Military Medical University, Shanghai, China

ARTICLE INFO

Article history:
Received 20 June 2015
Accepted 23 June 2015
Available online 9 July 2015

Keywords:
XCR1
Non-small cell lung cancer (NSCLC)
Bone metastasis
Cell proliferation
Cell migration
JAK2/STAT3 pathway

ABSTRACT

Bone metastasis occurs in approximately 30–40% patients with advanced non-small cell lung cancer (NSCLC), but the mechanism underlying this bone metastasis remains poorly understood. The chemokine super family is believed to play an important role in tumor metastasis in lung cancer. The chemokine receptor XCR1 has been identified to promote cell proliferation and migration in oral cancer and ovarian carcinoma, but the role of XCR1 in lung cancer has not been reported. In this study, we demonstrated for the first time that XCR1 was overexpressed in lung cancer bone metastasis as compared with that in patients with primary lung cancer. In addition, the XCR1 ligand XCL1 promoted the proliferation and migration of lung cancer cells markedly, and knockdown of XCR1 by siRNA abolished the effect of XCL1 in cell proliferation and migration. Furthermore, we identified JAK2/STAT3 as a novel downstream pathway of XCR1, while XCL1/XCR1 increased the mRNA level of the downstream of JAK2/STAT3 including PIM1, JunB, TTP, MMP2 and MMP9. These results indicate that XCR1 is a new potential therapeutic target for the treatment of lung cancer bone metastasis.

1. Introduction

Primary lung cancer is one of the most common malignancies, accounting for the leading cause of cancer-related death in men and the second leading cause in women [1]. Approximately 85% lung cancer cases are non-small cell lung cancer (NSCLC), whose histopathological types are typically composed of adenocarcinoma, squamous cell carcinoma and large cell carcinoma [2]. Bone metastasis is reported to occur in about 30–40% patients with advanced NSCLC, leading to regional pain, pathologic fracture and spinal cord compression [3,4]. The mean survival of patients after diagnosis of metastatic lung cancer to the bone is usually less than 6 months [5]. But there is no effective treatment that can prevent skeletal metastatic morbidity in lung cancer patients. And the mechanism underlying bone metastasis in lung cancer still remains unclear.

The chemokine super family has been reported to play an important role in tumor metastasis. C-X-C motif chemokine 12 (CXCL12) from neighboring stromal cells and other distant organs primarily binds to the chemokine receptor 4 (CXCR4) on tumor cells and subsequently induces intracellular signaling through several divergent pathways, which are involved in the progression and metastasis of several tumor types [6,7]. Lymphotactin receptor (XCR1) is also one of the members of the chemokine receptor family. Previous studies [8–10] showed that XCR1 was overexpressed in ovarian carcinoma, oral cancer and breast cancer. Activation of XCR1 by its ligand XCL1 could promote cancer cell proliferation, adhesion, migration and invasion [8,9,11]. Meanwhile, the XCL1/XCR1 axis also contributes to the progression of various diseases, including rheumatoid arthritis [12], Crohn's disease [13] and AIDS [14]. However, the role of XCL1/XCR1 in lung cancer has not been reported before, and the downstream pathway of XCR1 remains poorly understood.

* Corresponding author. Department of Bone Tumor Surgery, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China.

** Corresponding author. Department of Bone Tumor Surgery, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China.

*** Corresponding author. Department of Bone Tumor Surgery, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China.

E-mail addresses: brilliant212@163.com (W. Zhou), cnspineyang@163.com (X. Yang), jianruxiao83@163.com (J. Xiao).

[1] Ting Wang, Shuai Han and Zhipeng Wu contributed equally to this work, and all should be considered first author.

http://dx.doi.org/10.1016/j.bbrc.2015.06.175
0006-291X/

In this study, we detected the level of XCR1 mRNA and protein expression in clinical samples of lung cancer bone metastasis and primary lung cancer, and found that XCR1 was overexpressed in lung cancer bone metastasis compared with that in primary lung cancer. We also evaluated the role of XCL1/XCR1 in lung cancer cell proliferation and migration, and further attempted to explore the possible downstream of XCL1/XCR1 in lung cancer.

2. Materials and methods

2.1. Clinical samples

Five primary NSCLC tissue specimens and five lung cancer bone metastasis tissue specimens were collected from patients who underwent surgical resection in our hospital. The tissue specimens were snap-frozen and stored in liquid nitrogen within 2 h after surgical excision. This study was approved by the Ethnic Committee of Changzheng Hospital of the Second Military Medical University (Shanghai, China), and written informed consent was obtained from all participants.

2.2. Cell lines and culture

The lung cancer cell lines A549 and PC9 were used in this study. A549 was cultured in DMEM (GIBCO) supplemented with 10% fetal bovine serum (FBS, GIBCO) in the cell incubator (37 °C, 5% CO2). PC9 was maintained in RPMI-1640 (GIBCO) supplemented with 10% FBS.

2.3. qRT-PCR for mRNA analysis

qRT-PCR for mRNA was performed by TransStart Top Green qPCRSuperMix (TransGen Biotech, China) on 7900HT Fast Real-Time PCR System (Life Technologies Corporation, USA). All PCR primers are listed in Supplemental Table 1.

2.4. Western blot assay

Samples were resolved on SDS–PAGE gel. Proteins were transferred onto a PVDF membrane (Millipore, Germany) by conventional methods. The antibodies against XCR1 (sc-82428), JAK2 (sc-294), p-JAK2 (21870), STAT3 (sc-482) and p-STAT3 (sc-8059) were all purchased from Santa (Santa Cruz, USA). Western Blot was probed with indicated antibodies and the immunoreactive protein was visualized by using EasySee Western Blot Kit (TransGen Biotech, China).

2.5. Immunohistochemistry

Both the primary NSCLC and bone metastasis tissue specimens were sliced into 4-μm sections and paraffin-embedded. Endogenous peroxidase was blocked using 2% hydrogen peroxide in methanol and the sections were treated in citrate buffer (pH 6.0) in a microwave oven for 12 min for antigen retrieval before incubation with XCR1 antibody (sc-82428). Secondary antibody and avidin–biotin complex (ABC) provided with a Vectastain Elite ABC kit (Vector Labs, Burlingame, USA) were used in accordance with the manufacturer's instructions. The sections were then counterstained with haematoxylin, dehydrated, and mounted in DPX.

2.6. siRNA transfection

XCR1 siRNA(sc-76931) and siRNA control (sc-37007) were purchased from Santa. A total of 2 × 105 cells were plated in 6-well plates, allowed adhering for 24 h, and transfected using the HiPerFect Transfection Reagent (Qiagen, Germany) according to the manufacturer's instructions.

2.7. Cell proliferation assay

A total of 5 × 103 cells was plated in 96-well plates and cultured for 24 h. Then XCL1 protein (Peprotech, America) was added into the medium. After 48 h, cell-titer aqueous one proliferation reagent (Promega, America) was added to each well and the absorbance was recorded at 492 nm. All assays were performed in triplicate and a standard curve was constructed for each, enabling the cell number to be determined.

2.8. Cell migration assay

Chemotaxis assays were performed using Transwell insert polycarbonate membranes (8 μm pore size; Corning, The Netherlands) in 24-well plates. Generally, 1X105 cells were suspended in 200 μL serum free medium and seeded into the upper chamber. The lower chamber was filled with medium containing 10% FBS with or without XCL1. After 24-h incubation in a humidified incubator, non-migratory cells on the upper membrane surface were removed, and those on the bottom surface were fixed with 4% polyoxymethylene and stained with 0.1% crystal violet for 15 min. The acetic acid elution was next done and the absorbance was recorded at 570 nm.

2.9. Statistic analysis

SPSS 19.0 statistical software (SPSS Inc., Chicago, IL) was used for statistical analysis. All data are presented as mean ± standard error of the mean (SEM). Statistics were assessed using both Student t test and ANOVA, assuming double-sided independent variance. All experiments were repeated at least three times, and representative experiments are shown. P values of <0.05 were considered statistically significant.

3. Results

3.1. XCR1 overexpression is correlated with lung cancer bone metastasis

Previous studies [8,9] showed that XCR1 was overexpressed in oral cancer and ovarian carcinoma and enhanced the proliferation and migration of tumor cells. However, there is no study reporting the expression of XCR1 in NSCLC. In this study, we used qRT-PCR to detect the XCR1 mRNA level in five primary NSCLC and five bone metastasis tissue specimens, and found that the XCR1 mRNA level was evaluated significantly in the lung cancer bone metastasis tissue specimens as compared with that in primary NSCLC tissue specimens (Fig. 1A), while there was no significant difference in XCL1 mRNA level between them (Fig. 1B). We further used Western blot to detect the expression of XCR1 and found a similar change with the XCR1 mRNA level (Fig. 1C). The results of immunohistochemistry staining also showed the overexpression of XCR1 in the lung cancer bone metastasis tissue specimens as compared with primary lung cancer (Fig. 1D). We further detected the mRNA level of XCL1 in various organs of the mice, and found that the expression level of XCL1 was relatively high in the bone (Fig. 1E).

3.2. XCL1/XCR1 significantly increases lung cancer cell proliferation

XCR1 is reported to be the only receptor of XCL1 [11]. To explore the role of XCR1 in lung cancer cells, we stimulated A549 and PC9 cells with different concentrations of XCL1 (0, 5, 10 and 20 nmol/L) for 48 h and detected the cell proliferation rates by using the MTS

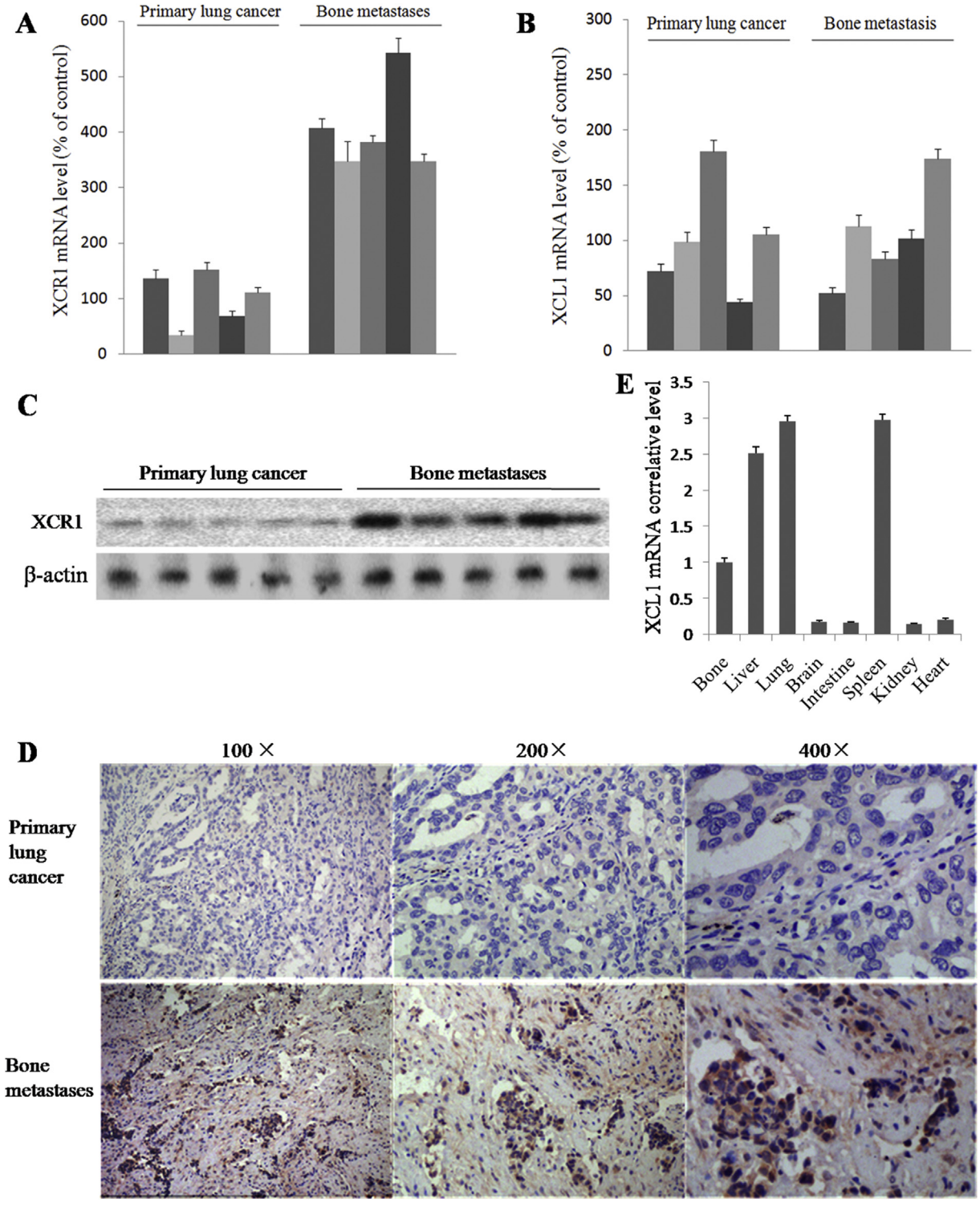

Fig. 1. XCR1 is overexpressed in lung cancer bone metastasis. (A) qRT-PCR assay of mRNA level of XCR1 in primary lung cancer tissues and lung cancer bone metastases tissues. (B) qRT-PCR assay of mRNA level of XCL1 in clinical samples. (C) Western blot assay of XCR1 expression in primary lung cancer tissues and lung cancer bone metastases tissues. (D) Immunohistochemistry staining of XCR1 in clinical samples. (E) qRT-PCR assay of mRNA level of XCL1 in various mouse organs.

assay (Fig. 2A). The results showed that XCL1 obviously increased the proliferation of A549 (Fig. 2B) and PC9 (Fig. 2C) cells in a dose-dependent manner. To further study whether XCL1 regulated cell proliferation through XCR1, we knocked down the expression of XCR1 by siRNA in both A549 and PC9 cells and cultured the cells with 20 nmol/L XCL1. The results showed that knockdown of XCR1 significantly decreased the proliferation rate of A549 and PC9 cells (Fig. 2D, E). These results indicated that XCL1 binding to XCR1 could significantly increase lung cancer cell proliferation.

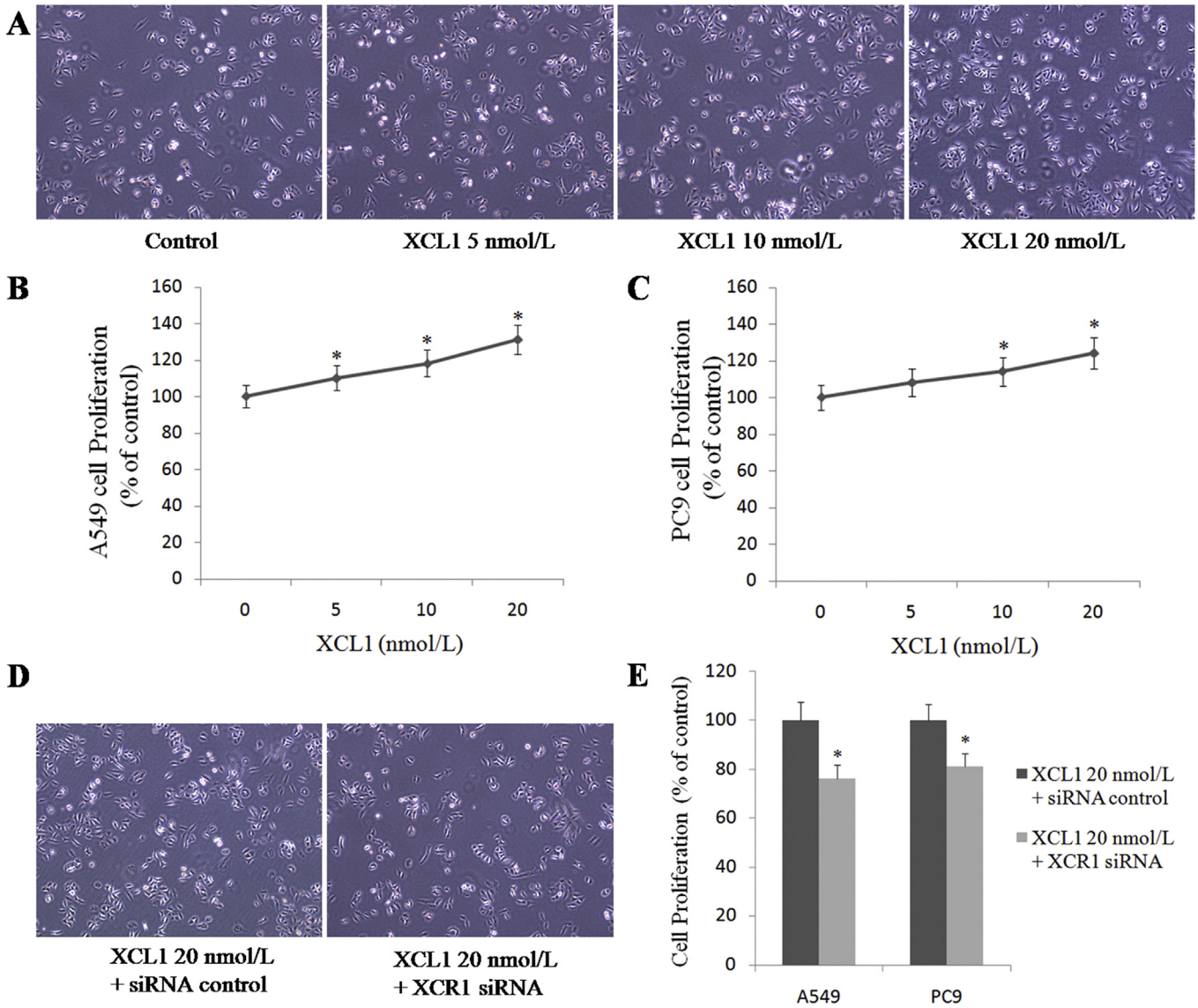

Fig. 2. XCL1/XCR1 promotes lung cancer cell proliferation. (A) The field of A549 cells under a light microscope (×40) after XCL1 stimulation. (B) MTS assay of the proliferation rate of A549 cells. (C) MTS assay of the proliferation rate of PC9 cells. (D) A549 cells were transfected with XCR1 siRNA and siRNA control and then stimulated with 20 nmol/L XCL1 for 48 h. The cells were observed under light microscopic (×40). (E) MTS assay of A549 and PC9 cells after transfection with XCR1 siRNA and siRNA control. * mean $p < 0.05$.

3.3. XCL1/XCR1 obviously increases lung cancer cell migration

To examine the role of XCR1 in lung cancer cell migration, we seeded A549 and PC9 cells on the upper membrane surface of the Transwell and stimulated them with different concentrations of XCL1 (0, 5, 10 and 20 nmol/L) for 24 h. The number of migrating cells was counted by crystal violet staining (Fig. 3A). The results showed that XCL1 significantly increased the migration of A549 and PC9 cells in a dose-dependent manner (Fig. 3B). In addition, the results of acetic acid elution and absorbance detection showed a similar fluctuation in the number of migrating cells (Fig. 3C). Meanwhile, knockdown of XCR1 expression using XCR1 siRNA in A549 and PC9 cells blocked XCR1 and abolished the role of XCL1 in cell migration (Fig. 3D, E). These results showed that XCL1 could promote lung cancer cell migration by activating XCR1.

3.4. XCL1/XCR1 activates JAK2/STAT3 pathway

Although several studies [8,9] have reported the role of XCR1 in cancer cell proliferation and migration, the downstream of XCR1 in tumors remains poorly understood. By using a pair of microarray assays, we found that a series of the downstream of signal transducer and activator of transcription 3 (STAT3) was markedly up-regulated in A549 cells after the transfection of plasmid carried XCL1 gene, including phosphatidylinositol mannoside 1 (PIM1), JunB and tristetraprolin (TTP, also named as ZFP36) (Supplemental Table 2). Through the qRT-PCR assay, we found that the mRNA level of PIM1, JUNB and TTP was significantly up-regulated after XCL1 stimulation with a dose dependent manner, while knockdown of XCR1 by siRNA decreased the mRNA level of PIM1, JunB and TTP (Fig. 4A–C). Further study showed that JAK2 mRNA level was slightly evaluated after XCL1 stimulation (Fig. 4D), while the mRNA level of STAT3 exhibited no obvious fluctuation (Fig. 4E).

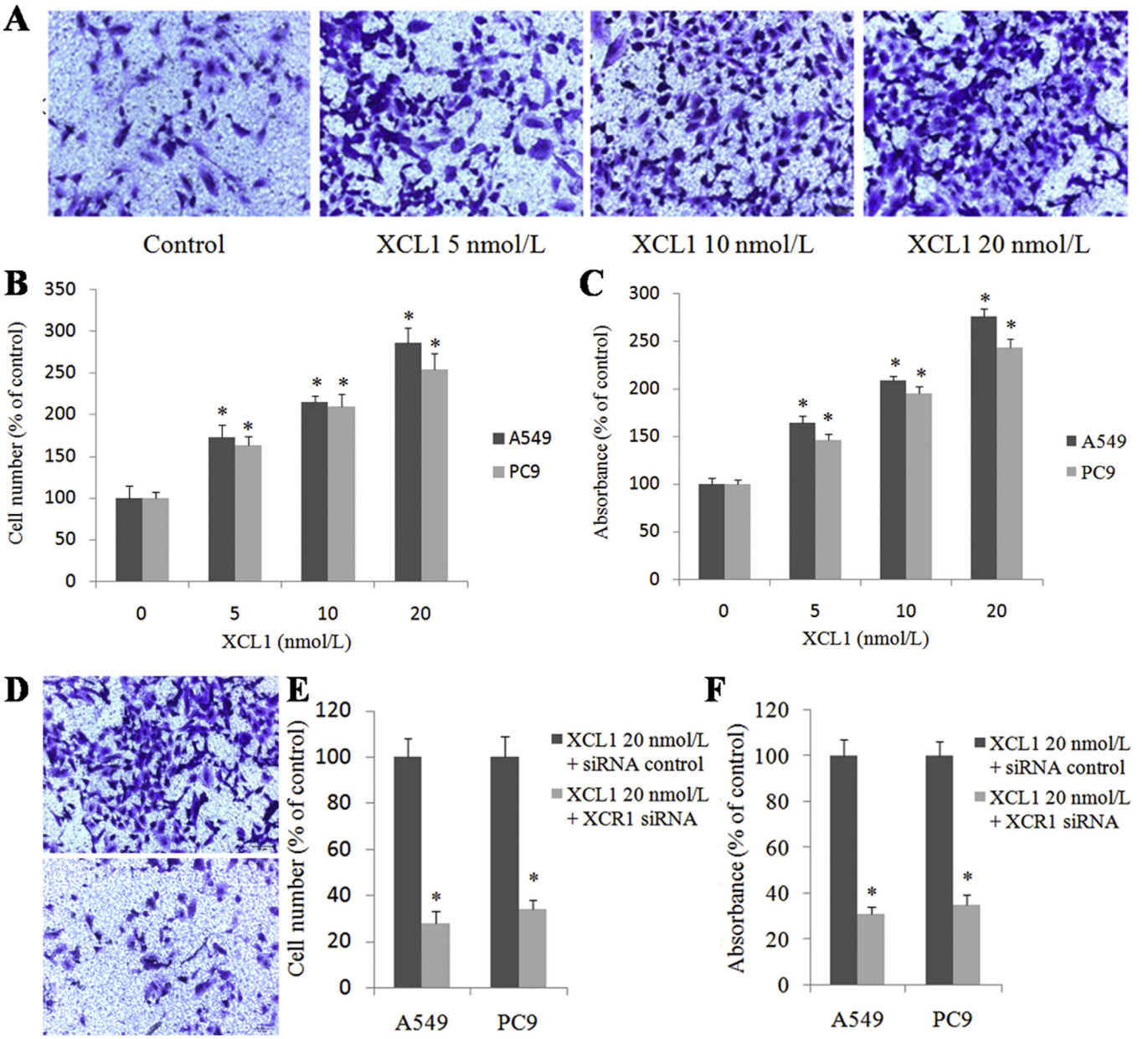

Fig. 3. XCL1/XCR1 promotes lung cancer cell migration. (A)Crystal violet staining of A549 migrating cells under a light microscope (×100) after XCL1 stimulation. (B) The number of A549 and PC9 migrating cells. (C) The absorbance of A549 and PC9 cells at 570 nm after acetic acid elution. (D, E) Knockdown of XCR1 by siRNA decreased the number of A549 and PC9 migrating cells. (F) The absorbance of A549 and PC9 cells after XCR1 siRNA transfection. * mean $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Meanwhile, activation of XCR1 was reported to increase the expression of matrix metalloproteinase 2 (MMP2) and MMP9 in oral cancer cells [9], which were also regulated by STAT3 [15,16]. We detected the role of XCL1/XCR1 in regulating MMP2 and MMP9 in lung cancer by qRT-PCR, and found that XCL1 up-regulated the mRNA level of MMP2 and MMP9 in a dose-dependent manner. Meanwhile, knockdown of XCR1 by siRNA obviously abolished the effect of XCL1 in regulating MMP2 and MMP9 (Fig. 4F, G).

By using the western blot assay, we further detected the protein level of JAK2, p-JAK2, STAT3 and p-STAT3 in both A549 and PC9 cells after XCL1 stimulation or knockdown of XCR1 by siRNA (Fig. 4H). The results showed that no significant fluctuation was observed in the expression of JAK2 and STAT3 after XCL1 stimulation. However, we found that XCL1 significantly promoted the phosphorylation of JAK2 and STAT3, while knockdown of XCR1 obviously decreased the expression of p-JAK2 and p-STAT3. These results suggested that JAK2/STAT3 was a possible downstream pathway of XCL1/XCR1.

4. Discussion

The XCL1-XCR1 axis was reported to play a key role in the immune system, including the regulation of the dendritic-cell-mediated cytotoxic immune response, the thymic establishment of self-tolerance and the generation of regulatory T cells [17]. Recent studies [8–11] also demonstrated the importance of XCL1/XCR1 in cancer cell proliferation, migration and invasion. However, the role of XCL1/XCR1 in lung cancer remains unknown. In this

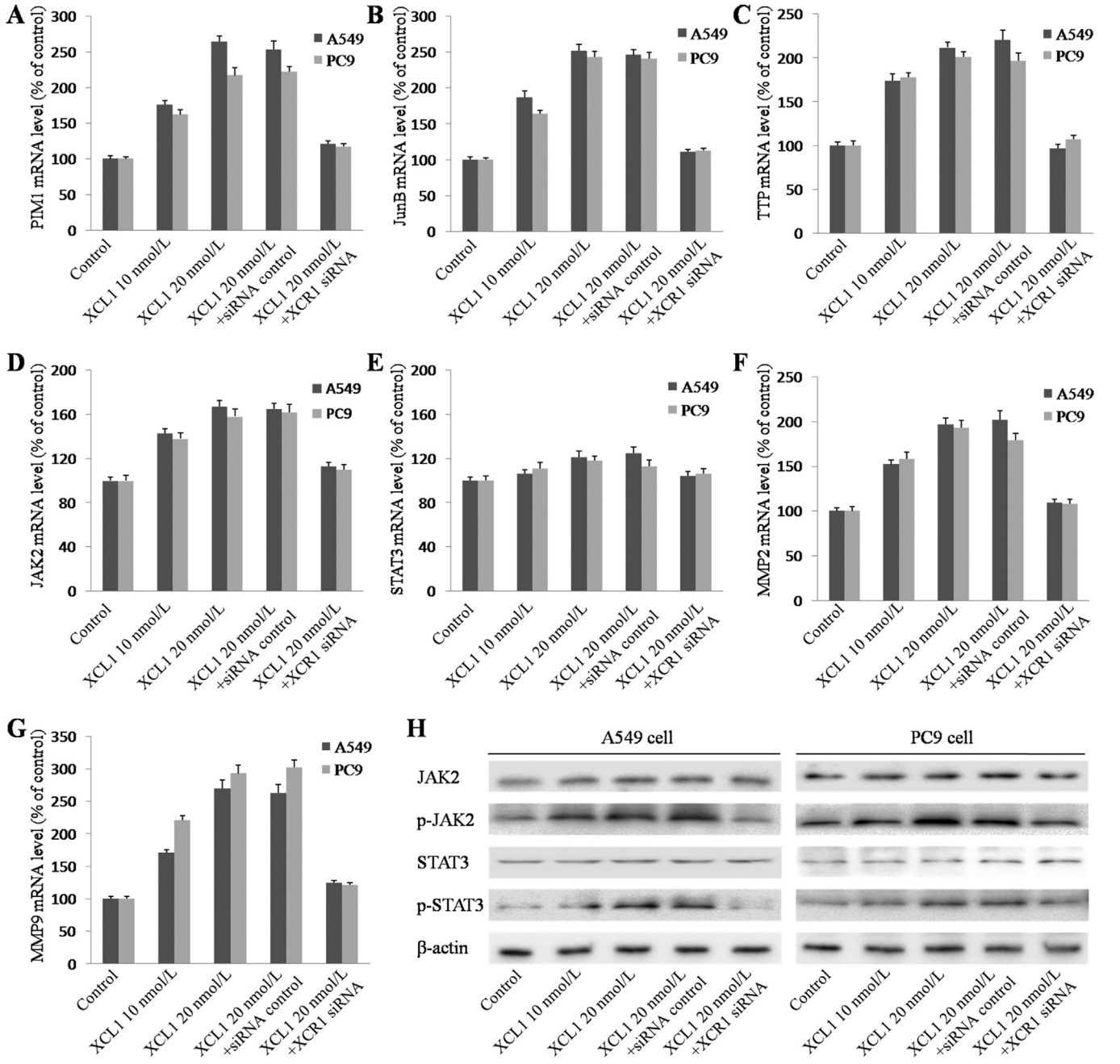

Fig. 4. Study of the possible downstream of XCR1. A549 and PC9 cells were cultured with the medium containing 0, 10 or 20 nmol/L XCL1 for 48 h, or knocked down of XCR1 and cultured with 20 nmol/L XCL1. (A) qRT-PCR assay of mRNA level of PIM1. (B) qRT-PCR assay of mRNA level of JunB. (C) qRT-PCR assay of mRNA level of TTP. (D) qRT-PCR assay of mRNA level of JAK2. (E) qRT-PCR assay of mRNA level of STAT3. (F) qRT-PCR assay of mRNA level of MMP2. (G) qRT-PCR assay of mRNA level of MMP9. (H) Western blot assay of JAK2, p-JAK2, STAT3, p-STAT3 in A549 and PC9 cells.

study, we demonstrated that XCR1 was overexpressed in lung cancer bone metastasis compared with primary NSCLC. Meanwhile, XCL1, the ligand of XCR1, was confirmed to be expressed in bone marrow cells [18]. In this study, we also found that the mRNA level of XCL1 was relatively highly expressed in the mouse bone tissue. Furthermore, the result of our in vitro experiment showed that XCL1 significantly promoted the proliferation and migration of lung cancer cells by activating XCR1. These results suggested a possible procedure in NSCLC bone metastasis that XCL1 in bone tissue activating XCR1 in cancer cells promoted tumor progression. However, this procedure still need further study. Our data indicated that the XCL1/XCR1 axis has the propensity to contribute to the progression of lung cancer by enhancing proliferation and facilitating migration and might play an important role in bone metastasis.

Though some studies showed the role of XCL1/XCR1 in cancer cell proliferation and migration, the mechanism of XCL1/XCR1 in tumors still remain poorly understood. The reported downstream of XCL1/XCR1 in cancer cells included ERK1/2 and MMPs [9]. In this study, we found that JAK2/STAT3 pathway was a novel possible downstream of XCL1/XCR1, for that XCL1/XCR1 could significantly up-regulate the expression of p-JAK2 and p-STAT3 and the mRNA level of the targets of JAK2/STAT3, including PIM1, JunB and TTP. JAK2/STAT3 pathway was known to be markedly activated in NSCLC

and promote cancer cell proliferation, invasion and metastasis [19,20]. Meanwhile, the overexpression of p-STAT3 was showed to be a strong predictor of poor prognosis among patients with NSCLC [21].

PIM1, JunB and TTP were reported as the direct targets of STAT3 [22]. PIM1 was known as an oncogene that could stimulate cancer cell proliferation, migration and invasion [23]. By targeting PRAS40, PIM1 also induced radioresistance of NSCLC [24]. JunB was reported to be overexpressed in lung cancer and promoted cell proliferation and cancer metastasis [25]. Meanwhile, TTP was also known as an oncogene that could promoted A549 cell proliferation [26]. In this study, we found that XCL1 could up-regulate PIM1, JunB and TTP while knockdown of XCR1 abolished the regulation role of XCL1 in these oncogenes, which indicated that XCL1/XCR1 might promote NSCLC progression by the regulation of PIM1, JunB and TTP.

MMPs were recognized to be derived from a number of cellular sources in the tumor–bone microenvironment, in particular the main cellular players involved in the vicious cycle, including metastatic cancer cells, osteoblasts and osteoclasts [27–29]. MMP2 and MMP9 were reported as the activators of osteoclast differentiation and tumor cell invasion [28,29]. Meanwhile, the overexpression of MMP2 and MMP9 in lung cancer has been confirmed previously [30]. Khurram et al. [9] reported that activation of the XCL1-XCR1 pathway could promote the expression of MMP2 and MMP9 in oral cancer cells. Furthermore, MMP2 and MMP9 were also considered as the downstream of JAK2/STAT3 pathway [15,16]. However, no correlation of XCL1/XCR1 and MMPs in lung cancer has been reported. In this study, we found that XCL1/XCR1 could possible activate JAK2/STAT3 pathway, which might explain the regulation role of XCR1 in MMP2 and MMP9 expression. In addition, the regulation of MMP2 and MMP9 might also partly explain the role of overexpressed XCR1 in lung cancer bone metastasis.

In conclusion, our study demonstrated for the first time that XCR1 was overexpressed in lung cancer bone metastasis compared with primary lung cancer. XCR1 activation significantly promoted the proliferation and migration of lung cancer cells. In addition, we identified the JAK2/STAT3 as a novel downstream pathway of XCR1, while XCL1/XCR1 increased the mRNA level of the downstream of JAK2/STAT3 including PIM1, JunB, TTP, MMP2 and MMP9. These results indicate that XCR1 is a novel potential therapeutic target for the treatment of lung cancer bone metastasis.

Acknowledgments

This work was supported by the grants from Shanghai Municipal Commission of Science and Technology (12ZR1439200), and the Training Program for Outstanding Young Talents in Shanghai Health System (XYQ2013099).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.175.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.175.

References

[1] T. Mitsudomi, T. Kosaka, H. Endoh, et al., Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence, J. Clin. Oncol. 23 (2005) 2513–2520.
[2] P.C. Hoffman, A.M. Mauer, E.E. Vokes, Lung cancer, Lancet 355 (2000) 479–485.
[3] R.E. Coleman, Metastatic bone disease: clinical features, pathophysiology and treatment strategies, Cancer Treat. Rev. 27 (3) (2001) 165–176.
[4] D.M. Sciubba, T. Nguyen, Z.L. Gokaslan, Solitary vertebral metastasis, Orthop. Clin. N. Am. 40 (1) (2009) 145–154.
[5] D. Rades, S. Douglas, T. Veninga, et al., Metastatic spinal cord compression in non-small cell lung cancer patients. Prognostic factors in a series of 356 patients, Strahlenther. Onkol. 188 (6) (2012) 472–476.
[6] Y.X. Liao, C.H. Zhou, H. Zeng, et al., The role of the CXCL12-CXCR4/CXCR7 axis in the progression and metastasis of bone sarcomas, Int. J. Mol. Med. 32 (6) (2013) 1239–1246.
[7] J. Shi, Y. Wei, J. Xia, et al., CXCL12-CXCR4 contributes to the implication of bone marrow in cancer metastasis, Futur. Oncol. 10 (5) (2014) 749–759.
[8] M. Kim, L. Rooper, J. Xie, et al., The lymphotactin receptor is expressed in epithelial ovarian carcinoma and contributes to cell migration and proliferation, Mol. Cancer Res. 10 (11) (2012) 1419–1429.
[9] S.A. Khurram, S.A. Whawell, L. Bingle, et al., Functional expression of the chemokine receptor XCR1 on oral epithelial cells, J. Pathol. 221 (2) (2010) 153–163.
[10] S.K. Gantsev, K. Umezawa, D.V. Islamgulov, et al., The role of inflammatory chemokines in lymphoid neoorganogenesis in breast cancer, Biomed. Pharmacother. 67 (5) (2013) 363–366.
[11] T. Yoshida, T. Imai, M. Kakizaki, et al., Identification of single C motif-1/lymphotactin receptor XCR1, J. Biol. Chem. 273 (1998) 16551–16554.
[12] S. Blaschke, P. Middel, B.G. Dorner, et al., Expression of activation-induced, T cell-derived, and chemokine-related cytokine/lymphotactin and its functional role in rheumatoid arthritis, Arthritis Rheum. 48 (2003) 1858–1872.
[13] P. Middel, P. Thelen, S. Blaschke, et al., Expression of the T-cell chemoattractant chemokine lymphotactin in Crohn's disease, Am. J. Pathol. 159 (2001) 1751–1761.
[14] C. Guzzo, J. Fox, Y. Lin, et al., The CD8-derived chemokine XCL1/lymphotactin is a conformation-dependent, broad-spectrum inhibitor of HIV-1, PLoS Pathog. 9 (12) (2013) e1003852.
[15] X. Xuan, S. Li, X. Lou, et al., Stat3 promotes invasion of esophageal squamous cell carcinoma through up-regulation of MMP2, Mol. Biol. Rep. 42 (5) (2015) 907–915.
[16] D.H. Jo, J.H. Kim, C.S. Cho, et al., STAT3 inhibition suppresses proliferation of retinoblastoma through down-regulation of positive feedback loop of STAT3/miR-17-92 clusters, Oncotarget 5 (22) (2014) 11513–11525.
[17] Y. Lei, Y. Takahama, XCL1 and XCR1 in the immune system, Microbes Infect. 14 (3) (2012) 262–267.
[18] X. Cao, W. Zhang, L. He, et al., Lymphotactin gene-modified bone marrow dendritic cells act as more potent adjuvants for peptide delivery to induce specific antitumor immunity, J. Immunol. 161 (11) (1998) 6238–6244.
[19] H.M. Yun, K.R. Park, M.H. Park, et al., PRDX6 promotes tumor development via the JAK2/STAT3 pathway in a urethane-induced lung tumor model, Free Radic. Biol. Med. 80 (2015) 136–144.
[20] R.Y. Liu, Y. Zeng, Z. Lei, et al., JAK/STAT3 signaling is required for TGF-β-induced epithelial-mesenchymal transition in lung cancer cells, Int. J. Oncol. 44 (5) (2014) 1643–1651.
[21] Y.H. Xu, S. Lu, A meta-analysis of STAT3 and phospho-STAT3 expression and survival of patients with non-small-cell lung cancer, Eur. J. Surg. Oncol. 40 (3) (2014) 311–317.
[22] P.Y. Bourillot, I. Aksoy, V. Schreiber, et al., Novel STAT3 target genes exert distinct roles in the inhibition of mesoderm and endoderm differentiation in cooperation with Nanog, Stem Cells 27 (8) (2009) 1760–1771.
[23] C.O. Leung, C.C. Wong, D.N. Fan, et al., PIM1 regulates glycolysis and promotes tumor progression in hepatocellular carcinoma, Oncotarget 6 (13) (2015) 10880–10892.
[24] W. Kim, H. Youn, K.M. Seong, et al., PIM1-activated PRAS40 regulates radioresistance in non-small cell lung cancer cells through interplay with FOXO3a, 14-3-3 and protein phosphatases, Radiat. Res. 176 (5) (2011) 539–552.
[25] A.S. Berghoff, P. Birner, B. Streubel, et al., ALK gene aberrations and the JUN/JUNB/PDGFR axis in metastatic NSCLC, APMIS 122 (9) (2014) 867–872.
[26] H.H. Lee, M.T. Vo, H.J. Kim, et al., Stability of the LATS2 tumor suppressor gene is regulated by tristetraprolin, J. Biol. Chem. 285 (23) (2010) 17329–17337.
[27] X. Lu, Q. Wang, G. Hu, C. Van Poznak, et al., ADAMTS1 and MMP1 proteolytically engage EGF-like ligands in an osteolytic signaling cascade for bone metastasis, Genes. Dev. 23 (2009) 1882–1894.
[28] S.M. Violette, J.L. Stein, G.S. Stein, et al., Integrin αvβ6 promotes an osteolytic program in cancer cells by upregulating MMP2, Cancer Res. 74 (5) (2014) 1598–1608.
[29] D. Chen, X. Zhang, Y. Guo, et al., MMP-9 inhibition suppresses wear debris-induced inflammatory osteolysis through downregulation of RANK/RANKL in a murine osteolysis model, Int. J. Mol. Med. 30 (6) (2012) 1417–1423.
[30] J. Liu, W. Ping, Y. Zu, W. Sun, Correlations of lysyl oxidase with MMP2/MMP9 expression and its prognostic value in non-small cell lung cancer, Int. J. Clin. Exp. Pathol. 7 (9) (2014) 6040–6047.