Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1623 (2003) 41–46
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Homocysteine induces vascular endothelial growth factor expression in differentiated THP-1 macrophages

Makiko Maeda, Isamu Yamamoto, Yasushi Fujio, Junichi Azuma*

Department of Clinical Evaluation of Medicines and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

Received 1 April 2003; received in revised form 29 July 2003; accepted 30 July 2003

Abstract

Hyperhomocysteinemia has been reported to be an independent risk factor for atherosclerosis and atherothrombosis. However, the molecular mechanism by which hyperhomocysteinemia can lead to atherosclerosis and atherothrombosis has not been completely described. Vascular endothelial growth factor (VEGF) has been proposed to play an important role in the progression of atherosclerosis. In the present study, we hypothesized that hyperhomocysteinemia might be associated with VEGF expression in atherosclerotic lesions. We investigated VEGF mRNA expression and VEGF secretion by homocysteine (Hcy) in differentiated THP-1 macrophages. As a result, it has been revealed that VEGF mRNA was upregulated by Hcy in a dose- and time-dependent manner in THP-1 macrophages with the increase in VEGF secretion. Importantly, other sulfur compounds, such as methionine and cysteine, showed no effect on VEGF expression, indicating that homocysteine specifically induced VEGF. Our findings suggest that hyperhomocysteinemia could promote the development of atherosclerotic lesions through VEGF induction in macrophages.

Keywords: Homocysteine; Vascular endothelial growth factor; THP-1; mRNA; Macrophage

1. Introduction

Hyperhomocysteinemia, elevated plasma homocysteine, was initially thought to be associated with advanced atherosclerosis in patients with homozygous defects in the enzymes responsible for homocysteine (Hcy) metabolism, such as cystathionine β-synthase [1,2]. The clinical observations from a patient not only with genetic defects, but also with abnormal cobalamine metabolism, a nongenetic factor, revealed that hyperhomocysteinemia is associated with vascular disease [3]. So far, accumulating evidence has confirmed that hyperhomocysteinemia can cause atherosclerotic vascular disease as an independent risk factor for atherosclerosis and atherothrombosis [4–6]. Hyperhomocysteinemia is associated with vascular endothelial cell injury [7], proliferation of vascular smooth muscle cells [8] and activation of the coagulation cascade [9]. Ross [10] proposed that dysfunction of endothelial cells, with subsequent infiltration of circulating monocytes/lymphocytes, is the primary event for atherogenesis. Thus, endothelial cell injury by hyperhomocysteinemia and consequent cellular responses to this injury can lead to the formation of atherosclerotic and atherothrombotic lesions.

Vascular endothelial growth factor (VEGF) has been known to induce migration and proliferation of endothelial cells, enhance vascular permeability, stimulate angiogenesis [11] and modulate thrombogenicity [12]. It has been demonstrated that VEGF activates monocytes and promotes their migration [13,14]. Importantly, VEGF was remarkably expressed in activated macrophages, endothelial cells, and smooth muscle cells in human coronary atherosclerotic lesions, but not in normal artery [15]. Moreover, Celletti et al. [16] proposed that VEGF caused an increase in atherosclerotic plaque size as well as the number of vascular cells associated with plaque. These data strongly suggest that VEGF plays a role in the chemotaxis of monocytes/macrophages in the process of inflammatory reactions of atherosclerosis.

In these contexts, we hypothesized that hyperhomocysteinemia might be associated with expression of VEGF in the accumulated macrophages of inflammatory lesions where atherogenesis occurs, resulting in the promotion of

* Corresponding author. Tel.: +81-6-6879-8258; fax: +81-6-6879-8259.
E-mail address: azuma@phs.osaka-u.ac.jp (J. Azuma).

0304-4165/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0304-4165(03)00161-2

atherosclerosis and thrombosis. In the present study, to reveal the involvement of Hcy in vascular inflammation, we investigated the effects of Hcy on VEGF expression in macrophages.

2. Materials and methods

2.1. Cell culture

THP-1 cells, a human monocytic cell line, were provided from RIKEN CELL BANK (Cell No. RCB1189). Cells were cultured in RPMI 1640 medium (ICN Biomedicals, Inc. Aurora, OH 44202) containing 10% fetal bovine serum (ICN Biomedicals, Inc.). With HPLC method, we could not detect Hcy in the culture medium. THP-1 cells were differentiated into macrophages with phorbol 12-myristate 13-acetate (PMA). Briefly, equal quantities of cells (approximately 1×10^6 cells/ml) were suspended into 6- or 12-well dishes. After suspension, cells were treated with 50 nM concentration of PMA (Sigma-Aldrich Japan K.K., Tokyo, Japan) at 37 °C for 48 h.

2.2. Purification of total RNA from THP-1 cells

Differentiated THP-1 cells were stimulated with the indicated concentrations of D,L-homocysteine (Sigma-Aldrich), D,L-homocystine (Wako, Japan), L-methionine (Nacalai Tesque, Japan), L-cysteine (Katayama Chemical, Japan) for indicated time. After treatments, total RNA was purified by the acid guanidium thiocyanate–phenol–chloroform (AGPC) method.

2.3. Determination of VEGF mRNA level by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

One-step RT-PCR assays for VEGF and β-actin, an internal control, mRNA were performed using the PLATINUM® Quantitative RT-PCR THERMOSCRIPT™ One-Step System (Invitrogen™, Life Technologies) according to the manufacturer's instruction. Oligonucleotide primers used for PCR were designed according to published sources to flank target sequences shown in Table 1. To detect all isoforms of VEGF, the primers were designed in exons 1 and 8 to flank all known human VEGF isoforms. cDNA was synthesized form 1 μg of total RNA and amplified by PCR according to the protocol as follows: denaturing at 95 °C for 15 s, annealing at 59 °C for 30 s and extension at 68 °C for 1 min. Reaction was repeated 34 cycles for VEGF and 18 cycles for β-actin. PCR products were confirmed by sequencing. For semiquantitative analysis of PCR products, PCR products were separated by electrophoresis in 3% agarose gels (ultra pure grade, Bio-Rad) and were stained with ethidium bromide. DNA bands were visualized with UV light (302 nm) and band intensities were measured by NIH image computerized densitometry program. Band intensities of the PCR products for VEGF were normalized with those for β-actin.

2.4. Quantification of VEGF mRNA by real-time RT-PCR analysis

One set of primers and the fluorescent TaqMan® probe for VEGF mRNA were designed using Primer Expression® version 1.0 (PE Applied Biosystems Inc.) based on the sequence from the GenBank database (Accession no. NM_003376). The oligonucleotide sequences of the primers and the probe for real-time RT-PCR analyses are shown in Table 1.

There are several isoforms for VEGF; however, exon 1 to exon 5 is common to all isoforms. In this study, forward primer was selected in exon 3, and the reverse primer was in exon 4. The probe spanned exon 3 to exon 4 in order to detect all isoforms. After amplification using these primers, the coding for VEGF (988–1062) was confirmed by sequencing.

VEGF mRNA and β-actin mRNA levels were determined by quantitative real-time RT-PCR using the ABI-PRISM® 7700 sequence detection system (PE Applied Biosystems Inc.). Relative quantification was performed using the standard curve method according to User Bulletin #2 for this detection system.

Five hundred nanograms of total RNA was used in this assay. Each reaction was performed in triplicate wells, using the following conditions: 30 min at 60 °C, followed by a total of 40 cycles of two temperature cycles 95 °C for 15 s and 60 °C for 30 s.

Table 1
Oligonucleotide sequence of VEGF mRNA and β-actin mRNA specific primers

Target gene		Sequence	Amplicon position
VEGF	Forward primer	5′-TCGGGCCTCCGAAACCATGA-3′	686–705
	Reverse primer	5′-CCTGGTGAGAGATCTGGTTC-3′	1315–1334
β-actin	Forward primer	5′-CAAGAGATGGCCACGGCTGCT-3′	746–760
	Reverse primer	5′-TCCTTCTGCATCCTGTCGGCA-3′	1000–1020
VEGF for TaqMan® analysis	Forward primer	5′-CCACTGACGAGTCCAACATCAC-3′	988–1009
	Reverse primer	5′-CATCTCTCCTATGTGCTGGCCT-3′	1040–1062
	TaqMan® probe	5′-(FAM)-TGCAGATTATGCGGATCAAACCTCACC-(TAMRA)-3′	1011–1038

Accession number for VEGF mRNA: NM_003376. Accession number for β-actin mRNA: NM_001101.

2.5. VEGF protein quantification

After incubation of the THP-1 macrophages under the indicated conditions, the conditioned medium was collected and centrifuged at 100 × *g* for 5 min to remove the cells and cell debris. The supernatant was then used for the measurement of VEGF protein with a QuantiGlo® Human VEGF ELISA Kit (R&D Systems, Inc., MN, USA). All samples were measured in duplicate.

2.6. Statistical analysis

All results were expressed as mean ± SE. Statistical analyses of the data were performed by one-way analysis of variance and Fisher's Protected Least Significant Difference (PLSD) test. A value of $P < 0.05$ was considered significant. The experiments presented are representative of at least three separate experiments.

3. Results

3.1. The effects of Hcy on VEGF mRNA levels in THP-1 macrophages

Three splicing variants of VEGF mRNA were observed in THP-1 macrophages. Splicing variants of VEGF mRNA, expressed in THP-1 macrophages, are controversial. To identify the splicing variants in THP-macrophages, we performed sequencing, and identified PCR products of

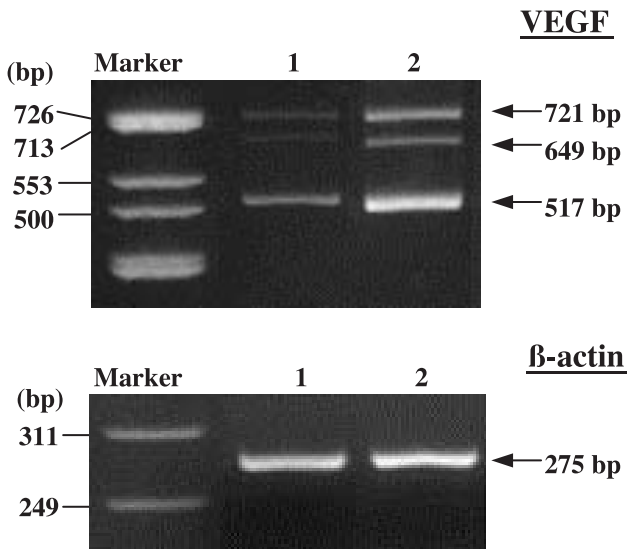

Fig. 1. Hcy upregulates $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ in THP-1 macrophages. THP-1 macrophages were incubated in the presence (lane 2) or absence (lane 1) of 200 μM Hcy for 24 h. After total RNA were prepared from THP-1 macrophages, RT-PCR was performed for VEGF (upper) and β-actin (lower). PCR products for VEGF (upper) showed 517, 649 and 721 bp, corresponding to $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$, respectively. All isoforms of the mRNA levels for VEGF were enhanced by Hcy treatment. PCR products of 275 bp corresponded to β-actin (lower).

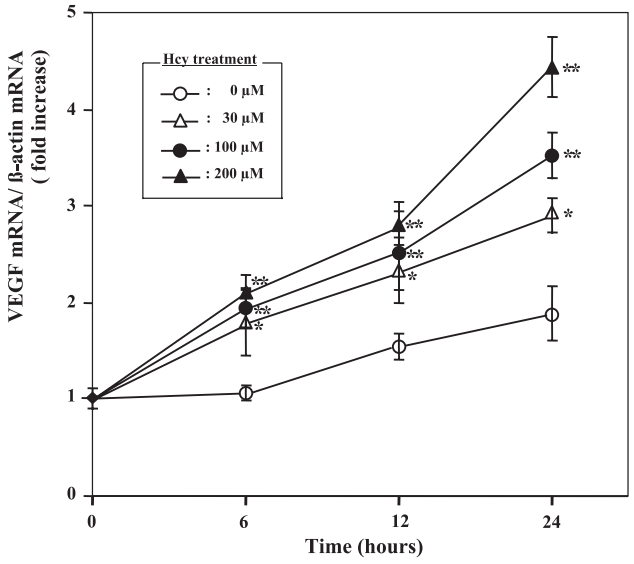

Fig. 2. Hcy induces VEGF mRNA expression in THP-1 macrophages in a dose- and time-dependent manner. THP-1 macrophages were incubated with various concentrations of Hcy for indicated time periods. VEGF mRNA expression was determined by an ABI PRISM® 7700 detection system using the relative standard curve method. Each of the VEGF mRNA levels were represented as fold induction relative to the VEGF mRNA level at time 0. The experiments were performed in triplicate. Results are expressed as mean ± S.E. of values from three independent experiments. *$P < 0.05$ and **$P < 0.01$—significantly different compared with nontreatment of each group (ANOVA, Fisher's PLSD).

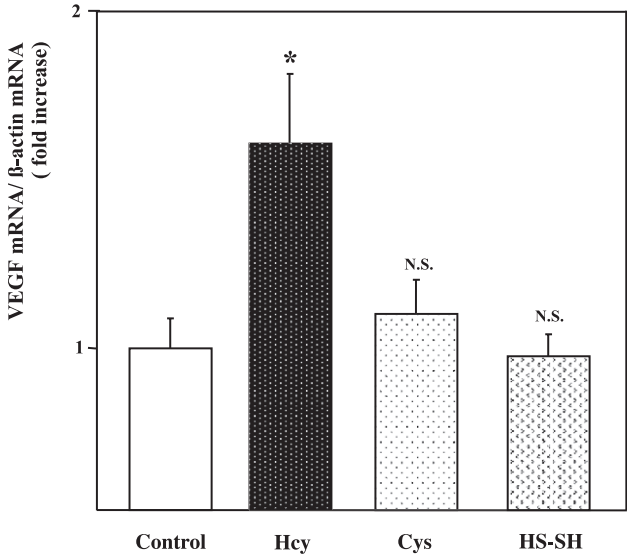

Fig. 3. Hcy specifically upregulates VEGF mRNA. To investigate the possibility of a thiol effect on the induction of VEGF mRNA, L-cysteine (Cys), L-homocysteine (Hcy) and L-homocystine (HS-SH) were tested for their ability to induce the VEGF expression. All compounds were studied at 100 μM. After 24 h of treatment, there was no increase in mRNA levels of VEGF with L-cysteine or L-homocystine. *$P < 0.01$, significantly different compared with nontreatment. N.S., not significantly different (ANOVA, Fisher's PLSD).

517, 649 and 721 bp corresponded to splicing variants $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$, respectively. We could also confirm the PCR product of β-actin, which had 275 bp (Fig. 1).

We then analyzed if VEGF mRNA level in THP-1 macrophages might be altered by Hcy (Fig. 1). As analyzed by semiquantitative RT-PCR, $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{185}$ splicing variants are constitutively expressed in THP-1 macrophages, and the intensity for $VEGF_{121}$ was the highest. All of the mRNA levels for $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ were enhanced by 200 μM of 24-h Hcy treatments.

Next we examined the effects of Hcy on VEGF mRNA expression from THP-1 macrophages by real-time RT-PCR. THP-1 macrophages were incubated with various concentrations (0, 30, 100 and 200 μM) of Hcy for 6, 12 and 24 h ($n=3$ for each set of conditions). Each of the VEGF mRNA levels was represented as value of fold induction relative to the VEGF mRNA level at time 0 h when Hcy treatments were started (Fig. 2). Hcy increased VEGF mRNA levels both in a dose- and time-dependent manner. VEGF mRNA increased significantly 6 h after Hcy stimulation.

3.2. Effects of other sulfur compounds on VEGF expression

It was reported that homocysteine is the only plasma thiol compound that is considered as a risk factor for preclinical cardiovascular disease [17]. Therefore, we evaluated the possible effects of plasma thiol compounds such as homocysteine, cysteine and homocystine on the induction of VEGF mRNA. D,L-homocysteine, D,L-homocystine and L-cysteine were tested at 100 μM for this study. After 24 h of treatment, there was no significant increase in mRNA levels of VEGF with cysteine or homocystine (Fig. 3).

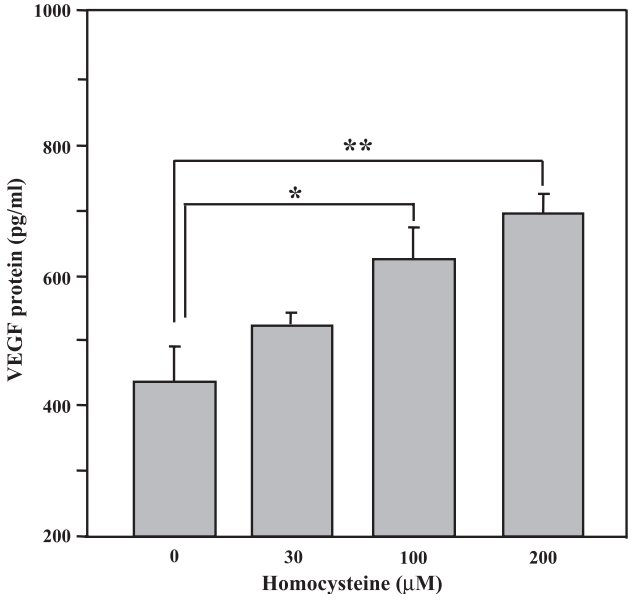

Fig. 4. Hcy upregulates VEGF protein from THP-1 macrophages. THP-1 macrophages were incubated with Hcy (0, 30, 100 and 200 μM) for 24 h ($n=3$ for each set of conditions). After the incubation of the THP-1 macrophages, the conditioned medium was collected and used for the measurement of VEGF protein with ELISA. The experiments were performed in triplicate. Results are expressed as mean ± S.E. of values from three independent experiments. *$P<0.01$ and **$P<0.001$, significantly different compared with nontreatment (ANOVA, Fisher's PLSD).

3.3. Effects of Hcy on the secretion of VEGF protein

VEGF levels were determined in culture medium by ELISA. VEGF concentration was 0 in medium prepared for this study (data not shown). As shown in Fig. 4, Hcy significantly increased VEGF secretion in a dose-dependent manner after treatment for 24 h. This study demonstrated that pathological concentrations of Hcy not only upregulated VEGF mRNA but also triggered the secretion of VEGF protein from THP-1 macrophages.

4. Discussion

In this study, we demonstrated that Hcy increased both the expression of VEGF mRNA and protein in THP-1 macrophages in a dose-dependent and a time-dependent manner. We also confirmed that among plasma thiol compounds, only Hcy exclusively induced VEGF mRNA in THP-1 macrophages. Our results suggest that hyperhomocysteinemia induces VEGF expression in macrophages in the process of arteriosclerosis. Many prospective epidemiological studies have indicated that hyperhomocysteinemia is involved in atherogenesis. Because VEGF has been reported to be abundant in atherosclerotic lesions [15] and involved in the progression of atherosclerosis [16], it is supposed that hyperhomocysteinemia may be associated with the induction of VEGF expression, which contributes to atherosclerotic plaque progression.

Monocytes/macrophages play a central role in atherogenesis and the development of atherosclerotic plaque progression [18]. In the present study, we used THP-1 cells as a macrophage model because more than 90% of THP-1 cells become terminally differentiated with PMA activation and demonstrate macrophage-like characteristics [19–23]. We then showed here that the stimulation of THP-1 macrophages with Hcy, but not with other sulfur compounds, such as homocystine, methionine and cysteine, leads to VEGF induction. Taken together with the previous report that macrophages are one of the major sources for producing VEGF in atherosclerotic plaque [24], our present data proposed that macrophages could be major target of Hcy, in atherosclerosis, leading to VEGF expression, as well as oxidized low-density lipoprotein [25] and prostaglandin E_2 [26]. The previous study demonstrated that Hcy activates NF-κB in THP-1 cells in vitro [27]. Furthermore, recent data have provided the evidence for the in vivo activation of NF-κB and downstream inflammatory marker expression in

atherosclerotic lesion in hyperhomocysteinemic apolipoprotein E (apoE)-deficient mice [28], indicating the pathological significance of Hcy in NF-κB activation. Notably, in human macrophages, VEGF expression is regulated by NF-κB, a proinflammatory transcriptional factor [29]. Collectively, these contexts could support our hypothesis that Hcy is involved in vascular inflammation with VEGF expression in macrophages.

Hyperhomocysteinemia is a condition where total plasma Hcy levels in the fasting state are higher than normal (5–15 μM) [30,31]. Plasma Hcy level is affected not only by genetic defects of Hcy metabolism but also by various nongenetic factors such as nutritional deficiency of cobalamin, folate, pyridoxine or choline as well as other disease and some medications [31–34]. Fifteen to thirty micromolars of plasma Hcy is described as moderate hyperhomocysteinemia, 30–100 μM as intermediate, and more than 100 μM as severe hyperhomocysteinemia [30,31,35]. Importantly, our results showed that VEGF was upregulated in THP-1 macrophages by concentrations as low as 30 μM of Hcy, that is the same concentrations of patients with moderate hyperhomocysteine. Consistently, accumulating epidemiological evidence has demonstrated that moderate hyperhomocysteinemia is an independent risk factor for atheromatous vascular disease [32,33,36]. Therefore, our data biologically support the possibility that macrophage-derived VEGF by Hcy stimulation promotes atherosclerosis in patients with moderate to intermediate hyperhomocysteinemia.

In summary, we have demonstrated for the first time that Hcy induces VEGF expression in macrophages. VEGF then may contribute to drive plaque formation by its various effects on the vascular endothelium in atherosclerotic lesion. Our findings suggest the possible involvement of hyperhomocysteinemia in the development of atherosclerosis lesions through VEGF induction in macrophages. It is proposed that pharmacological intervention in Hcy/VEGF signaling pathway is promising therapeutics against the progression of atherosclerotic lesion.

Acknowledgements

This study was supported in part by a grant from the Organization for Pharmaceutical Safety and Research (OPSR) in Japan and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology.

References

[1] S.H. Mudd, J.D. Finkelstein, F. Irreverre, L. Laster, Homocystinuria: an enzymatic effect, Science 143 (1964) 1443–1445.
[2] M.M. Ress, G.M. Rodgers, Homocysteinemia: association of a metabolic disorder with vascular disease and thrombosis, Thromb. Res. 71 (1993) 337–359.
[3] K.S. McCully, Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis, Am. J. Pathol. 56 (1969) 111–128.
[4] M.R. Malinow, Homocyst(e)ine and arterial occlusive diseases, J. Intern. Med. 236 (1994) 603–617.
[5] P. Durand, S. Lussier-Cacan, D. Blache, Acute methionine load-induced hyperhomocysteinemia enhances platelet aggregation, thromboxane biosynthesis, and macrophage-derived tissue factor activity in rats, FASEB J. 11 (1997) 1157–1168.
[6] P.B. Duell, M.R. Malinow, Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease, Curr. Opin. Lipidol. 8 (1997) 28–34.
[7] L.A. Harker, S.J. Slichter, C.R. Scott, R. Ross, Homocysteinemia vascular injury and arterial thrombosis, N. Engl. J. Med. 291 (1974) 537–543.
[8] J. Tsai, M.A. Perrella, M. Yoshizumi, C. Hsieh, E. Harber, R. Shlegel, M. Lee, Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis, Proc. Natl. Acad. Sci. 91 (1994) 6369–6373.
[9] M.K. Al-Obaidi, H. Philippou, P.J. Stubbs, A. Adami, R. Amersey, M.M. Noble, D.A. Lane, Relationships between homocysteine, factor VIIa, and thrombin generation in acute coronary syndrome, Circulation 4 (2000) 372.
[10] R. Ross, The pathogenesis of atherosclerosis: a perspective for the 1990s, Nature 362 (1993) 801–809.
[11] D.T. Connolly, D.M. Heuvelman, R. Nelson, J.V. Olander, B.L. Eppley, J.J. Delfino, N.R. Siegel, R.M. Leimgruber, J. Feder, Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis, J. Clin. Invest. 84 (1989) 1470–1478.
[12] H.M. Verheul, A.S. Jorna, K. Hoekman, H.J. Broxterman, M.F. Gebbink, H.M. Pinedo, Vascular endothelial growth factor-stimulated endothelial cells promote adhesion and activation of platelets, Blood 96 (2000) 4216–4221.
[13] B. Barleon, S. Sozzani, D. Zhou, H.A. Weich, A. Mantovani, D. Marme, Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1, Blood 87 (1996) 3336–3343.
[14] M. Clauss, M. Gerlach, H. Gerlach, J. Brett, F. Wang, P.C. Familletti, Y.C. Pan, J.V. Olander, D.T. Connolly, D. Stern, Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration, J. Exp. Med. 172 (1990) 1535–1545.
[15] M. Inoue, H. Itoh, M. Ueda, K. Nakao, Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions—possible pathophysiological significance of VEGF in progression of atherosclerosis, Circulation 98 (1998) 2108–2116.
[16] F.L. Celletti, J.M. Waugh, P.G. Amabile, A. Brendolan, P.R. Hilfiker, M.D. Dake, Vascular endothelial growth factor enhances atherosclerotic plaque progression, Nat. Med. 7 (2001) 425–429.
[17] K. Demuth, S. Drunat, X. Girerd, N. Moatti, J.L. Paul, M. Safar, P. Boutouyrie, Homocysteine is the only plasma thiol associated with carotid artery remodeling, Atherosclerosis 165 (2002) 167–174.
[18] A.M. Gown, T. Tsukada, R. Ross, Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions, Am. J. Pathol. 125 (1986) 191–207.
[19] S. Tsuchiya, M. Yamabe, Y. Yoshihiko, Y. Kobayashi, T. Konno, K. Tada, Establishment and characterization of a human acute monocytic leukemia cell line (THP-1), Int. J. Cancer 26 (1980) 171–176.
[20] S. Tsuchiya, Y. Kobayashi, Y. Goto, H. Okumura, S. Nakae, T. Konno, K. Tada, Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester, Cancer Res. 42 (1982) 1530–1536.
[21] A. Ueda, Y. Ishigatsubo, T. Okubo, T. Yoshimura, Transcriptional regulation of the human monocyte chemoattractant protein-1 gene. Cooperation of two NF-κB sites and NF-κB/Rel subunit specificity, J. Biol. Chem. 272 (1997) 31092–31099.
[22] D.P. Via, L. Pons, D.K. Dennison, A.E. Franslow, F. Bernini, Induction of acetyl-LDL receptor activity by phorbol ester in human monocyte cell line THP-1, J. Lipid Res. 30 (1989) 1515–1524.

[23] R.K. Tangirala, K. Murao, O. Quehenberger, Regulation of expression of the human monocyte chemotactic protein-1 receptor (hCCR2) by cytokines, J. Biol. Chem. 272 (1997) 8050–8056.
[24] B. Berse, L.F. Brown, L. Van de Water, H.F. Dvorak, D.R. Senger, Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors, Mol. Biol. Cell 3 (1992) 211–220.
[25] MA. Ramos, M. Kuzuya, T. Esaka, S. Miura, S. Satake, T. Asai, S. Kanda, T. Hayashi, A. Iguchi, Induction of macrophage VEGF in response to oxidized LDL and VEGF accumulation in human atherosclerotic lesions, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 1188–1196.
[26] M. Mukutmoni, N.E. Hubbard, K.L. Erickson, Prostaglandin E_2 modulation of vascular endothelial growth factor production in murine macrophages, Prostaglandins Leukot. Essent. Fat. Acids 65 (2001) 123–131.
[27] G. Wang, Y.L. Siow, K. O, Homocysteine induces monocyte chemoattractant protein-1 expression by activating NF-κB in THP-1 macrophages, Am. J. Physiol., Heart Circ. Physiol. 280 (2001) H2840–H2847.
[28] M.A. Hofmann, E. Lalla, Y. Lu, M.R. Gleason, B.M. Wolf, N. Tanji, L.J. Ferran Jr., B. Kohl, V. Rao, W. Kisiel, D.M. Stern, A.M. Schmidt, Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model, J. Clin. Invest. 107 (2001) 675–683.
[29] S. Kiriakidis, E. Andreakos, C. Monaco, B. Foxwell, M. Feldmann, E. Paleolog, VEGF expression in human macrophages is NF-κB-dependent: studies using adenoviruses expressing the endogenous NF-κB inhibitor IκBalpha and a kinase-defective form of the IκB kinase 2, J. Cell. Sci. 116 (2003) 665–674.
[30] G.N. Welch, J. Loscalzo, Homocysteine and atherothrombosis, N. Engl. J. Med. 338 (1998) 1042–1050.
[31] S.S. Kang, P.W.K. Wang, M.R. Malinow, Hyperhomocysteinemia as a risk factor for occlusive vascular disease, Annu. Rev. Nutr. 12 (1992) 279–298.
[32] P.M. Ueland, H. Refsum, Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy, J. Lab. Clin. Med. 114 (1989) 473–501.
[33] K.S. McCully, Homocysteine and vascular disease, Nat. Med. 2 (1996) 386–389.
[34] R. Bissonnette, E. Treacy, R. Rozen, B. Boucher, J.S. Cohn , J. Genest Jr., Fenofibrate raises plasma homocysteine levels in the fasted and fed states, Atherosclerosis 155 (2001) 455–462.
[35] U. Ikeda, M. Ikeda, S. Minota, K. Shimada, Homocysteine increases nitric oxide synthesis in cytokine-stimulated vascular smooth muscle cells, Circulations 99 (1999) 1230–1235.
[36] M.J. Stampfer, M.R. Malinow, W.C. Willett, L.M. Newcomer, B. Upson, D. Ullmann, P.V. Tishler, C.H. Hennekens, A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians, JAMA 268 (1992) 877–881.