Biochemical and Biophysical Research Communications 382 (2009) 124–128

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

CCL23 up-regulates expression of KDR/Flk-1 and potentiates VEGF-induced proliferation and migration of human endothelial cells

Kyu Yeon Han [1], Chan Woo Kim [1], Tae Hoon Lee, Youngsook Son, Jiyoung Kim *

Graduate School of Biotechnology, College of Life Science, Kyung Hee University, 1 Seocheon-Ri Giheung-Eup, Yongin 446-701, Republic of Korea

ARTICLE INFO

Article history:
Received 3 February 2009
Available online 3 March 2009

Keywords:
CCL23
KDR/Flk-1
VEGF
Endothelial cells
Angiogenesis

ABSTRACT

CCL23 is a CC chemokine and exerts its biological activities on endothelial cells as well as on immune cells through CCR1. We investigated the potential effect of CCL23 on expression of KDR/Flk-1 receptor in endothelial cells. PCR, confocal microscope and Western blot analysis revealed that CCL23 up-regulated KDR/Flk-1 mRNA and protein levels in endothelial cells. A reporter assay indicated that CCL23-induced KDR/Flk-1 expression primarily occurred at the transcriptional level. In addition, CCL23 stimulated phosphorylation of SAPK/JNK, and an inhibitor of SAPK/JNK blocks the CCL23-induced KDR/Flk-1 expression. Furthermore, VEGF-induced ERK phosphorylation was stimulated by CCL23. Finally, CCL23 promoted VEGF-induced endothelial proliferation and migration, which were correlated with the maximal stimulation of KDR/Flk-1 expression by CCL23. Taken together, these findings suggest that CCL23 results in up-regulation of KDR/flk-1 receptor gene transcription and protein expression and that KDR/Flk-1 up-regulation induced by CCL23 may contribute to potentiation of VEGF action in angiogenesis.

Introduction

Angiogenesis occurs in a variety of physiological and pathological processes such as embryonic development, wound healing, and tumor growth. Angiogenic processes involve activation, migration, and proliferation of endothelial cells [1–3]. A number of cytokines and growth factors play roles in the control of angiogenesis [4,5]. Of the many proangiogenic mediators, VEGF is one of the most potent factors [6,7]. VEGF, which is known to induce proliferation, shape change, and migration during angiogenesis, has two known receptors, KDR/Flk-1 and Flt-1. VEGF exerts mitogenic and chemotactic effects in endothelial cells via KDR/Flk-1 but not Flt-1 [8–11]. In addition, up-regulation of VEGF and its receptors has been observed in tumors and in response to various conditions such as hypoxia and wound healing [12]. Furthermore, activation of KDR/Flk-1 stimulates the migration of endothelial cells during embryogenesis and adulthood [13]. Moreover, changes in VEGF receptor expression have been shown to result in altered cell proliferation and survival rates both in vitro and in vivo [14].

A number of chemokines modulate angiogenesis and are known to be involved in tumor growth and metastatic cell invasion [15]. CCL23 (also known as MPIF-1, MIP-3, or Ckb8) is one of CC chemokines that was initially characterized as a chemoattractant for monocytes and dendritic cells [16–18]. In addition, it has recently been reported that CCL23 induces endothelial cell migration, tube formation and promotes angiogenesis via CCR1, which is a seven-transmembrane G-protein-coupled receptor [19,20]. In this study, we investigated the possibility that CCL23 treatment could regulate KDR/Flk-1 receptor expression in endothelial cells. Here we showed new evidence that CCL23 was capable of directly up-regulating KDR/Flk-1 receptor gene transcription and subsequently increasing KDR/Flk-1 mRNA and protein expression. Furthermore, up-regulation of KDR/Flk-1 induced by CCL23 resulted in potentiation of VEGF-induced proliferation and migration in human umbilical vein endothelial cells (HUVECs). Our finding may represent an indirect mechanism for the angiogenic responses of endothelial cells induced by CCL23.

Materials and methods

Cell cuture. Human vascular endothelial cells (HUVECs) were cultured in M199 (Invitrogen, Grand Island, NY) supplemented with 20% fetal bovine serum (Invitrogen, Grand Island, NY), 2 mM glutamine, 100 U/ml penicillin and streptomycin, 5 ng/ml of bFGF and were incubated at 37 °C in humidified atmosphere of

Abbreviations: VEGF, vascular endothelial growth factor; KDR/Flk-1, kinase insert domain-containing receptor/fms-like tyrosine kinase 1; ERK, extracellular signal regulated kinase; MAPK, mitogen activated protein kinase; PMA, phorbol 12-myristate 13-acetate.

* Corresponding author. Fax: +82 31 203 4969.
E-mail address: jkim@khu.ac.kr (J. Kim).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.02.149

5% CO_2. Cells were pretreated with various concentration of CCL23 (R&D Systems, Minneapolis, MN) for 12 h as indicated.

Reverse transcriptase-polymerase chain reaction analysis and real-time PCR. Total RNA was purified from HUVECs using modified Trizol reagent (Invitrogen, Grand Island, NY). Total RNA (2 μg) was reverse transcribed using M-MuLV reverse transcriptase (MBI Fermentas, Amherst, NY) and PCR was performed as previously described using specific primers [21]. Real-time PCRs were performed using the fluorescence detection method using the Light Cycler System with a First-Start DNA Master SYBR Green I kit (Roche Diagnostics, Indianapolis, IN). The cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 amplification cycles of 95 °C for 15 s, 56 °C for 5 s and 72 °C for 15 s. After amplification, the temperature was slowly elevated above the melting temperature of the PCR product to measure the fluorescence and thereby to determine the melting curve. A negative control without cDNA template was performed to assess the overall specificity.

Western blot analysis. Briefly, HUVECs were lysed in RIPA buffer (Amersham Corporation, Uppsala, Sweden) containing proteinase inhibitors. Protein was quantified with a protein assay kit. Equal amounts of protein (20 μg/lane) were resolved with SDS–polyacrylamide gel electrophoresis and transferred to Hybond C nitrocellulose membranes (Amersham Corporation, Uppsala, Sweden). The membranes were probed with rabbit anti-KDR/Flk-1 (Research Santa Cruz, Santa Cruz, CA) and then incubated for 1 h with secondary peroxidase-conjugated antibody. The membranes were developed with an enhanced chemiluminescence system (Amersham Corporation, Uppsala, Sweden) and exposed to X-ray film.

Confocal laser-scanning microscope. HUVECs were fixed with 3.7% paraformaldehyde, washed in PBS, and incubated for overnight with FITC-conjugated polyclonal anti-KDR/Flk-1 antibody. To examine the distribution of KDR/Flk-1, immunostained cells were mounted, cells were observed using a laser-scanning confocal microscope (Carl Zeiss, Germany).

Cell proliferation assay. The assay was conducted by using BrdU and a Detection kit (Roche, Indianapolis, IN). In brief, HUVECs were seeded in 0.1% gelatin-coated 96-well plates. After 12 h of CCL23 (R&D Systems, Minneapolis, MN) treatment, VEGF (10 ng/ml) was added. The BrdU-integrated DNA was quantitated by the relative luminescence unit (RLU) of each well using a Wallace Victor^2 1420 Multilabel counter (Perkin-Elmer, Norwalk, CT).

Cell migration and scratch wound assays. Cell migration assays were performed in 48-well microchemotaxis chambers (Neuro Probe, Inc., Cabin John, MD). The bottom chamber was loaded with 30,000 cells, and polyester membrane was laid over the cells. The microchamber was then inverted and incubated at 37 °C for 2 h. The upper wells were then loaded with M199 containing 0.1% BSA and CCL23. The chamber was reincubated at 37 ºC for 2 h, and the filters were fixed and stained using Diff-Quick (Baxter Healthcare Corporation, McGraw Park, IL). Each condition was studied in triplicate wells, and each experiment was performed three times. Scratch wounds were generated in confluent HUVEC cultures with a sterile 200-μl pipette tip. Cells were treated with CCL23 for 12 h prior to VEGF (10 ng/ml) treatment. After 12 h, HUVECs were stained with Diff-Quick and photographed.

Transfection and luciferase reporter assays. Transient transfections were carried out by Lipofectamine method as described by manufacturer (Invitrogen, Grand Island, NY). Reporter containing KDR/Flk-1 promoter plasmids were transient transfected to HUVECs and treated with various concentrations of CCL23 for 36 h, harvested and lysed in Report lysis buffer (Promega). Luciferase activities were measured by the method using enhanced luciferase assay kit (Promega). Experiments were repeated at least three times with duplicate samples, and values are expressed as means ± standard deviations (SD).

Statistical analysis. Unless otherwise stated, all experiments were performed with triplicate sample and repeated at least three times. The data are presented as means ± SD and statistical comparisons between groups were performed using one-way ANOVA followed by Student's *t*-test.

Results and discussions

CCL23 induces KDR/Flk-1 mRNA and protein expression in HUVECs

RT-PCR revealed that treatment of HUVECs with CCL23 resulted in induction of KDR/Flk-1 mRNA expression at a maximal level of approximately 10 ng/ml (Fig. 1A). It has been reported that CCL23 displayed its biological activities via binding to CCR1 [16–19,22] and human endothelial cells expressed CCR1 [23]. Treatment of HUVECs with anti-CCR1 effectively reduced CCL23-induced up-regulation of KDR/Flk-1 mRNA expression, indicating that CCL23 may up-regulate KDR/Flk-1 via CCR1.

Quantitative real-time PCR analysis demonstrated that the KDR/Flk-1 mRNA levels increased by approximately 2-fold following CCL23 treatment (Fig. 1B). Furthermore, KDR/Flk-1 promoter/Luc reporter assays demonstrated that KDR/Flk-1 treatment specifically increased the KDR/Flk-1 promoter activity by approximately 2-fold, which was similar to the increase in mRNA levels that was observed (Fig. 1C). Taken together, these results indicate that

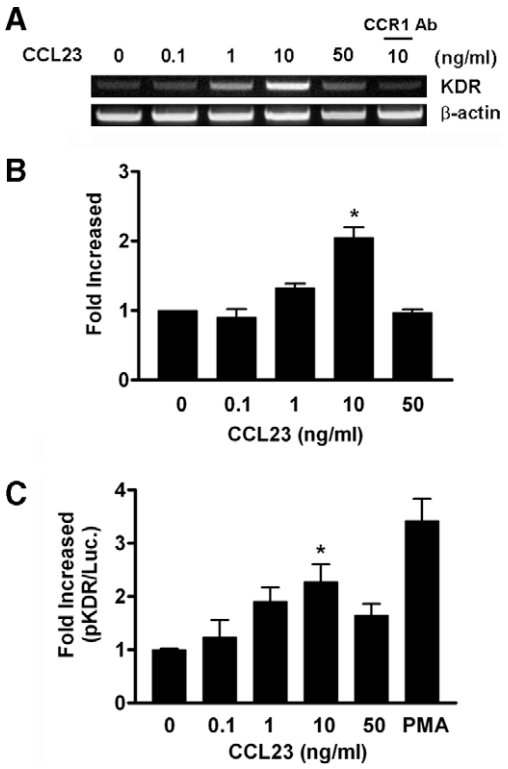

Fig. 1. CCL23 up-regulates mRNA expression of KDR/Flk-1 in HUVECs. (A) HUVECs were incubated for 12 h with the indicated concentrations of CCL23. RT-PCR was performed as described in Materials and methods. CCL23 increased expression of KDR/Flk-1 mRNA dose-dependently. CCL23 treated with neutralizing antibody against CCR1 significantly reduced mRNA expression of KDR/Flk-1 compared to that of CCL23 alone. (B) Real-time quantitative RT-PCR of KDR/Flk-1. The levels of KDR/Flk-1 transcripts were measured in the presence of the primers and SYBR Green I dye in real-time quantitative PCR. (C) HUVECs were transiently transfected with 0.1 μg of KDR/Flk-1 promoter/Luc constructs and 0.1 μg of the control pGL3-basic plasmid and incubated with various concentration of CCL23 for 36 h. CCL23 increased the promoter activity of the KDR/Flk-1 promoter/Luc fusion gene. $^*P < 0.01$ versus negative control. The data are mean values ± SD from quadruplicates and are representative of at least three experiments.

the up-regulation of KDR/Flk-1 mRNA expression was largely accounted for by an increase in KDR/Flk-1 gene transcription.

Next, we measured the protein levels by Western blot analysis and confirmed that CCL23 induced an increase in the levels of KDR/Flk-1 protein (Fig. 2A). Blockage of CCL23 with a neutralizing anti-CCL23 antibody or heat-inactivated CCL23 abolished the CCL23-induced KDR/Flk-1 up-regulation (Fig. 2B). These results demonstrate that CCR1 is indeed functional receptors important for up-regulation of KDR/Flk-1 expression induced by CCL23 in endothelial cells. In addition, confocal microscopic analysis revealed the presence of KDR/Flk-1 proteins in immunofluorescent-stained cells. As expected, the fluorescence intensity of KDR/Flk-1 expression was higher in CCL23-treated cells than in control cells (Fig. 2C). These results demonstrate that functional CCL23 is required to induce the up-regulation of KDR/Flk-1.

CCL23 up-regulates KDR expression in endothelial cells via the SAPK/JNK signal pathway

CCL23 specifically binds to CCR1 on the surface of monocytes and dendritic cells [17]. CCR1 has been detected in endothelial cells [23], and its signaling pathways are known to consist of seven-transmembrane G-protein-coupled receptors that are sensitive to inhibition by pertussis toxin. However, these pathways have not yet been elucidated in endothelial cells, although the transduction of CCR1 signals is known to occur through the ERK MAP kinase pathway in HOS cells that overexpress CCR1 [24]. Therefore, we evaluated CCL23 to determine if it activated the ERK, SAPK/JNK, and p38 MAP kinase pathways. To accomplish this, subconfluent HUVECs were treated with various concentrations of CCL23 for 30 min and subsequently analyzed for the phosphorylation status of the three MAP kinases by immunodetection using specific anti-phospho ERK, SAPK/JNK, and p38 antibodies. As shown in Fig. 3A, CCL23 significantly stimulated the phosphorylation of SAPK/JNK, with the peak effects being observed in response to treatment with 10 ng/ml; however, it caused little or no activation of the ERK1/2 and p38 MAP kinases. These results suggest that CCL23 induces KDR/Flk-1 expression via the SAPK/JNK pathway.

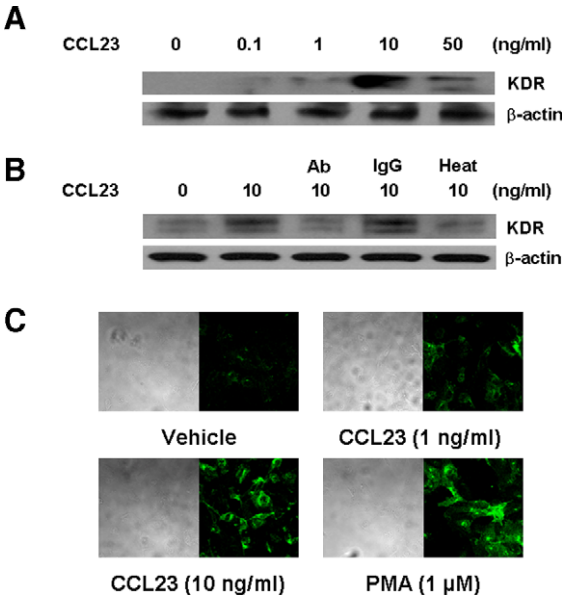

Fig. 2. CCL23 up-regulates protein expression of KDR/Flk-1 in HUVECs. (A) HUVECs were treated with various concentration of CCL23 for 12 h. Western blot analysis showed that CCL23 significantly increased KDR/Flk-1 protein levels in HUVECs. (B) HUVECs were incubated with CCL23 in the presence of polyclonal anti-CCL23 antibody (Ab) or with heat-inactivated CCL23 (Heat). Western blot analysis was carried out as described in Materials and methods. A control isotype-matched IgG1 was used as a control. (C) KDR/Flk-1 protein levels were increased in HUVECs treated with CCL23 in immunofluorescent staining of cells with FITC–anti-KDR/Flk-1 antibodies. The photo figures in the left panels are photomicrographs and those in the right are immunofluorescence-stained cells. Magnification: 400×.

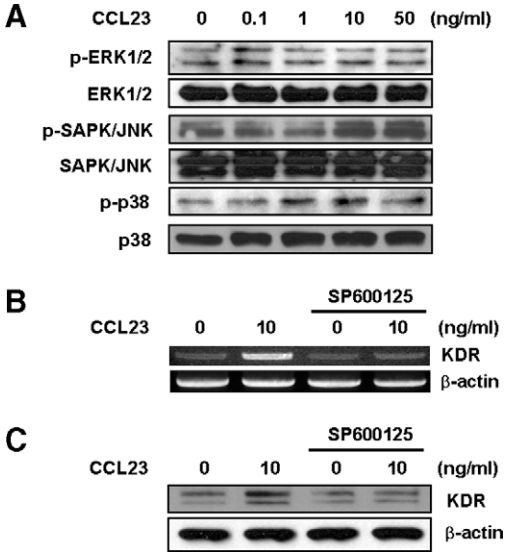

Fig. 3. CCL23 stimulates phosphorylation of SAPK/JNK and inhibition of SAPK/JNK blocks CCL-stimulated KDR/Flk-1 up-regulation. (A) Western blot of phosphorylated MAPK in cultured HUVECs in presence of CCL23. Cells were incubated with various concentration of CCL23 (0.1, 1, 10, 50 ng/ml) for 30 min, and phosphorylated ERK1/2, p38 MAPK and SAPK/JNK were determined using specific antibodies. CCL23 stimulated phosphorylation of SAPK/JNK significantly but affect little phosphorylation of ERK1/2 and p38. (B, C) HUVECs were cultured in medium with CCL23 in the presence or absence of SAPK/JNK inhibitor, SP600125 at 10 μM. RT-PCR analysis (B) and Western blot analysis (C) showed that co-treatment of HUVECs with CCL23 and SP600125 significantly inhibited CCL23-induced KDR/Flk-1 mRNA and protein expression.

Inhibition of the signaling molecules was used to further elucidate the mechanism responsible for KDR/Flk-1 up-regulation. When cells were co-incubated with SP600125, a specific inhibitor of SAPK/JNK kinase, during CCL23 exposure, inhibition of the CCL23-induced KDR/Flk-1 expression was observed at the mRNA and protein levels (Fig. 3B and C). These results demonstrate that activation of SAPK/JNK is necessary for CCL23-induced KDR/Flk-1 up-regulation.

CCL23 potentiates the VEGF-induced proliferation and migration of endothelial cells

Proliferation and migration of endothelial cells can be stimulated by a variety of growth factors, including VEGF [6,25]. We found that treatment with CCL23 alone did not induce a significant increase in endothelial cell growth. However, when HUVECs were pretreated with CCL23 for 12 h and subsequently exposed to VEGF, CCL23 enhanced the stimulation of cell proliferation that was induced by VEGF (Fig. 4A). Specifically, CCL23 enhanced VEGF-induced cell proliferation significantly when compared to treatment with VEGF alone. In addition, these findings were correlated with the CCL23-induced increase in KDR/Flk-1 mRNA and protein levels.

VEGF is known to induce ERK via a PKC-dependent pathway, and this pathway is essential for VEGF stimulation of endothelial cell proliferation [26]. In this study, we demonstrated that VEGF-induced ERK1/2 phosphorylation increased markedly when cells were pretreated with CCL23 (Fig. 4B). These results suggest that the cell proliferation induced by VEGF and CCL23 was associated with sensitization of the MAPK pathway in HUVECs.

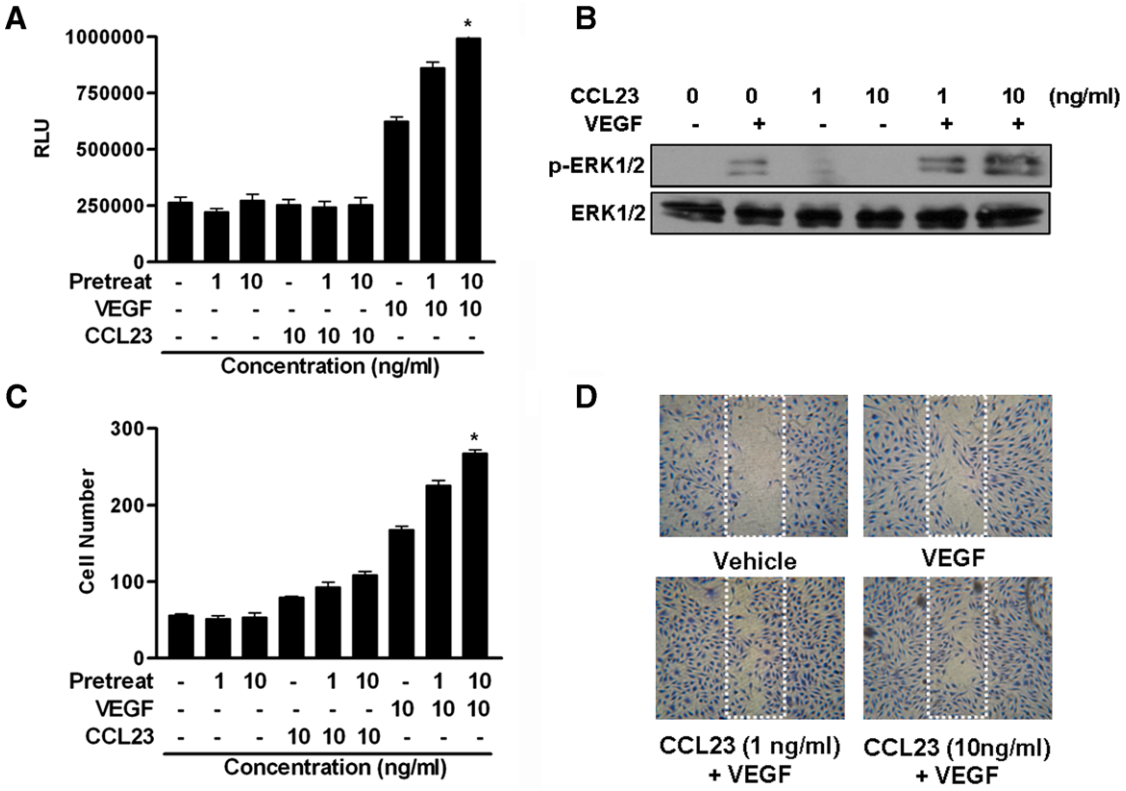

Fig. 4. CCL23 stimulates VEGF-induced proliferation and migration of endothelial cells. (A) HUVECs were treated with CCL23 at the indicated concentrations for 12 h and then treated with or without VEGF or CCL23 (10 ng/ml) as indicated. Proliferation of HUVECs pretreated with CCL23 at the indicated concentrations was compared with that observed in the presence of VEGF alone. (B) Pretreatment of HUVECs with CCL23 increased the VEGF-induced phosphorylation of ERK1/2 in a dose-dependent manner. (C) HUVECs were treated as described in (A). Human CCL23 significantly stimulated the VEGF-mediated migration of HUVECs over the presence of VEGF alone. (D) HUVECs were pretreated with CCL23 at the indicated concentrations for 12 h and then treated VEGF (10 ng/ml). CCL23 enhanced VEGF-induced endothelial cell migration in scratch wound assay. Magnification: 40×. $^{*}P < 0.01$ versus VEGF alone. The data are mean values ± SD from quadruplicates and are representative of at least three experiments. RLU: relative luminescence unit.

We previously reported that CCL23 induces endothelial cell migration only slightly [19]. Therefore, we evaluated CCL23 to determine if it potentiates VEGF-induced cell migration to a greater degree than VEGF alone. Using a Boyden Chamber assay, we found that pretreatment with CCL23 followed by VEGF induced a greater degree of migration among HUVECs than treatment with either CCL23 or VEGF alone (Fig. 4C). This increase in VEGF-mediated endothelial cell migration by CCL23 was also demonstrated by scratch wound assays (Fig. 4D). The maximum stimulatory activity of CCL23 was observed at a concentration of approximately l0 ng/ml. Taken together, these results suggest that potentiation of VEGF-induced cell migration by CCL23 coincided with an increase in KDR/Flk-1 protein levels.

In this study, we demonstrated that CCL23 up-regulated KDR/Flk-1 receptor expression in endothelial cells and potentiated VEGF-induced endothelial cell proliferation and migration, which are important steps in the angiogenic processes. Regulation of KDR/Flk-1 expression can occur at various levels of expression. Therefore, we used RT-PCR to monitor the effect of VEGF on KDR/Flk-1 gene expression and real-time PCR analysis to quantify the observed changes in gene expression. The results of these analyses demonstrated that treatment of HUVECs with CCL23 resulted in an approximately 2-fold increase in KDR/Flk-1 mRNA. Furthermore, the results of a luciferase reporter assay demonstrated that CCL23 treatment induced an approximately 2-fold increase in KDR/Flk-1 promoter activity, which indicates that the up-regulation of KDR/Flk-1 occurred at the transcriptional level.

The signaling pathway that involves the CCL23 interaction with CCR1, a seven-transmembrane G-coupled receptor, is not well understood in endothelial cells. In this study, we demonstrated that the SAPK/JNK MAP kinase pathway is activated after treatment with CCL23, which indicates that CCL23 transduces its signal through the SAPK/JNK MAP kinase pathway in endothelial cells. Furthermore, SP600125, a potent SAPK/JNK kinase inhibitor, completely blocked the CCL23-induced KDR/Flk-1 up-regulation. These findings indicate that activation of SAPK/JNK kinase is involved in the signaling cascade that leads to KDR/Flk-1 up-regulation by CCL23.

Angiogenesis is regulated by a variety of growth factors and cytokines, including VEGF, FGF, and chemokines such as IL-8 and CCL23 [4,15]. Angiogenic factors including bFGF and VEGF have also been reported to increase KDR/Flk-1 receptor expression [27–30]. However, the mechanism responsible for the up-regulation of KDR/Flk-1 expression by these other growth factors is not well understood. Based on the results of this study, CCL23 up-regulates the expression of KDR/Flk-1. This up-regulation, in turn, renders the cells more sensitive to the action of VEGF, as demonstrated by a significant potentiation of the VEGF-induced proliferation and migration of HUVECs. Most importantly, the increased activity of the KDR/Flk-1 is directly linked to angiogenesis and tumor metastasis. Taken together, these data strongly support the notion that up-regulation of KDR/Flk-1 expression, and consequently the sensitization of endothelial cells to VEGF are at least partially responsible for CCL23-mediated angiogenesis. These findings can be used for future fine tuning of VEGF-based therapies that exert their effects through modulation of KDR/Flk-1 expression.

Acknowledgment

This research was supported by the Kyung Hee University Research Fund in 2003 (KHU-20031128) and by the Korea Research Foundation Grant funded by the Korean Government.

References

[1] J. Folkman, Angiogenesis in cancer, vascular, rheumatoid and other disease, Nat. Med. 1 (1995) 27–31.
[2] G. Breier, A. Damert, K.H. Plate, W. Risau, Angiogenesis in embryos and ischemic diseases, Thromb. Haemost. 78 (1997) 678–683.
[3] J.M. Isner, T. Asahara, Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization, J. Clin. Invest. 103 (1999) 1231–1236.
[4] R. Benelli, G. Lorusso, A. Albini, D.M. Noonan, Cytokines and chemokines as regulators of angiogenesis in health and disease, Curr. Pharm. Des. 12 (2006) 3101–3115.
[5] M. Nomi, H. Miyake, Y. Sugita, M. Fujisawa, S. Soker, Role of growth factors and endothelial cells in therapeutic angiogenesis and tissue engineering, Curr. Stem Cell Res. Ther. 1 (2006) 333–343.
[6] D.W. Leung, G. Cachianes, W.J. Kuang, D.V. Goeddel, N. Ferrara, Vascular endothelial growth factor is a secreted angiogenic mitogen, Science 246 (1989) 1306–1309.
[7] H.F. Dvorak, L.F. Brown, M. Detmar, A.M. Dvorak, Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis, Am. J. Pathol. 146 (1995) 1029–1039.
[8] A.A. Parikh, L.M. Ellis, The vascular endothelial growth factor family and its receptors, Hematol. Oncol. Clin. North. Am. 18 (2004) 951–971.
[9] M. Shibuya, S. Yamaguchi, A. Yamane, T. Ikeda, A. Tojo, H. Matsushime, M. Sato, Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family, Oncogene 5 (1990) 519–524.
[10] B.I. Terman, M.E. Carrion, E. Kovacs, B.A. Rasmussen, R.L. Eddy, T.B. Shows, Identification of a new endothelial cell growth factor receptor tyrosine kinase, Oncogene 6 (1991) 1677–1683.
[11] J. Waltenberger, L. Claesson-Welsh, A. Siegbahn, M. Shibuya, C.H. Heldin, Different signal transduction properties of KDR and Flt-1, two receptors for vascular endothelial growth factor, J. Biol. Chem. 269 (1994) 26988–26995.
[12] N. Ferrara, T. Davis-Smyth, The biology of vascular endothelial growth factor, Endocr. Rev. 18 (1997) 4–25.
[13] S. Hiratsukam, Y. Kataoka, K. Nakao, K. Nakamura, S. Morikawa, S. Tanaka, M. Katsuki, Y. Maru, M. Shibuya, Vascular endothelial growth factor A (VEGF-A) is involved in guidance of VEGF receptor-positive cells to the anterior portion of early embryos, Mol. Cell. Biol. 25 (2005) 355–363.
[14] N.E. Tsopanoglou, M.E. Maragoudakis, On the mechanism of thrombin-induced angiogenesis. Potentiation of vascular endothelial growth factor activity on endothelial cells by up-regulation of its receptors, J. Biol. Chem. 274 (1999) 23969–23976.
[15] M.M. Rowenkilde, T.W. Schwartz, The chemokine system—a major regulator of angiogenesis in health and disease, APMIS 112 (2004) 481–495.
[16] U. Forssmann, M.B. Delgado, M. Uguccioni, P. Loetscher, G. Garotta, M. Baggiolini, CKbeta8, a novel CC chemokine that predominantly acts on monocytes, FEBS Lett. 408 (1997) 211–216.
[17] B. Nardelli, D.K. Morahan, G.W. Bong, M.A. Semenuk, B.L. Kreider, G. Garotta, Dendritic cells and MPIF-1: chemotactic activity and inhibition of endogenous chemokine production by IFN-gamma and CD40 ligation, J. Leukoc. Biol. 65 (1999) 822–828.
[18] B.S. Youn, S.M. Zhang, H.E. Broxmeyer, S. Cooper, K. Antol, M. Fraser Jr., B.S. Kwon, Characterization of CKbeta8 and CKbeta8-1: two alternatively spliced forms of human beta-chemokine, chemoattractants for neutrophils, monocytes, and lymphocytes, and potent agonists at CC chemokine receptor 1, Blood 91 (1998) 3118–3126.
[19] J. Hwang, K.N. Son, C.W. Kim, J. Ko, D.S. Na, B.S. Kwon, Y.S. Gho, J. Kim, Human CC chemokine CCL23, a ligand for CCR1, induces endothelial cell migration and promotes angiogenesis, Cytokine 30 (2005) 254–263.
[20] K.N. Son, J. Hwang, B.S. Kwon, J. Kim, Human CC chemokine CCL23 enhances expression of matrix metalloproteinase-2 and invasion of vascular endothelial cells, Biochem. Biophys. Res. Commun. 340 (2006) 498–504.
[21] C.W. Kim, K.N. Son, S.Y. Choi, J. Kim, Human lactoferrin upregulates expression of KDR/Flk-1 and stimulates VEGF-A-mediated endothelial cell proliferation and migration, FEBS Lett. 580 (2006) 4332–4336.
[22] T.A. Berkhout, J. Gohil, P. Gonzalez, C.L. Niclos, K.E. Moores, C.H. Macphee, J.R. White, P.H. Groot, Selective binding of the truncated form of the chemokine CKbeta8 (25–99) to CC chemokine receptor 1 (CCR1), Biochem. Pharmacol. 59 (2000) 591–596.
[23] R. Salcedo, H.A. Young, M.L. Ponce, J.M. Ward, H.K. Kleinman, Eotaxin (CCL11) induces in vivo angiogenic responses by human CCR3$^+$ endothelial cells, J. Immunol. 166 (2001) 7571–7578.
[24] I.S. Kim, Y.S. Ryang, Y.S. Kim, S.W. Jang, H.J. Sung, Y.H. Lee, J. Kim, D.S. Na, J. Ko, Leukotactin-1-induced ERK activation is mediated via Gi/Go protein/PLC/PKC delta/Ras cascades in HOS cells, Life Sci. 73 (2003) 447–459.
[25] B. Millauer, S. Wizigmann-Voos, H. Schnürch, R. Martinez, N.P. Møller, W. Risau, A. Ullrich, High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis, Cell 72 (1993) 835–846.
[26] C. Wong, Z.G. Jin, Protein kinase C-dependent protein kinase D activation modulates ERK signal pathway and endothelial cell proliferation by vascular endothelial growth factor, J. Biol. Chem. 280 (2005) 33262–33269.
[27] M.A. Hervé, H. Buteau-Lozano, S. Mourah, F. Calvo, M. Perrot-Applanat, VEGF189 stimulates endothelial cells proliferation and migration in vitro and up-regulates the expression of Flk-1/KDR mRNA, Exp. Cell Res. 309 (2005) 24–31.
[28] M.L. Petreaca, M. Yao, Y. Liu, K. Defea, M. Martins-Green, Transactivation of vascular endothelial growth factor receptor-2 by interleukin-8 (IL-8/CXCL8) is required for IL-8/CXCL8-induced endothelial permeability, Mol. Biol. Cell 18 (2007) 5014–5023.
[29] B.Q. Shen, D.Y. Lee, H.P. Gerber, B.A. Keyt, N. Ferrara, T.F. Zioncheck, Homologous up-regulation of KDR/Flk-1 receptor expression by vascular endothelial growth factor in vitro, J. Biol. Chem. 273 (1998) 29979–29985.
[30] M.S. Pepper, S.J. Mandriota, Regulation of vascular endothelial growth factor receptor-2 (Flk-1) expression in vascular endothelial cells, Exp. Cell Res. 241 (1998) 414–425.