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IL-8 Increases Integrin Expression and Cell Motility in Human Chondrosarcoma Cells

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ABSTRACT

Chondrosarcoma is a type of highly malignant tumor with a potent capacity to invade locally and cause distant metastasis. Chondrosarcoma shows a predilection for metastasis to the lungs. Interleukin-8 (IL-8), a chemokine with a defining CXC amino acid motif, is known to possess tumorigenic and proangiogenic properties. Over-expression of IL-8 has been detected in many human tumors. However, the effects of IL-8 in migration and integrin expression in chondrosarcoma cells are largely unknown. In this study, we found that IL-8 increased the migration and the expression of αvβ3 integrin in human chondrosarcoma cells. Activations of phosphatidylinositol 3-kinase (PI3K), Akt, and AP-1 pathways after IL-8 treatment were demonstrated, and IL-8-induced expression of integrin and migration activity was inhibited by the specific inhibitor and mutant of PI3K, Akt, and AP-1 cascades. Taken together, our results indicated that IL-8 enhances the migration of chondrosarcoma cells by increasing αvβ3 integrin expression through the PI3K/Akt/AP-1 signal transduction pathway. J. Cell. Biochem. 112: 2549–2557, 2011.

KEY WORDS: IL-8; MIGRATION; CHONDROSARCOMA; INTEGRIN; AP-1

Chondrosarcoma is a malignant primary bone tumor with a poor response to currently used chemotherapy or radiation treatment, making the management of chondrosarcomas a complicated challenge [Terek et al., 1998]. Clinically, surgical resection remains the primary mode of therapy for chondrosarcoma. Due to the absence of an effective adjuvant therapy, this mesenchymal malignancy has a poor prognosis and, therefore, it is important to explore a novel and adequate remedy [Yuan et al., 2005].

Interleukin-8 (IL-8), a member of the neutrophil-specific CXC subfamily of chemokines with ELR (Glu-Leu-Arg) motif, can act not only on leukocyte chemotaxis and inflammatory responses and infectious diseases but also on endothelial cells via their receptors to promote migration, invasion, proliferation, and in vivo angiogenesis [Xie, 2001; Lee et al., 2005; Raman et al., 2007]. The biological action of IL-8 is mediated through binding to its receptors, CXCR1 (IL-8RA) and CXCR2 (IL-8RB), which are members of the seven transmembrane G-protein-coupled receptor family [Rollins, 1997; Heidemann et al., 2003]. It has been suggested that tumor cells produce IL-8 as an autocrine growth factor, which promote tumor growth, tissue invasion, and metastatic spread [Xie, 2001]. Previous studies have revealed that highly metastatic solid tumors such as prostate, breast, melanoma, and ovarian cancer constitutively express IL-8 [Xu and Fidler, 2000; Huang et al., 2002]. Moreover, IL-8 expression correlates with tumor cell growth and vascularity in gastric carcinoma [Kido et al., 2001]. However, the exact role of IL-8 in the progressive growth of chondrosarcoma remains unclear.

Decades of scrutiny into the molecular bases of cancer have largely focused on what causes oncogenic transformation and the incipient emergence of tumors [Gupta and Massague, 2006]. The invasion of tumor cells is a complex, multistage process. To facilitate cell motility, invading cells need to change the cell–cell adhesion

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properties, rearrange the extracellular matrix (ECM) environment, suppress anoikis, and reorganize their cytoskeletons [Woodhouse et al., 1997]. Integrins are a family of transmembrane adhesion receptors comprising 19α and 8β subunits that interact non-covalently to form up to 24 different heterodimeric receptors. The combination of different integrin subunits on the cell surface allows cells to recognize and respond to a variety of different ECM proteins including fibronectin, laminin, collagen, and vitronectin [Humphries, 2000]. Because integrins are the primary receptors for cellular adhesion to ECM molecules, they act as crucial transducers of bidirectional cell signaling, regulating cell survival, differentiation, proliferation, migration, and tissue remodeling [Stupack, 2007]. Activation and elevated expression of integrin-coupled signaling effectors have been implicated in the induction of a wide variety of human cancers, including those of the breast, colon, prostate, and ovaries [White et al., 2004]. In addition, integrin has also been implicated in metastasis of lung, breast, bladder, and colon cancers [Takenaka et al., 2000; Heyder et al., 2005; Seales et al., 2005].

Previous studies have shown that IL-8 modulates cell migration and invasion in human cancer cells [Kido et al., 2001; Xie, 2001]. However, the effect of IL-8 on integrin expression and migration activity in human chondrosarcoma cells is mostly unknown. In this study, we explored whether IL-8 increased the migration and integrin expression in human chondrosarcoma cells. In addition, phosphatidylinositol 3-kinase (PI3K), Akt, and AP-1 signaling pathways may be involved in the increase of integrin expression and cell migration by IL-8.

MATERIALS AND METHODS

MATERIALS

Protein A/G beads, anti-mouse, and anti-rabbit IgG-conjugated horseradish peroxidase, rabbit polyclonal antibodies specific for p-p85, p85, p-Akt, Akt, p-c-Jun, c-Jun, β-actin, c-Jun siRNA, control siRNA, control shRNA plasmid, and IL-8 shRNA plasmid were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal antibody (mAb) specific for αvβ3 integrin was purchased from Chemicon (Temecula, CA). Ly294002, wortmannin, Akt inhibitor, curcumin, and tanshinone IIA were purchased from Calbiochem (San Diego, CA). The recombinant human IL-8, CXCR1 and CXCR2 mAb were purchased from R&D Systems (Minneapolis, MN). The p85 (Δp85; deletion of 35 amino acids from residues 479 to 513 of p85) and Akt (Akt K179A) dominant-negative mutants were gifts from Dr. W.M. Fu (National Taiwan University, Taipei, Taiwan). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

CELL CULTURE

The human chondrosarcoma cell line (JJ012) was kindly provided by the laboratory of Dr. Sean P. Scully (University of Miami School of Medicine, Miami, FL). The human chondrosarcoma cell line (SW1353) was obtained from the American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium/α-minimum essential medium supplemented with 10% fetal bovine serum and maintained at 37°C in a humidified atmosphere of 5% CO_2.

MIGRATION AND INVASION ASSAY

The migration assay was performed using Transwell (Costar, NY; pore size, 8 μm) in 24-well dishes. For invasion assay, filters were precoated with 25 μl Matrigel basement membrane matrix (BD Biosciences, Bedford, MA) for 30 min. The following procedures were the same for both migration and invasion assays. Cells were pretreated for 30 min with different concentrations of inhibitors, including the Ly294002, wortmannin, Akt inhibitor, curcumin, tanshinone IIA, or vehicle control (0.1% DMSO). Approximately 1×10^4 cells in 200 μl of serum-free medium were placed in the upper chamber, and 300 μl of the same medium containing IL-8 was placed in the lower chamber. The plates were incubated for 24 h at 37°C in 5% CO_2, then cells were fixed in methanol for 15 min and stained with 0.05% crystal violet in PBS for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope. Each clone was plated in triplicate in each experiment, and each experiment was repeated at least three times. The number of invading cells in each experiment was adjusted by the cell viability assay to correct for proliferation effects of IL-8 treatment (corrected invading cell number = counted invading cell number/percentage of viable cells) [Fong et al., 2008; Lu et al., 2010].

WOUND-HEALING MIGRATION ASSAY

For the wound-healing migration assay, cells were seeded on 12-well plates at a density of 1×10^5 cells/well in culture medium. At 24 h after seeding, the confluent monolayer of culture was scratched with a fine pipette tip, and migration was visualized by microscope and magnification. The rate of wound closure was observed at the indicated time.

FLOW CYTOMETRIC ANALYSIS

Human chondrosarcoma cells were plated in six-well dishes. The cells were then washed with PBS and detached with trypsin at 37°C. Cells were fixed for 10 min in PBS containing 1% paraformaldehyde. After being rinsed in PBS, the cells were incubated with mouse anti-human antibody against integrin (1:100) for 1 h at 4°C. Cells were then washed again and incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit secondary IgG (1:100; Leinco Tec. Inc., St. Louis, MO) for 45 min and analyzed by flow cytometry using FACS Calibur and CellQuest software (BD Biosciences).

WESTERN BLOT ANALYSIS

The cellular lysates were prepared as described previously [Tang et al., 2008]. Proteins were resolved on SDS–PAGE and transferred to Immobilon polyvinyldifluoride (PVDF) membranes. The blots were blocked with 4% BSA for 1 h at room temperature and then probed with rabbit anti-human antibodies against p-p85, p85, p-Akt, Akt, or p-c-Jun (1:1,000) for 1 h at room temperature. After three washes, the blots were subsequently incubated with a donkey anti-rabbit peroxidase-conjugated secondary antibody (1:1,000) for 1 h at room temperature. The blots were visualized by enhanced chemiluminescence using Kodak X-OMAT LS film (Eastman Kodak, Rochester, NY).

QUANTITATIVE REAL-TIME PCR

Total RNA was extracted from chondrosarcomas using a TRIzol kit (MDBio Inc., Taipei, Taiwan). Two micrograms of total RNA was reverse transcribed into cDNA using oligo(dT) primer. The quantitative real-time PCR (qPCR) analysis was carried out using Taqman® one-step PCR Master Mix (Applied Biosystems, CA). One hundred nanograms of total cDNA were added per 25 μl reaction with sequence-specific primers and Taqman® probes. Sequences for all target gene primers and probes were purchased commercially (GAPDH was used as internal control; Applied Biosystems). qPCR assays were carried out in triplicate on a StepOnePlus sequence detection system. The cycling conditions were 10-min polymerase activation at 95°C followed by 40 cycles at 95°C for 15 s and 60°C for 60 s. The threshold was set above the non-template control background and within the linear phase of target gene amplification to calculate the cycle number at which the transcript was detected (denoted C_t).

ESTABLISHMENT OF STABLY TRANSFECTED CELLS

IL-8 shRNA or control shRNA plasmids are transfected into cancer cells with Lipofetamine 2000 transfection reagent. Twenty-four hours after transfection, stable transfectants are selected in puromycin (Life Technologies) at a concentration of 10 μg/ml. Thereafter, the selection medium is replaced every 3 days. After 2 weeks of selection in puromycin, clones of resistant cells are isolated. Integration of transfected shRNA was confirmed by Western blot analysis.

STATISTICS

The values given are means ± SEM. The significance of differences between the experimental groups and controls was assessed by Student's *t*-test. *P*-values < 0.05 were considered significant.

RESULTS

IL-8 DIRECTED MIGRATION OF CHONDROSARCOMA CELLS

IL-8 has been suggested to stimulate directional migration and invasion of human cancer cells [Kido et al., 2001; Xie, 2001]. IL-8-triggered migration in chondrosarcoma cells was examined using the Transwell assay with correction of IL-8-induced proliferation effects on human chondrosarcoma cells [Fong et al., 2008]. IL-8-directed human chondrosarcoma cell (JJ012 and SW1353 cells) migration (Fig. 1A). It was also found that the invasive ability of chondrosarcoma cells through the Matrigel basement membrane matrix was increased by IL-8 stimulation (Fig. 1B). On the other hand, IL-8 also increased wound-healing activity in human chondrosarcoma cells (Fig. 1C). Interaction of IL-8 with its specific receptor CXCR1 and CXCR2 on the surface of cancer cells has been reported to induce cancer invasion [Rollins, 1997; Heidemann et al., 2003]. Pretreatment of cells for 30 min with anti-CXCR1 and CXCR2 antibody (5 μg/ml) markedly inhibited the IL-8-induced cell migration (Fig. 1D). These data suggest that IL-8-induced cancer migration may occur via CXCR1 and CXCR2 receptor.

INVOLVEMENT OF αVβ3 INTEGRIN IN THE IL-8-DIRECTED MIGRATION OF CHONDROSARCOMA

Previous studies have shown significant expression of αvβ3 integrin in human chondrosarcoma cells [Chen et al., 2009; Lai et al., 2009]. We therefore, hypothesized that αvβ3 integrin may be involved in IL-8-directed chondrosarcoma cell migration. Flow cytometry analysis showed that IL-8 induced the cell surface expression of αvβ3 integrin dose-dependently (Fig. 2A). To confirm this finding, expression of mRNA for the integrins in response to IL-8 was analyzed by qPCR. Treatment of JJ012 cells with IL-8 induced the mRNA expression of αv and β3 integrin (Fig. 2B). Pretreatment of cells for 30 min with anti-αvβ3 mAb (10 μg/ml) markedly inhibited the IL-8-induced cell migration (Fig. 2C). The cyclic RGD peptide (cyclo-RGDfV) has been reported to bind αvβ3 at high affinity and block its function effectively at low concentrations [Brooks et al., 1996]. Treatment of cells with cyclic RGD, but not cyclic RAD, inhibited the IL-8-induced migration of chondrosarcoma (Fig. 2C). In addition, CXCR1 and CXCR2 mAb also reduced IL-8-enhanced integrin expression (Fig. 2D). These data suggest that IL-8/CXCR-induced cancer migration may occur via up-regulation of αvβ3 integrin receptor.

PI3K AND AKT SIGNALING PATHWAYS ARE INVOLVED IN THE IL-8-INDUCED INTEGRIN UP-REGULATION AND MIGRATION OF HUMAN CHONDROSARCOMA CELLS

PI3K/Akt signaling pathway can be activated by a variety of growth factors, such as insulin and nerve growth factors [Horowitz et al., 2004]. Treatment of cells with IL-8 led to a significant increase of phosphorylation of p85 subunit of PI3K (Fig. 3A). To explore whether PI3K is involved in IL-8-induced cells migration, PI3K inhibitor Ly294002 and wortmannin were used. As shown in Figure 3B,C, pretreatment of cells with Ly294002 or wortmannin inhibited IL-8-induced migration activity and integrin expression of chondrosarcoma. Transfection of cells with p85 mutant also antagonized the potentiating effect of IL-8 (Fig. 3B,C). We then directly measured the Akt phosphorylation in response to IL-8 stimulation. Figure 4A shows that IL-8 increased Akt phosphorylation in a time-dependent manner. Furthermore, Akt inhibitor or mutant also antagonized IL-8-induced cell migration and integrin expression (Fig. 4B,C). Taken together, these results indicate that the PI3K and Akt pathway are involved in IL-8-induced migration and αvβ3 integrin expression of human chondrosarcoma cells.

AP-1 SIGNALING PATHWAY IS INVOLVED IN IL-8-INDUCED INTEGRIN UP-REGULATION AND MIGRATION ACTIVITY

As mentioned above, AP-1 activation is necessary for the migration and invasion of human chondrosarcoma cells [Hou et al., 2009; Tan et al., 2009]. To examine whether AP-1 activation is involved in IL-8-induced cancer migration, an AP-1 inhibitor, curcumin, and tanshionone IIA were used. Figure 5A shows that chondrosarcoma cells pretreated with curcumin and tanshionone IIA inhibited IL-8-induced chondrosarcoma cell migration. In addition, treatment of cells with curcumin or tanshionone IIA also antagonized IL-8-induced expression of αvβ3 integrin (Fig. 5B). AP-1 activation was further evaluated by analyzing the accumulation of phosphorylated c-Jun in the nucleus. Treatment of cells with IL-8 resulted in a

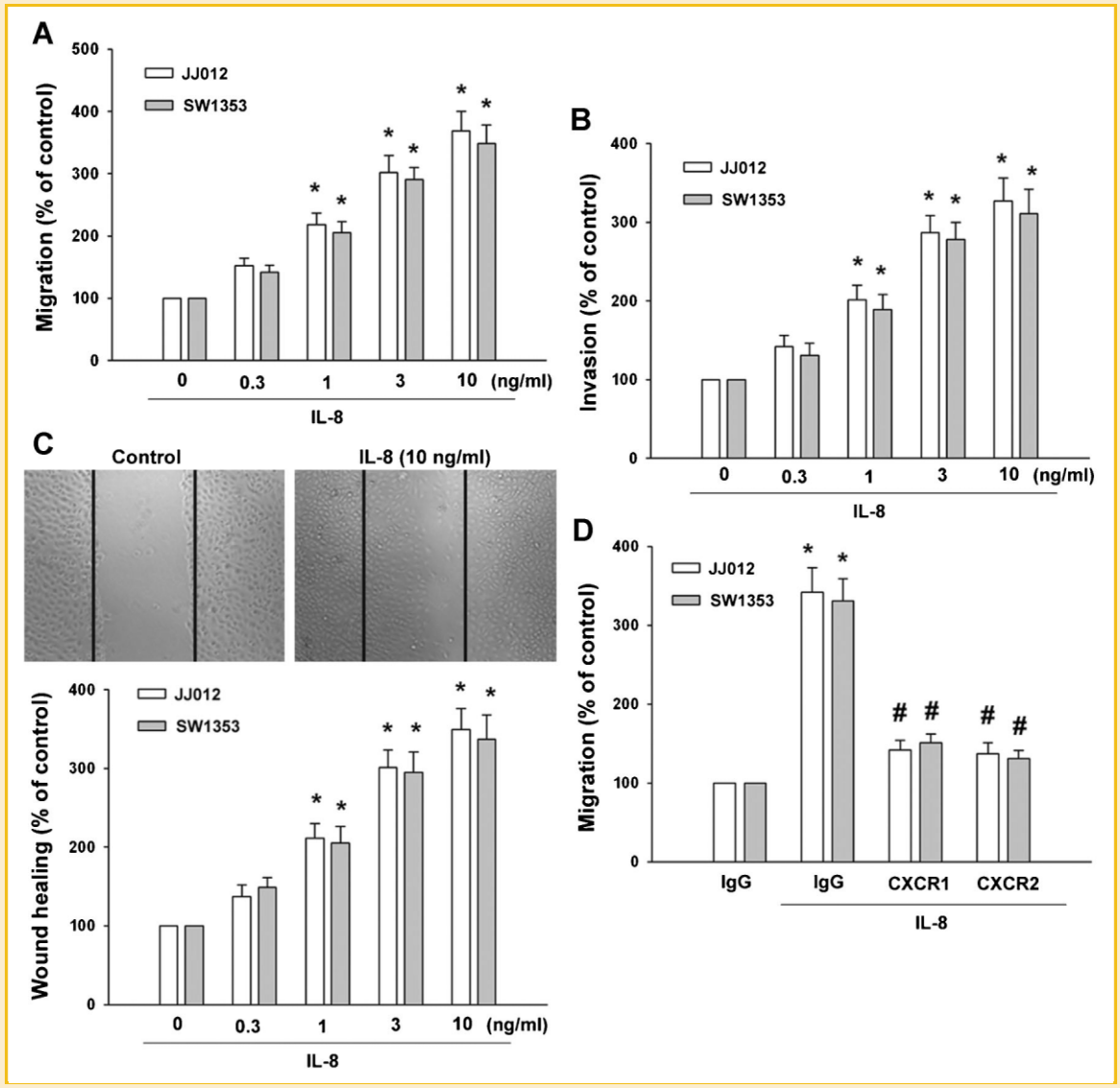

Fig. 1. IL-8-directed migration of human chondrosarcoma cells (A,B) JJ012 or SW1353 cells were incubated with IL-8 for 24 h, and in vitro migration and invasion was measured by Transwell after 24 h ($n = 4$). C: Cells were treated with IL-8 for 48 h, the wound-scratching assay was performed ($n = 5$). D: Cells were pretreated with CXCR1 or CXCR2 Ab (5 μg/ml) for 30 min followed by stimulation with IL-8 for 24 h, and in vitro migration was measured with the Transwell after 24 h ($n = 4$). Results are expressed as the mean ± SE. $^{*}P < 0.05$ compared with control.

marked accumulation of phosphorylated c-Jun in the nucleus (Fig. 5C). Transfection of cells with c-Jun siRNA suppressed the expression of c-Jun (data not shown). IL-8-induced cell migration and integrin expression was also inhibited by c-Jun siRNA but not by control siRNA (Fig. 5D). Taken together, these data suggest that activation of the CXCR1/CXCR2, PI3K, Akt, c-Jun, and AP-1 pathways are required for the IL-8-induced increase of cell migration and integrin expression in human chondrosarcoma cells.

DECREASED CELL MOTILITY IN IL-8-SHRNA OVER-EXPRESSION CLONE

To further confirm IL-8-mediated cell migration and αvβ3 integrin expression in human chondrosarcoma cells, IL-8-shRNA expression cells were established. The IL-8 expression level in stable transfectants was compared using Western blotting. The expression of IL-8 was dramatically inhibited by IL-8-shRNA orientation in JJ012/IL-8-shRNA cells (Fig. 6B). We next characterized the cellular growth rate of control cells and transfectants by performing the MTT assay 1–6 days after cell seeding. No appreciable difference in cell growth ability was evident in these cells (data not shown), suggesting that IL-8 does not have any mitogenic effect in human chondrosarcoma cells. Furthermore, the migratory ability of these transfectants was analyzed using a Transwell migration assay. Knockdown of IL-8 expression inhibited the migratory ability by approximately 73% in JJ012 cells (Fig. 6A). In addition, knockdown IL-8 also reduced αv and β3 integrin expression in JJ012 cells (Fig. 6B). Therefore, human chondrosarcoma cells with a higher tendency to migrate expressed more IL-8 and αvβ3 integrin.

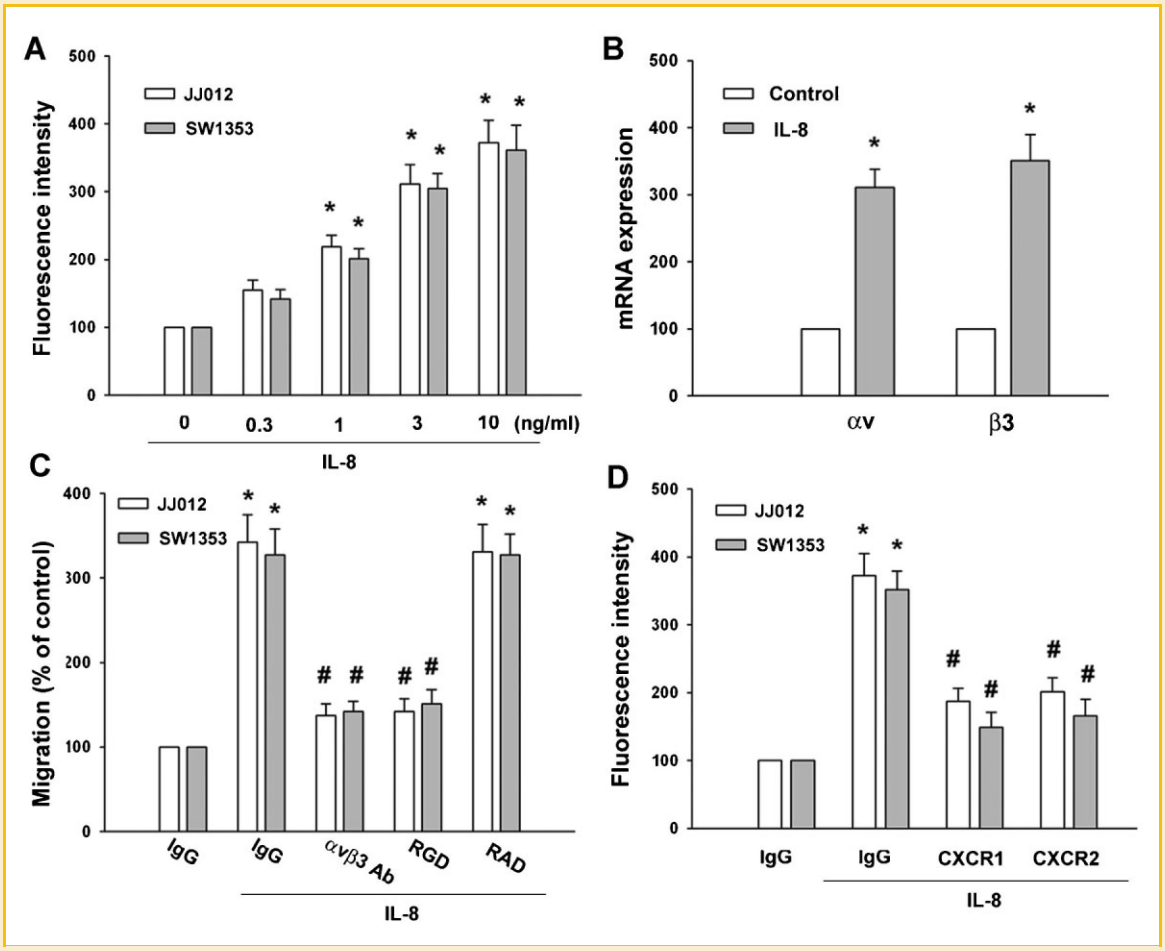

Fig. 2. IL-8-directed migration activity of human chondrosarcoma cells involves up-regulation of αvβ3 integrin. A: Cells were incubated with IL-8, and the cell surface expression of αvβ3 integrin was determined using flow cytometry (n = 5). B: JJ012 cells were incubated with IL-8, and mRNA expression of αv and β3 integrin was determined using qPCR (n = 4). C: Cells were pretreated with αvβ3 monoclonal antibody (10 μg/ml), cyclic RGD (100 nM), or cyclic RAD (100 nM) for 30 min followed by stimulation with IL-8. The in vitro migration activity was measured after 24 h (n = 5). D: Cells were pretreated with CXCR1 or CXCR2 Ab (5 μg/ml) for 30 min followed by stimulation with IL-8 for 24 h, and cell surface αvβ3 integrin was measured using flow cytometry (n = 4). Results are expressed as the mean ± SE. $^{*}P < 0.05$ compared with control; $\#P < 0.05$ compared with IL-8-treated group.

DISCUSSION

IL-8 activates target genes involved in differentiation, survival, apoptosis, and proliferation. It also manipulates the process of tumorigenesis and tumor progression [Xie, 2001; Raman et al., 2007]. However, the effect of IL-8 on migration activity in chondrosarcoma is mostly unknown. Using migration, invasion, and would healing analysis, we found that IL-8 increased cell motility in human chondrosarcoma cells. Moreover, over-expression of IL-8 shRNA inhibited the migratory ability by approximately 73% in JJ012 cells. Our data provided the evidence that the expression of IL-8 is associated with a metastatic phenotype of chondrosarcoma cells. IL-8 exert it effects through interaction with specific IL-8 receptors [Rollins, 1997; Heidemann et al., 2003]. We found that the CXCR1 and CXCR2 Ab reduced IL-8-mediated migration and integrin expression. Therefore, our data suggest a critical role for CXCR1 and CXCR2 receptor in the IL-8-mediated cell migration and integrin expression in human chondrosarcoma cells.

Integrins link the ECM to intracellular cytoskeletal structures and signaling molecules and are implicated in the regulation of a number of cellular processes, including adhesion, signaling, motility, survival, gene expression, growth, and differentiation [Shattil et al., 2010]. Here, we found that IL-8 increased αvβ3 integrin expression by using flow cytometry analysis, which plays an important role during tumor metastasis [Beer and Schwaiger, 2008]. Furthermore, IL-8 also increased the mRNA levels of αv and β3 integrins. In the present study, we used αvβ3 integrin antagonist (cyclic RGD) to determine the role of αvβ3 integrin and found that it inhibited IL-8-induced cancer migration, indicating the possible involvement of αvβ3 integrin in IL-8-induced migration in chondrosarcoma cells. This was further confirmed by the result that the αvβ3 integrin antibody inhibited the enhancement of migration activity by IL-8, indicating the involvement of αvβ3 integrin in IL-8-mediated induction of cancer migration.

A variety of growth factors stimulate cancer metastasis via signal-transduction pathways that converge to activate AP-1

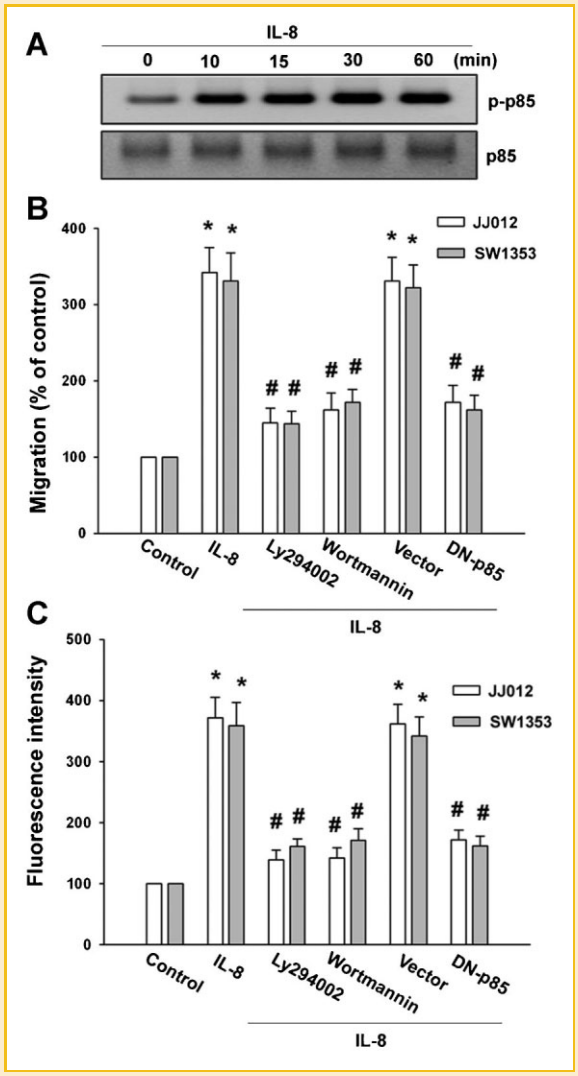

Fig. 3. PI3K is involved in IL-8-induced migration and integrin up-regulation in human chondrosarcoma cells. A: Cells were incubated with IL-8 for indicated time intervals, and p-p85 was examined by Western blot analysis ($n = 4$). B,C: Cells were pretreated for 30 min with Ly294002 (10 μM) and wortmannin (1 μM) or transfected with dominant negative (DN) mutant of p85 for 24 h followed by stimulation with IL-8, and in vitro migration and cell surface αvβ3 integrin was measured by Transwell and flow cytometry ($n = 5$). Results are expressed as the mean ± SE. $^{*}P < 0.05$ compared with control; $\#P < 0.05$ compared with IL-8-treated group.

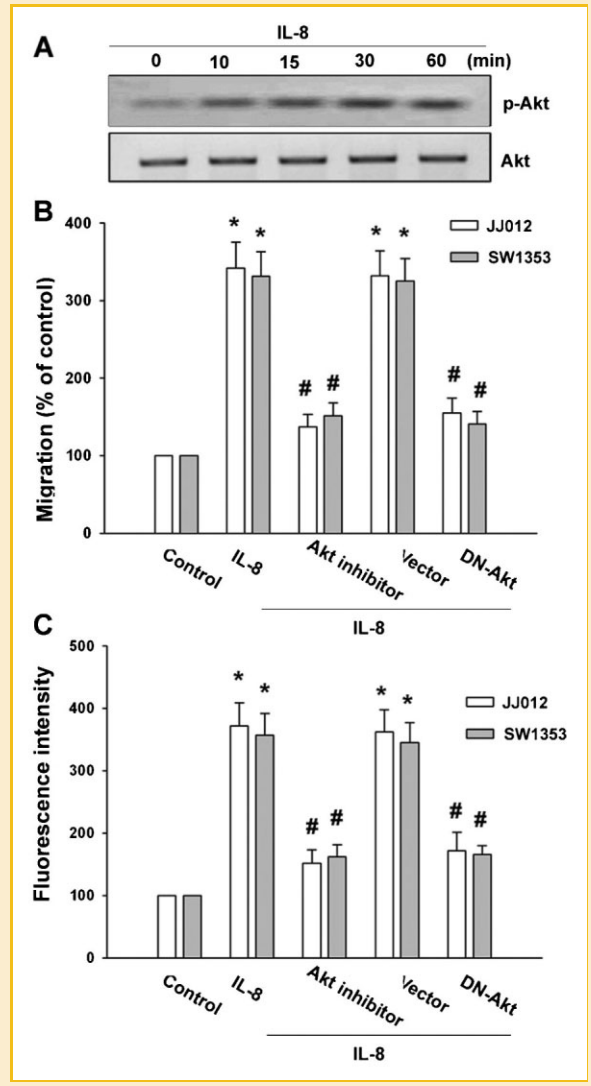

Fig. 4. Akt is involved in IL-8-induced migration and integrin up-regulation in human chondrosarcoma cells. A: Cells were incubated with IL-8 for indicated time intervals, and Akt phophorylation was examined by Western blot analysis ($n = 4$). B,C: Cells were pretreated for 30 min with Akt inhibitor (10 μM) or transfected with dominant negative (DN) mutant of Akt for 24 h followed by stimulation with IL-8, and in vitro migration and cell surface αvβ3 integrin was measured by Transwell and flow cytometry ($n = 5$). Results are expressed as the mean ± SE. $^{*}P < 0.05$ compared with control; $\#P < 0.05$ compared with IL-8-treated group.

complex of transcription factors [Ozanne et al., 2007]. The PI3K/Akt pathway is a major cascade mediating activation of the AP-1 signaling pathway in human cancer cells [Ozanne et al., 2007]. Phosphorylation of the p85 subunit is required for activation of the p110 catalytic subunit of PI3K [Qureshi et al., 2007]. We found IL-8-enhanced the p85 subunit phosphorylation in human chondrosarcoma cells. Pretreatment of cells with PI3K inhibitors Ly294002 or wortmannin antagonized an increase in migration and integrin expression by IL-8 stimulation. This was further confirmed by the result that the dominant-negative mutant of p85 inhibited the enhancement of migration by IL-8. Moreover, we also found that IL-8-activated Akt Ser473 phosphorylation, while Akt inhibitor and Akt mutant inhibited IL-8-mediated cell migration and integrin expression. Our data indicate that PI3K/Akt could play an important role in the expression of integrin and migration of human chondrosarcoma cells. We also examined the interaction of PI3K with CXCR or integrin after IL-8 stimulation. Treatment of cells with IL-8 increased p85 associated with CXCR1 and CXCR2 but not αv and β3 integrin (Supplementary Fig. S1A,B). Therefore, PI3K is associated with CXCR receptor but not integrin after IL-8 stimulation. Transfection of cells with IL-8 siRNA and p85 mutant but not Akt mutant and c-Jun siRNA reduced p85 associated with

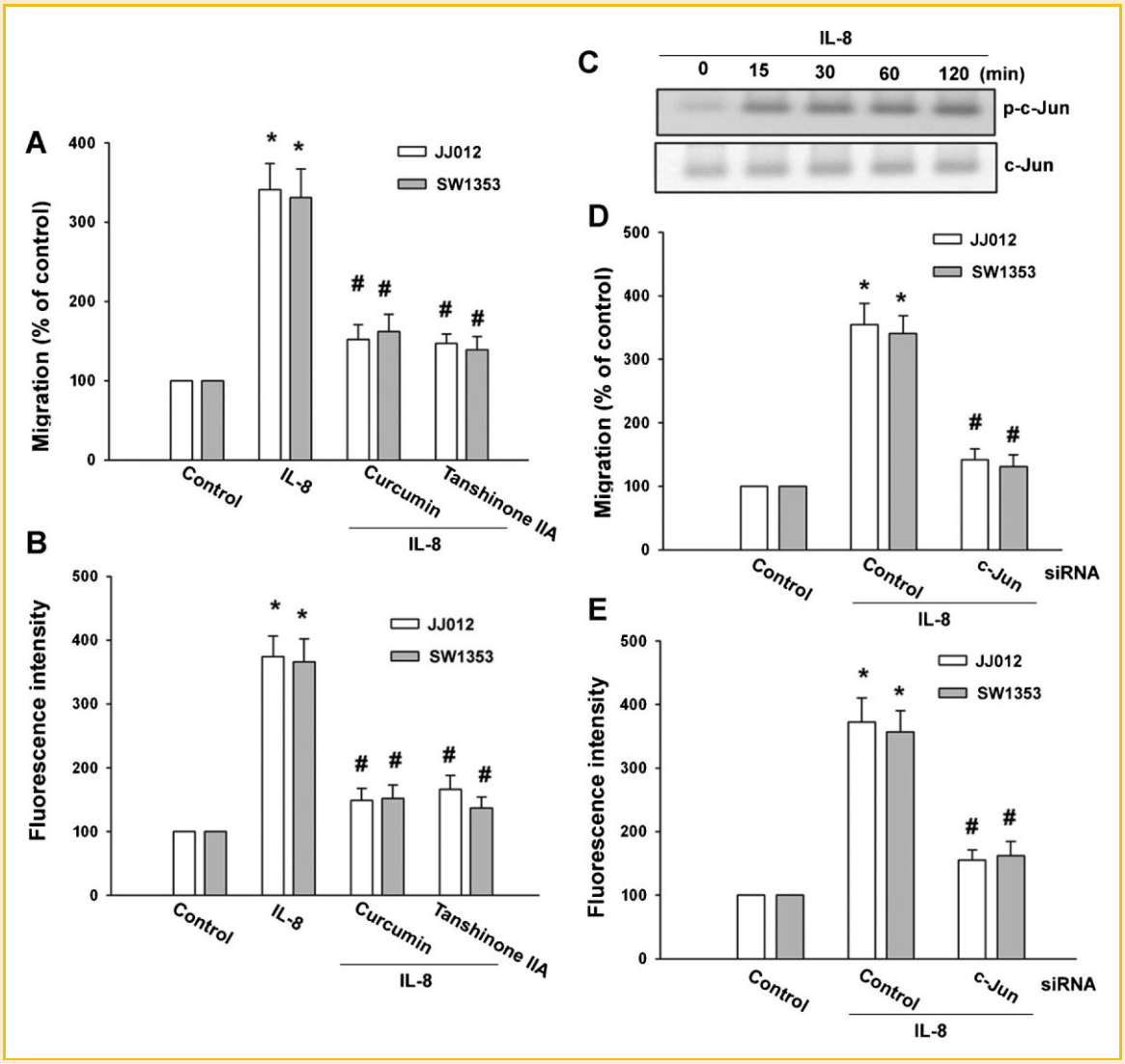

Fig. 5. IL-8 induces cells migration and integrin up-regulation through AP-1. A,B: Cells were pretreated for 30 min with curcumin (10 μM) or tanshinone IIA (10 μM) followed by stimulation with IL-8, and in vitro migration and cell surface αvβ3 integrin was measured by Transwell and flow cytometry (n = 4). C: JJ012 cells were incubated with IL-8 for indicated time intervals, and c-Jun phosphorylation in nucleus was determined by Western blot (n = 4). D,E: Cells were transfected with c-Jun or control siRNA for 24 h followed by stimulation with IL-8, and in vitro migration and cell surface αvβ3 integrin was measured by Transwell and flow cytometry (n = 5). Results are expressed as the mean ± SE. *$P < 0.05$ compared with control; #$P < 0.05$ compared with IL-8-treated group.

CXCR1 and CXCR2 (Supplementary Fig. S1C,D). Therefore, Akt and c-Jun are downstream molecules of PI3K. Studies have shown that CXCR1 and CXCR2 have different internalization properties [Rollins, 1997; Heidemann et al., 2003]. Here, we found that IL-8 increased p85 associated with CXCR1 and CXCR2 (Supplementary Fig. S1A,B). Transfection of cells with CXCR1 and CXCR2 siRNA reduced IL-8-induced cell migration, respectively (Fig. 1D). Therefore, p85 associated with CXCR1 and CXCR2 and formatted a molecule complex after IL-8 stimulation.

Unlike other mesenchymal malignancies, such as osteosarcoma and Ewing's sarcoma, which have seen dramatic increases in long-term survival with the advent of systemic chemotherapy, chondrosarcoma continues to have a poor prognosis due to absence of an effective adjuvant therapy [Fong et al., 2007]. The metastatic potential for conventional chondrosarcomas correlates well with the histologic grade of the tumor. But due to the relatively indolent growth rates of many low- and moderate-grade chondrosarcomas, ~15% of patients who die from metastatic disease do so >5 years after initial diagnosis [Fong et al., 2007]. Therefore, it is important to develop effective adjuvant therapy for preventing chondrosarcoma metastasis. We hypothesized that IL-8 would help to direct the metastasis of chondrosarcoma cells. We found that IL-8 increased the migration of chondrosarcoma cells. One of the mechanisms underlying IL-8-directed migration was up-regulation of αvβ3 integrin and activation of CXCR1/2, PI3K, Akt, and AP-1 pathways (Fig. 6C). Furthermore, the discovery of IL-8-mediated signaling

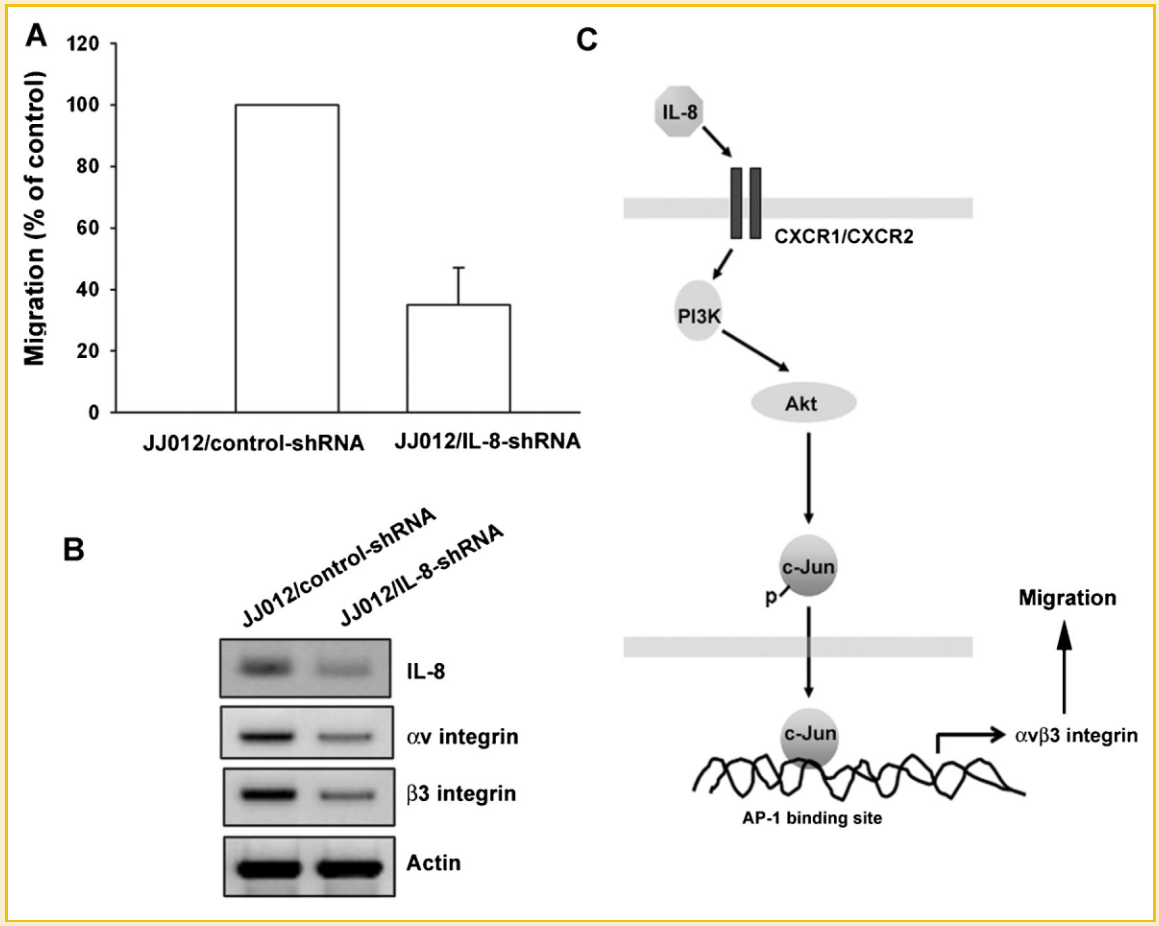

Fig. 6. Knockdown of IL-8 inhibited the migratory ability in chondrosarcoma cells. A: The in vitro migration activity of JJ012, JJ012/control-shRNA, and JJ012/IL-8-shRNA cells was measured with the Transwell ($n = 4$). B,C: The protein levels of IL-8, αv, and β3 integrin in JJ012/control-shRNA and JJ012/IL-8-shRNA cells was examined by Western blot analysis ($n = 4$). Results are expressed as the mean ± SE. Schematic presentation of the signaling pathways involved in IL-8-induced migration and αvβ3 integrin expression of chondrosarcoma. IL-8 and CXCR receptor interaction activates PI3K and Akt pathways, which in turn induces AP-1 activation, which leads to αvβ3 integrin expression and increases the migration of human chondrosarcoma.

pathway helps us understand the mechanism of human chondrosarcoma metastasis. IL-8 inhibitor or gene therapy may lead us to effective therapy in the future.

ACKNOWLEDGMENTS

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