Stromal cell-derived factor 1 α (SDF-1 α) induces gene-expression of early growth response-1 (Egr-1) and VEGF in human arterial endothelial cells and enhances VEGF induced cell proliferation

Thomas Neuhaus*, Sebastian Stier*, Gudrun Totzke†,
Elisabeth Gruenewald*, Stefan Fronhoffs*, Agapios Sachinidis‡,
Hans Vetter* and Yon D. Ko*

*Medizinische Universitäts-Poliklinik Bonn, Bonn, Germany †Institut für Molekulare Medizin, University of Düsseldorf, Düsseldorf, Germany and ‡Center of Physiology and Pathophysiology, Köln, Germany

Received 31 October 2002; revision 10 March 2003; accepted 11 March 2003

Abstract. Stromal cell-derived factor-1 (SDF-1), mainly known as a chemotactic factor for haematopoietic progenitor cells, also provides angiogenic potency. Since the intracellular signalling of SDF-1-induced neovascularization remains unclear, we studied in human umbilical arterial endothelial cells (HUAEC) the influence of SDF-1 α on induction of the genes of early growth response-1 (Egr-1) and VEGF, as well as the activation of extracellular regulated kinases (ERK) 1/2, which are all known to be involved in endothelial cell proliferation. We found a time-dependent induction of Egr-1 and VEGF mRNA expression and phosphorylation of ERK1/2 by SDF-1 α . Furthermore, we demonstrated that Egr-1 expression is dependent on ERK 1/2 activation. Finally, we tried to confirm the relevance of the induced gene expression by detecting the [3H]thymidine incorporation as a marker for cell proliferation in HUAEC after stimulation with SDF-1 α alone or together with VEGF. This particular test showed, that SDF-1 α alone has no effect, but is able to significantly enhance VEGF induced DNA synthesis. In summary, SDF-1 α is involved in different steps of endothelial cell proliferation, but, since Egr-1 and VEGF offer different functions, it may also play a so far undefined role on other conditions of the endothelium.

INTRODUCTION

Stromal cell-derived factor-1 (SDF-1) is a CXC-chemokine and is a ligand for the receptor CXCR-4 (Loetscher *et al.* 1994; Bleul *et al.* 1996). Two isoforms are described: SDF-1 α and SDF-1 β . Both are encoded by one gene and develop from different splicing (Shirozu *et al.* 1995). SDF-1 was first characterized as a pre-B cell growth-stimulating factor in mouse bone

marrow, but as a chemotactic factor for CD34+ human progenitor cells (Aiuti *et al.* 1997; Naiyer *et al.* 1999) it is also involved in the homing of haematopoietic stem cells (Peled *et al.* 2000).

While the interaction of SDF-1 with different haematopoietic cells is well characterized, only very little is known about the influence of SDF-1 on endothelial cells (EC), though they also express the CXCR-4 receptor (Volin *et al.* 1998) and show the haematopoietic surface marker CD34. SDF-1 α leads to neovascularization *in vivo* (Salcedo *et al.* 1999; Mirshahi *et al.* 2000) and the CXCR-4 receptor is essential for vascularization of the gastrointestinal tract (Tachibana *et al.* 1998); however, the intracellular pathways of SDF in EC, which are related to angiogenesis, are not well defined.

Thus, we focused in our study on genes or proteins, which are known to be coupled with endothelial cell proliferation. Regarding the signalling cascade, this is true for the extracellular signal-regulated kinases (ERK)1/2 (Yu & Sato 1999) and, looking downstream, for the transcription-factor early growth response 1 (Egr-1) (Biesiada *et al.* 1996; Hofer *et al.* 1996). Egr-1 expression is associated with endothelial injury (Khachigian *et al.* 1997; Silverman & Collins 1999) and can be activated by growth factors like basic fibroblast growth factor (bFGF) (Ko *et al.* 1995). In addition, Egr-1 up-regulates the expression of platelet-derived growth factor (PDGF), the VEGF-receptor Flt-1 (Khachigian *et al.* 1996; Vidal *et al.* 2000) and it triggers the expression of VEGF itself (Yan *et al.* 2000).

Since VEGF is the key growth factor inducing neovascularization (Neufeld *et al.* 1999), we examined a possible linkage between SDF-1 α and VEGF. Recently a SDF-1 α induced VEGF expression and production was described in microvascular endothelial cells (Mirshahi *et al.* 2000), but this group failed to demonstrate a proliferative potency of SDF-1 α in those cells. We repeated these experiments in HUAEC and furthermore analysed the influence of SDF-1 α in combination with VEGF on cell proliferation, using the [3H]thymidine incorporation.

MATERIALS AND METHODS

Materials

SDF-1 α was obtained from R & D systems (Minneapolis, MN). Fetal calf serum (FCS) and Dispace II was obtained from Roche Diagnostics (Mannheim, Germany). Collagenase I and TRI-reagent were obtained from Sigma Chemical (Deisenhofen, Germany). Moloney murine leukaemia virus (MMLV) reverse transcriptase was obtained from Life Technologies (Karlsruhe, Germany) and Taq polymerase, random primers and dNTP's were obtained from Perkin Elmer (Weiterstadt, Germany). RNA-Guard and all chemicals for oligonucleotide synthesis were obtained from Pharmacia (Freiburg, Germany). ERK1/2 antibodies, p38, the secondary antibody and the p38 positive control were obtained from New England BioLabs (Beverly, MA). The ECL Western blotting detection system was obtained from Amersham (Little Chalfont, UK).

Culture and stimulation of human umbilical arterial endothelial cells (HUAEC)

Endothelial cells were isolated from human umbilical cord arteries, cultured on human fibronectin-coated culture dishes in Medium 199 supplemented with 20% v/v fetal calf serum, 10 μ g/mL heparin and 30 μ g/mL crude endothelial cell growth factor, and characterized as described in (Ko *et al.* 1995). Only umbilical cords from subjects who had a normal pregnancy and birth were used. For gene-expression studies, confluent HUAEC of the third or fourth passage were washed twice with PBS and then (for the purpose of starving) exposed for 4 h to Medium 199

without serum or growth factor substitution. For Western-blotting, EC of the third passage were transferred on 6-well-plates, grown until confluence and then exposed to starvation-medium for 4 h. The cells were then stimulated with *SDF-1* α for various time intervals.

RNA-Extraction

Total RNA was extracted from cells with TRI reagent according to the manufacturer's protocol. RNA quantification was performed by spectrophotometer. The integrity of RNA was analysed by electrophoresis of 4 μ g of total RNA on a 1% (w/v) agarose gel stained with ethidium bromide.

Primers

In the case of *Egr-1*, the following primers were used: 5'-primer: 5'-cagcagtcctccattactcag-3', 3'-primer: 5'-gactgtagctggtattg-3'. The resulting PCR product had a length of 345 bp. For *VEGF*, the primers were: 5'-primer: gcagaatcatcacgaagtgg-3', 3'-primer: 5'-gcaacgcgagctctgtgttttg-3', resulting in a PCR product with a length of 414 bp. For both primers, Southern blot analysis as a control of specificity and the determination of the exponential phase of the reverse transcriptase/PCR (RT/PCR) were performed as previously described (Ko *et al.* 1995; Ko *et al.* 1999). For standardization, each RT/PCR experiment was followed by a PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5'-primer, 5'-gccaaaagggtcatcatctc-3'; 3'-primer, 5'-gtagaggcaggatgatgttc-3'), a constitutively expressed gene in HUAEC.

Reverse transcription/polymerase chain reaction (RT/PCR)

A 4- μ g portion of total RNA was incubated with moloney murine leukaemia virus (MMLV) reverse transcriptase (200 U/ μ L) for 5 min at 25 °C, 5 min at 30 °C, 90 min at 37 °C and 5 min at 95 °C in a total reaction volume of 40 μ L containing 1 $\times$ RT-buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂), 10 mM dithiothreitol, 0.5 mM of each dNTP, 50 U of RNA-guard and 100 pmol of random hexamer primer. A 4- μ L aliquot of the RT sample was used for PCR-reaction, which contained (in a final volume of 50 μ L) 1 $\times$ PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ and 0.001% gelatin), 0.2 mM concentration of each dNTP, 20 pmol of each primer and 2.5 units of Taq polymerase. The samples were covered with 40 μ L of mineral oil and PCR cycles were then performed in an automated DNA thermal cycler (model 480: Perkin Elmer, Weiterstadt, Germany) with the following temperature profile: denaturation at 94 °C for 1 min, primer annealing at 60 °C for 1 min, and primer extension at 72 °C for 1.5 min. After an initial denaturation step (94 °C for 5 min), the cycle was repeated 33 times using the *VEGF* primer, 27 times using the *Egr-1* primer and 25 times for the GAPDH primer. The last cycle was followed by a final extension step of 20 min at 72 °C. Portions of 20 μ L of each sample were electrophoresed on a 1.5% (w/v) agarose gel and stained with ethidium bromide. Each PCR experiment was performed with a negative control (lacking MMLV reverse transcriptase) to detect DNA contamination. In order to assess the product size, ϕ X-174-RF DNA *Hae*III digest was used as a marker.

Western-Blotting

Cells were lysed with SDS sample buffer containing 20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 50 mM NaF, 10 mM EDTA, 20 mM napyrophosphate, 1 mM sodium orthovanadate, and 1% Triton X-100. Aliquots were used for protein determinations using the Bio-Rad protein assay as previously described (Bradford 1976). Then, 0.1% bromphenol blue (w/v) was added to the aliquots. Thirty μ g of protein were analysed by SDS-PAGE in a 10% acrylamide gel with a thickness of 0.75 mm using the Mini Gel Protein system (Bio-Rad). Proteins were transferred overnight to a cellulose nitrate membrane (Schleicher and Schuell, Dassel, Germany) by 100 mA with a

buffer containing 25 mM Tris-base, 192 mM glycine and 20% methanol, pH 8.3. The protein transfer was checked using Ponceau S. The membrane was then washed three times with 50 mM Tris-Cl and 150 mM NaCl, pH 7.5. Saturation was performed with 50 mM Tris-Cl, 150 mM NaCl, pH 7.5, containing 2% BSA (w/w), and 0.1% Tween 20. The sheets were incubated overnight at 8 °C with the various antibodies. Following six rinses with washing buffer containing 50 mM Tris-Cl, 150 mM NaCl, pH 7.5 and 0.1% Tween 20, sheets were incubated for 1 h at room temperature in saturation buffer containing the antirabbit secondary antibody. Again, the sheets were washed six times. For detection, the ECL Western blotting detection system by Amersham was used.

Determination of the DNA Synthesis in HUAEC

The effect of VEGF and/or SDF-1 α on [3H]thymidine incorporation into cell DNA was assessed as performed previously (Sachinidis *et al.* 1995). HUAEC were seeded in 24-well culture plates and grown to 70% confluence. The medium was then replaced by serum-free Medium 199. Cultures were then exposed to 50 ng/mL VEGF and/or SDF-1 α 1, 10, 50 and 100 ng/mL for 20 h before 3 μ Ci/mL [3H]thymidine were added to the serum-free medium. Four hours later, experiments were terminated by aspirating the medium and subjecting the cultures to sequential washes with PBS containing 1 mM CaCl₂, 1 mM MgCl₂, 10% trichloroacetic acid, and ethanol:ether (2 : 1, v/v). Acid-insoluble [3H]thymidine was extracted into 250 μ L/dish 0.5 M NaOH, and 100 μ L of this solution were mixed with 5 mL scintillant (Ultimagold; Packard, Meriden, CT) and quantified using a liquid scintillation counter, model Beckman LS 3801 (Düsseldorf, Germany). Fifty microliters of the residual solution were prepared for the determination of protein using the Bio-Rad protein assay as described previously (Bradford 1976).

Densitometric analysis

Densitometric analysis was performed on a two-dimensional scanning densitometer (BIOMETRA, Göttingen, Germany) using the 'ScanPack'-software version 14.1 A 27. The ethidium bromide stained agarose gels were photographed. The densitometric results of gene-expression were standardized to that of GAPDH expression from the same reverse-transcribed mRNA sample.

Statistical analysis

Data were presented as mean $\pm$ standard deviation. Statistical differences were determined using analysis of variance with repeated measures. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Induction of Egr-1 and VEGF expression

After 4 h in starvation, EC were stimulated with 50 ng/mL of SDF-1 α for different time intervals. SDF-1 α induced a time-dependent increase in Egr-1 mRNA levels (Fig. 1). Egr-1 mRNA expression was highest at 60 min and returned to basal levels 2 h after stimulation. In contrast to Egr-1, VEGF mRNA was found to be expressed in unstimulated EC. However, stimulation of EC with SDF-1 α , 50 ng/mL, resulted in an increase of VEGF mRNA above baseline levels (Fig. 2). Interestingly, the time course of SDF-1 α induced VEGF mRNA expression was different from Egr-1 mRNA expression. The VEGF signal intensity peaked at 4 h after stimulation and returned to control levels 8 h after cell stimulation.

Figure 1. (a) and (b): Effects of *SDF-1* α (50 ng/mL) on the expression of *Egr-1* mRNA in endothelial cells. Cells were serum-deprived for 4 h prior to stimulation with *SDF-1* α for the indicated time-intervals. Expression of *Egr-1* and *GAPDH* mRNA was analysed by RT-PCR. Analysis of *GAPDH* mRNA expression was performed as a control for the same amount of RNA. One representative experiment out of $n = 3$ with similar results is shown (a). The densitometric results of these experiments are shown in parallel (b).

Phosphorylation of ERK1/2

After 6 h in starvation, EC were stimulated with 50 ng/mL of *SDF-1* α for different time intervals. The primary antibodies used recognized p38^{mapk}, ERK-1 and ERK-2 only when they were catalytically activated by phosphorylation. There was a significant base-line phosphorylation of ERK-1 and ERK-2. However, stimulation with *SDF-1* α , 50 ng/mL, resulted in a time-dependent increase of ERK1/2 phosphorylation with a peak at 10 min. In contrast, a phosphorylation of p38^{mapk} by *SDF-1* α , 50 ng/mL, could not be detected (Fig. 3).

Figure 2. a and b: Effects of SDF-1α (50 ng/mL) on the expression of VEGF mRNA in endothelial cells. The procedures were similar to Figure 1.

Effects of ERK1/2 or p38^{mapk} Inhibition on Egr-1 mRNA expression

For inhibition experiments, EC were set on starvation medium for 3 h. They were then incubated with PD098059, which specifically inhibits phosphorylation of ERK1/2, or SB203580, which has been shown to inhibit phosphorylation p38^{mapk}, for 1 h in different concentrations. This was followed by a stimulation with SDF-1α, 50 ng/mL for 60 min for Egr-1 mRNA expression.

As shown in Fig. 4, preincubation with PD098059 resulted in a concentration-dependent decrease of Egr-1 mRNA expression. In contrast, SB203580 failed to suppress Egr-1 mRNA expression, demonstrating an ERK1/2 mediated expression of Egr-1 by SDF-1α in EC.

Figure 3. Effects of *SDF-1α* (50 ng/mL) on the phosphorylation of ERK1/2 and MAP kinase p38 in endothelial cells. Cells were serum-deprived for 4 h prior to stimulation with *SDF-1α* for the indicated time-intervals. Lysates were prepared and subjected to gel electrophoresis. Western blotting was performed with antibodies that just recognize phosphorylated ERK1/2 and p38. In an experiment regarding phosphorylation of p38, a positive control was added. One representative experiment out of $n = 3$ with similar results is shown.

Determination of the DNA Synthesis in HUAEC

As demonstrated in Fig. 5, stimulation of HUAEC with 50 ng/mL VEGF caused an increase of [3H]thymidine incorporation from 51 ± 8 to 183 ± 45 cpm/ μ g protein. In opposite, *SDF-1α* did not influence the [3H]thymidine incorporation in neither concentration, but it significantly enhanced the VEGF induced DNA synthesis, leading to a [3H]thymidine incorporation of 265 ± 55 cpm/ μ g protein in case of a combination of VEGF with *SDF-1α* 100 ng/mL.

DISCUSSION

This study is the first to demonstrate a stimulation of *Egr-1* mRNA expression in human endothelial cells by *SDF-1α*. The presented kinetics of induced *Egr-1* or VEGF mRNA expression are comparable with known data in human EC (Choi *et al.* 1994; Ko *et al.* 1995; Ko *et al.* 1999), which showed a maximal expression of *Egr-1* within 30–60 min after stimulation and of VEGF within 4 h and a return to basal levels after 2 h for *Egr-1* and 8 h for VEGF.

Furthermore, we demonstrated a time-dependent phosphorylation of the extracellular signal-regulated kinases (ERK)1/2. This is in accordance with previous studies that observed an ERK 1/2 phosphorylation after stimulation with *SDF-1α* in haematopoietic cells (Ganju *et al.* 1998; Popik *et al.* 1998) and in EC (Molino *et al.* 2000). The kinetics described in these studies also show a peak expression of phosphorylated ERK1/2 at about 5–10 min after stimulation and a return to basal levels within 15–30 min. The comparatively strong signal of phosphorylated ERK1/2 we found at the base-line was probably due to the long period EC were kept in starvation medium, which is a highly nonphysiological procedure.

Figure 4. a and b: Effects of inhibition of ERK1/2 and MAP kinase p38 on mRNA expression of Egr-1 1 h after stimulation with SDF-1 α (50 ng/mL). Cells were serum-deprived for 4 h prior to stimulation and preincubated with PD098059 and SB203580 with the indicated concentrations. Expression of Egr-1 and GAPDH mRNA, was analysed by RT-PCR. Analysis of GAPDH mRNA expression was performed as a control for the same amount of RNA. One representative experiment out of $n = 3$ with similar results is shown (a). The densitometric results of these experiments are shown in parallel (b).

In contrast to ERK1/2, we did not observe a phosphorylation of MAP kinase p38 in EC after stimulation with SDF-1 α . The data concerning a SDF-induced phosphorylation of p38 are contradictory. While some studies described such a phosphorylation in T-cells or neuronal cells (Kaul & Lipton 1999; Misse *et al.* 2001), others did not (Ganju *et al.* 1998; Bajetto *et al.* 2001).

It is widely accepted that phosphorylation of MAP kinases like ERK1/2, correlates with their activation (Seger & Krebs 1995; Bokemeyer *et al.* 1996). Thus, ERK1/2 should be involved in the intracellular signalling in EC after stimulation with SDF-1 α and mediate the expression of certain genes. In fact, we found a reduction of Egr-1 mRNA expression dependent upon the

Figure 5. Endothelial cell proliferation in the presence of VEGF alone and with SDF-1 α in different concentrations. HUAEC were seeded in 24-well culture plates and grown to 70% confluence. The medium was then replaced by serum-free Medium 199. Cultures were then exposed to 50 ng/mL VEGF and/or SDF-1 α 1, 10, 50 and 100 ng/mL for 20 h before [3H]thymidine were added to the serum-free medium. Four hours later, experiments were terminated and 100 μ L of this solution were mixed with 5 mL scintillant and quantified using a liquid scintillation counter. Fifty microliters of the residual solution were prepared for the determination of protein using the Bio-Rad protein assay. The results are shown as mean $\pm$ SD of 4 different experiments. * P < 0.05 compared with VEGF alone.

concentration of the ERK1/2 inhibitor PD098059, while an inhibition of p38 MAP kinase with SB203580 had no effect on the amount of *Egr-1* mRNA expression. The dependency of *Egr-1* mRNA expression on activation of ERK1/2 was previously described in different kinds of cells like monocytes or EC (Schwachtgen *et al.* 1998; Guha *et al.* 2001; Morimoto *et al.* 2001), but a p38 mediated expression of *Egr-1* in Jurkat T-cells was also reported (Rolli *et al.* 1999).

Since *Egr-1* and VEGF evoke a wide variety of responses, the results presented here indicate that SDF-1 α may participate in different physiological and pathophysiological processes of endothelial cells. For example, *Egr-1* mRNA expression can be enhanced by certain growth factors like bFGF or VEGF (Ko *et al.* 1995; Liu, Tsai & Aird 2000) and, in turn, *Egr-1* itself results in an expression of growth factors like VEGF, PDGF or transforming growth factors (Khachigian & Collins 1997; Houston *et al.* 2001), but also alters transcription of genes like adhesion molecules, cytokines and plasminogen activator (Silvermann & Collino 1999). Thus, *Egr-1* is considered to be involved in angiogenesis and inflammation or in the pathogenesis of vascular diseases like arteriosclerosis (McCaffrey, Fu & Du 2000). A participation in angiogenesis also seems to be true for ERK1/2 (Redlitz *et al.* 1999; Yu & Sato 1999).

VEGF mainly acts via its receptors KDR and Flt-1. While KDR seems to be the receptor which is responsible for the angiogenetic potency of VEGF, Flt-1, in addition to other functions, mediates VEGF-induced migration in human monocytes and macrophages (Barleon *et al.* 1996; Hiratsuka *et al.* 1998; Neufeld *et al.* 1999; Hiratsuka *et al.* 2001). At that time the significance of the SDF induced expression of *Egr-1* and VEGF as well as its function in endothelial cells remains unclear, but further studies focusing on this point seem to be reasonable.

Because of the induction of VEGF mRNA expression by SDF-1 α we analysed the influence of this chemokine on proliferation of HUAEC. Using the method of [3H]thymidine incorporation as a marker of cell proliferation we found, that SDF-1 α alone did not affect the DNA synthesis rate, but it significantly enhanced the [3H]thymidine incorporation by VEGF.

SDF-1 α is known to influence the proliferation of certain cells, but while the proliferation in astrocytes is due to SDF alone (Bajetto *et al.* 2001), in other cells like granule precursor cells or haematopoietic progenitor cells it just, comparable to our data, enhances the proliferative effects of cytokines or Sonic hedgehog (Lataillade *et al.* 2000; Klein *et al.* 2001). As mentioned initially, the only study examining the influence of SDF-1 α on proliferation in endothelial cells by using a cell counter failed to demonstrate an effect (Mirshahi *et al.* 2000), but this group did not use a combination of SDF-1 α with a growth factor.

The mentioned enhancement of VEGF induced proliferation in EC by proteins with no or only less own effect was already described for, e.g. angiopoietins and insulin-like growth factor (Huang *et al.* 1999; Castellon *et al.* 2002), for SDF-1 α this synergism has not been shown. It has to be elucidated if the proliferative potency of SDF is just caused by the induction of VEGF expression or if it is based on another pathway. However, since SDF-1 α fails to offer a proliferative effect in the absence of exogenous VEGF, the signification of the induced VEGF expression concerning the mitogenic potency of SDF-1 α seems to be rather low.

In conclusion, we were able to show, that SDF-1 α offers different effects in human endothelial cells and that it seems to be involved in certain physiological and pathophysiological conditions like, e.g. cell proliferation, thus playing a so far underestimated role in vascular biology.

ACKNOWLEDGEMENTS

We thank Professor Werner Stolpe and his staff from the Johanniter-Krankenhaus, Bonn, Germany, for continuously providing umbilical cords. We thank Dr Artur-Aron Weber from the Institut für Pharmakologie, Düsseldorf, for his review of the paper and helpful comments. We thank Dipl.-Stat. C. Nicolay from the Institut für Medizinische Biometrie for her advice in statistics. This work was supported by a grant from BONFOR, Bonn, Germany (110/12).

REFERENCES

- AIUTI A, WEBB IJ, BLEUL C, SPRINGER T, GUTIERREZ-RAMOS JC (1997) The chemokine SDF-1 is a chemoattractant for human CD34⁺ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34⁺ progenitors to peripheral blood. *J. Exp. Med.* **185**, 111.
- BAJETTO A, BARBERO S, BONAVIA R, PICCIOLI P, COSTA A, FLORIO T, SCHETTINI G (2001) Stromal cell derived factor-1 α induces astrocyte proliferation through the activation of extracellular signal-regulated kinases 1/2 pathway. *J. Neurochem.* **77**, 1226.
- BARLEON B, SOZZANI S, ZHOU D, WEICH HA, MANTOVANI A, MARME D (1996) Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. *Blood* **87**, 3336.
- BIESIADA E, RAZANDI M, LEVIN ER (1996) Egr-1 activates basic fibroblast growth factor transcription. Mechanistic implications for astrocyte proliferation. *J. Biol. Chem.* **271**, 18576.
- BLEUL CC, FARZAN M, CHOE H, PAROLIN C, CLARK-LEWIS I, SODROSKI J, SPRINGER TA (1996) The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **382**, 829.
- BOKEMEYER D, SOROKIN A, DUNN MJ (1996) Multiple intracellular MAP kinase signaling cascades. *Kidney Int.* **49**, 1187.
- BRADFORD MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **7**, 248.
- CASTELLON R., HAMDI HK, SACERIO I, AOKI AM, KENNEY MC, LJUBIMOV AV (2002) Effects of angiogenic growth factor combinations on retinal endothelial cells. *Exp Eye Res.* **74**, 523.

- CHOI AM, TUCKER RW, CARLSON SG, WEIGAND G, HOLBROOK NJ (1994) Calcium mediates expression of stress-response genes in prostaglandin A₂-induced growth arrest. *FASEB J.* **8**, 1048.
- GANJU RK, BRUBAKER SA, MEYER J, DUTT P, YANG Y, QIN S, NEWMAN W, GROOPMAN JE (1998) The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. *J. Biol. Chem.* **273**, 23169.
- GUHA M, O'CONNELL MA, PAWLINSKI R, HOLLIS A, MCGOVERN P, YAN SF, STERN D, MACKMAN N (2001) Lipopolysaccharide activation of the MEK-ERK1/2 pathway in human monocytic cells mediates tissue factor and tumor necrosis factor alpha expression by inducing Elk-1 phosphorylation and *Egr-1* expression. *Blood* **98**, 1429.
- HIRATSUKA S, MARU Y, OKADA A, SEIKI M, NODA T, SHIBUYA M (2001) Vascular endothelial growth factor (VEGF) and its receptors. *Cancer Res.* **61**, 1207.
- HIRATSUKA S, MINOWA O, KUNO J, NODA T, SHIBUYA M (1998) Flt-1 lacking the tyrosine kinase domain is sufficient for the normal development and angiogenesis in mice. *Proc. Natl. Acad. Sci. (USA)* **95**, 9349.
- HOFER G, GRIMMER C, SUKHATME VP, STERZEL RB, RUPPRECHT HD (1996) Transcription factor *Egr-1* regulates glomerular mesangial cell proliferation. *J. Biol. Chem.* **271**, 28306.
- HOUSTON P, CAMPBELL CJ, SVAREN J, MILBRANDT J, BRADDOCK M (2001) The transcriptional corepressor NAB2 blocks *Egr-1*-mediated growth factor activation and angiogenesis. *Biochem. Biophys. Res. Commun.* **283**, 480.
- HUANG XL, TAKAKURA N, SUDA T (1999) In vitro effects of angiopoietins and VEGF on hematopoietic and endothelial cells. *Biochem. Biophys. Res. Commun.* **264**, 133.
- KAUL M, LIPTON SA (1999) Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc. Natl. Acad. Sci. (USA)* **96**, 8212.
- KHACHIGIAN LM, ANDERSON KR, HALNON NJ, GIMBRONE MA JR, RESNICK N, COLLINS T (1997) *Egr-1* is activated in endothelial cells exposed to fluid shear stress and interacts with a novel shear-stress-response element in the PDGF A-chain promoter. *Arterioscler. Thromb. Vasc. Biol.* **17**, 2280.
- KHACHIGIAN LM, COLLINS T (1997) Inducible expression of *Egr-1*-dependent genes. A paradigm of transcriptional activation in vascular endothelium. *Circ. Res.* **81**, 457.
- KHACHIGIAN LM, LINDNER V, WILLIAMS AJ, COLLINS T (1996) *Egr-1*-induced endothelial gene expression: a common theme in vascular injury. *Science* **271**, 1427.
- KLEIN RS, RUBIN JB, GIBSON HD, DEHAAN EN, ALVAREZ-HERNANDEZ X, SEGAL RA, LUSTER AD (2001) SDF-1 alpha induces chemotaxis and enhances Sonic hedgehog-induced proliferation of cerebellar granule cells. *Development* **128**, 1971.
- KO Y, TOTZKE G, GOUNI-BERTHOLD I, SACHINIDIS A, VETTER H (1999) Cytokine-inducible growth factor gene expression in human umbilical endothelial cells. *Mol. Cell. Probes.* **13**, 203.
- KO Y, TOTZKE G, SCHIERMEYER B, ZEITLER H, SCHMITZ U, VETTER H, SACHINIDIS A (1995) Reverse transcriptase-polymerase chain reaction (RT-PCR): a sensitive method to examine basic fibroblast growth factor-induced expression of the early growth response gene-1 (*egr-1*) in human umbilical arterial endothelial cells. *Mol. Cell. Probes.* **9**, 215.
- LATAILLADE JJ, CLAY D, DUPUY C, RIGAL S, JASMIN C, BOURIN P, LE BOUSSE-KERDILES MC (2000) Chemokine SDF-1 enhances circulating CD34(+) cell proliferation in synergy with cytokines: possible role in progenitor survival. *Blood* **95**, 756.
- LIU L, TSAI JC, AIRD WC (2000) *Egr-1* gene is induced by the systemic administration of the vascular endothelial growth factor and the epidermal growth factor. *Blood* **96**, 1772.
- LOETSCHER M, GEISER T, O'REILLY T, ZWAHLEN R, BAGGIOLINI M, MOSER B (1994) Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. *J. Biol. Chem.* **269**, 232.
- MCCAFFREY TA, FU C, DU B (2000) High-level expression of *Egr-1* and *Egr-1*-inducible genes in mouse and human atherosclerosis. *J. Clin. Invest.* **105**, 653.
- MIRSHAHI F, POURTAU J, LI H, MURAIN M, TROCHON V, LEGRAND E, VANNIER J, SORIA J, VASSE M, SORIA C (2000) SDF-1 activity on microvascular endothelial cells: Consequences on angiogenesis in in vitro and in vivo models. *Thromb. Res.* **99**, 587.
- MISSE D, ESTEVE PO, RENNEBOOG B, VIDAL M, CERUTTI M, ST PIERRE Y, YSSEL H, PARMENTIER M, VEAS F (2001) HIV-1 glycoprotein 120 induces the MMP-9 cytopathogenic factor production that is abolished by inhibition of the p38 mitogen-activated protein kinase signaling pathway. *Blood* **98**, 541.
- MOLINO M, WOOLKALIS MJ, PREVOST N, PRATICO D, BARNATHAN ES, TARABOLETTI G, HAGGARTY BS, HESSELGESSER J, HORUK R, HOXIE JA, BRASS LF (2000) CXCR4 on human endothelial cells can serve as both a mediator of biological responses and as a receptor for HIV-2. *Biochim. Biophys. Acta* **1500**, 227.
- MORIMOTO M, KUME N, MIYAMOTO S, UENO Y, KATAOKA H, MINAMI M, HAYASHIDA K, HASHIMOTO N, KITA T (2001) Lysophosphatidylcholine induces early growth response factor-1 expression and activates the core promoter of PDGF-A chain in vascular endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **21**, 771.

- NAIYER AJ, JO DY, AHN J, MOHLE R, PEICHEV M, LAM G, SILVERSTEIN RL, MOORE MA, RAFII S (1999) Stromal derived factor-1-induced chemokinesis of cord blood CD34(+) cells (long-term culture-initiating cells) through endothelial cells is mediated by E-selectin. *Blood* **94**, 4011.
- NEUFELD G, COHEN T, GENGRI NOVITCH S, POLTORAK Z (1999) Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* **13**, 9.
- PELED A, KOLLET O, PONOMARYOV T, PETIT I, FRANTITZ S, GRABOVSKY V, SLAV MM, NAGLER A, LIDER O, ALON R, ZIPORI D, LAPIDOT T (2000) The chemokine SDF-1 activates the integrins LFA-1, VLA-4 and VLA-5 on immature human CD34(+) cells: Role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood* **95**, 3289.
- POPIK W, HESSELGESSER JE, PITHA PM (1998) Binding of human immunodeficiency virus type 1 to CD4 and CXCR4 receptors differentially regulates expression of inflammatory genes and activates the MEK/ERK signaling pathway. *J. Virol.* **72**, 6406.
- REDLITZ A, DAUM G, SAGE EH (1999) Angiostatin diminishes activation of the mitogen-activated protein kinases ERK-1 and ERK-2 in human dermal microvascular endothelial cells. *J. Vasc. Res.* **36**, 28.
- ROLLI M, KOTLYAROV A, SAKAMOTO KM, GAESTEL M, NEININGER A (1999) Stress-induced stimulation of early growth response gene-1 by p38/stress activated protein kinase 2 is mediated by a cAMP-responsive promoter element in a MAPKAP kinase 2-independent manner. *J. Biol. Chem.* **274**, 19559.
- SACHINIDIS A, FLESCH M, KO Y, SCHRÖR K, BÖHM M, DÜSING R, VETTER H (1995) Thromboxane A2 and vascular smooth muscle cell proliferation. *Hypertension* **26**, 771.
- SALCEDO R, WASSERMAN K, YOUNG HA, GRIMM MC, HOWARD OM, ANVER MR, KLEINMAN HK, MURPHY WJ, OPPENHEIM JJ (1999) Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1alpha. *Am. J. Pathol.* **154**, 1125.
- SCHWACHTGEN JL, HOUSTON P, CAMPBELL C, SUKHATME V, BRADDOCK M (1998) Fluid shear stress activation of egr-1 transcription in cultured human endothelial and epithelial cells is mediated via the extracellular signal-related kinase 1/2 mitogen-activated protein kinase pathway. *J. Clin. Invest.* **101**, 2540.
- SEGER R, KREBS EG (1995) The MAPK signaling cascade. *FASEB J.* **9**, 726.
- SHIROZU M, NAKANO T, INAZAWA J, TASHIRO K, TADA H, SHINOHARA T, HONJO T (1995) Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics* **28**, 495.
- SILVERMAN ES, COLLINS T (1999) Pathways of Egr-1-mediated gene transcription in vascular biology. *Am. J. Pathol.* **154**, 665.
- TACHIBANA K, HIROTA S, IIZASA H, YOSHIDA H, KAWABATA K, KATAOKA Y, KITAMURA Y, MATSUSHIMA K, YOSHIDA N, NISHIKAWA S, KISHIMOTO T, NAGASAWA T (1998) The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* **393**, 591.
- VIDAL F, ARAGONES J, ALFRANCA A, DE LANDAZURI MO (2000) Up-regulation of vascular endothelial growth factor receptor Flt-1 after endothelial denudation: role of transcription factor Egr-1. *Blood* **95**, 3387.
- VOLIN MV, JOSEPH L, SHOCKLEY MS, DAVIES PF (1998) Chemokine receptor CXCR4 expression in endothelium. *Biochem. Biophys. Res. Commun.* **242**, 46.
- YAN SF, FUJITA T, LU J, OKADA K, SHANZOU Y, MACKMAN N, PINSKY DJ, STERN DM (2000) Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress. *Nat. Med.* **6**, 1355.
- YU Y, SATO JD (1999) MAP kinases, phosphatidylinositol 3-kinase, and p70, S6 kinase mediate the mitogenic response of human endothelial cells to vascular endothelial growth factor. *J. Cell. Physiol.* **178**, 235.