Original Article

Suppression of Oxidized LDL-Induced PDGF Receptor β Activation by Ginkgo Biloba Extract Reduces MMP-1 Production in Coronary Smooth Muscle Cells

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Aim: An extract of *Ginkgo Biloba L.* was shown to have preventive effects on cardiovascular disorders, but the molecular mechanisms of its actions remain to be elucidated. Since matrix metalloproteinases (MMPs) are implicated in the rupture of atherosclerotic plaques and the subsequent occurrence of acute coronary syndrome, we examined the effects of a leaf extract (Ginkgolon-24) on the production of MMP-1 in human coronary smooth muscle cells stimulated with oxidized low-density lipoprotein (oxLDL) and 4-hydroxynonenal, which are factors proposed to play a pivotal role in atherogenesis.

Methods: The production of MMP-1 and phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 were estimated by immunoblotting. The tyrosine-phosphorylated form of platelet-derived growth factor receptor β (PDGFR- β) was analyzed by immunoprecipitation of the receptor followed by immunoblotting.

Results: oxLDL and 4-hydroxynonenal accelerated the production of MMP-1 with the preceding phosphorylation of ERK1/2 and PDGFR- β . Pretreatment with Ginkgolon-24 inhibited the production of MMP-1 and phosphorylation of ERK1/2 induced by oxLDL and 4-hydroxynonenal, but did not affect the production and phosphorylation induced by phorbol ester. Furthermore, Ginkgolon-24 prevented tyrosine phosphorylation of the receptor induced by oxLDL and 4-hydroxynonenal.

Conclusion: These results suggest that Ginkgo Biloba extract suppresses the oxLDL- and 4-hydroxynonenal-induced production of MMP-1, probably through the inhibition of PDGFR- β activation in human coronary smooth muscle cells.

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Key words; Ginkgo Biloba extracts, Metalloproteinase-1, Oxidized low-density lipoprotein, Smooth muscle cells

Introduction

The progression of atherosclerosis is a complex process, which is mediated by numerous cellular responses in atheroma-associated cells, such as macrophages and vascular smooth muscle cells (SMCs), stimulated with cytokines, atherogenic lipoproteins including oxidized low-density lipoprotein (oxLDL), or bioactive oxidized lipids including oxysterols and 4-hy-

droxynonenal (4-HNE). Among the cellular responses, the production of matrix metalloproteinases (MMPs) is involved in the degradation of extracellular matrix proteins, such as collagen and fibronectin, leading to the migration of SMCs into the intima and to the rupture of atherosclerotic plaques¹⁻⁴.

The more than 20 types of MMPs identified to date have been classified into groups including collagenases, gelatinases, stromelysins, membrane-type MMPs, and matrilysins⁵. The expression of collagenases (MMP-1, -8, and -13) is increased in atherosclerotic lesions, especially the shoulder region of vulnerable plaques, in humans and animal models^{2, 6-10}. MMP-1 localizes mainly with SMCs^{6, 7} and macrophages^{6, 8}, while MMP-8 and -13 are expressed by

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neutrophils⁹⁾ and macrophages⁸⁾, respectively. Several cytokines have been shown to stimulate the expression of MMP-1 in SMCs and macrophages¹¹⁻¹⁴⁾. Recently, we reported that oxLDL and 4-HNE accelerated the production of MMP-1 through the activation of platelet-derived growth factor receptor β (PDGFR- β) in human coronary SMCs (hCSMCs)¹⁵⁾. Since oxLDL and bioactive oxidized lipids are well known to mediate the progression and rupture of atherosclerotic lesions^{1, 16, 17)}, it is conceivable that prevention of atherogenic factor-induced MMP-1 production could be beneficial in atherosclerosis.

An extract of *Ginkgo Biloba L.*, a mixture mainly composed of flavonoid glycosides (glycosides of quercetin and kaempferol) and terpenoides (ginkgolides and bilobalide), is shown to suppress the generation of reactive oxygen species (ROS)¹⁸⁻²¹⁾ as well as the oxidation of LDL^{19, 22)}, which is a critical event in atherogenesis. The extract and its ingredients, especially ginkgolides, also exhibit an antagonistic effect on platelet-activating factor²³⁾. These effects of the extract are responsible, in part, for its protective effects on tissue abnormalities including myocardial ischemia-reperfusion injury²⁴⁾ and ischemic brain damage^{25, 26)}, being beneficial to patients with cardiovascular, cerebrovascular, and neurological disorders^{27, 28)}. A previous study showed that *Ginkgo Biloba* extracts inhibit serum-induced proliferation of rat vascular SMCs, and prevent intimal hyperplasia in balloon-injured abdominal aorta in rabbits fed with high-cholesterol diet²⁹⁾; however, it is unclear whether these inhibitory effects are ascribable only to the antioxidative effects or antagonistic effects on platelet-activating factor. Thus, the molecular mechanisms underlying the beneficial effects on cardiovascular disorders, especially atherosclerosis, remain to be fully elucidated. Flavonoids including quercetin and kaempferol, of which glycosides are major constituents of *Ginkgo Biloba* extracts, are shown to exhibit inhibitory effects on tyrosine kinases, including growth factor receptors^{30, 31)}. Furthermore, our recent study showed that oxLDL-induced production of MMP-1 is mediated by the activation of PDGFR- β , but occurs independently of the generation of ROS in hCSMCs¹⁵⁾. Taking these findings into account, in the present study, we examined the effects of the leaf extract on the production of MMP-1 in hCSMCs stimulated with oxLDL and 4-HNE.

Materials and methods

Materials

Antibodies against extracellular signal-regulated kinase (ERK) 1/2, phosphorylated ERK1/2, PDGFR- β

(sc-432), and the phosphorylated tyrosine residue of PDGFR- β (sc-17173), and protein A-agarose were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). MCDB131 medium, basic fibroblast growth factor, epidermal growth factor, insulin, and the anti-MMP-1 antibody were from Sigma (St. Louis, MO, USA). PD98059, 4-HNE, and AG1295 were from Calbiochem (La Jolla, CA, USA). Human native LDL (BT-903) was from Biomedical Technologies Inc. (Stoughton, MI, USA). The leaf extract of *Ginkgo Biloba L.* (Ginkgolon-24), a mixture containing flavonoid glycosides (14.2% quercetin-3-*O*-rutinoside, 11.3% kaempferol-3-*O*-rutinoside, and 2.0% isorhamnetin-3-*O*-rutinoside) and terpene lactones (2.3% bilobalide, and 3.8% ginkgolide A, B, and C), was donated by Tokiwa Phytochemical Co., Ltd. (Chiba). All other reagents were obtained from Wako Pure Chemical Industries (Osaka).

Cell Culture

hCSMCs (Cambrex Bio Science Walkersville, Inc., Walkersville, MD, USA) were cultured in MCDB131 medium supplemented with 5% heat-inactivated fetal bovine serum, 1 ng/mL basic fibroblast growth factor, 0.5 ng/mL epidermal growth factor, 5 μ g/mL insulin, 100 units/mL penicillin, and 100 μ g/mL streptomycin in collagen (type I)-coated culture dishes (Asahi Techno Glass, Corp. Tokyo). The cells, between the 6th to 8th passage, were plated in 35-mm dishes at 2×10^5 cells, and made quiescent by incubating with MCDB131 medium for 24h.

Preparation of oxLDL

Human native LDL was dialyzed against phosphate-buffered saline at 4°C, and oxidized as described previously³²⁾. Briefly, LDL (2.5 mg protein/mL) was oxidized with 10 μ M CuSO₄ at 37°C for 3h. The oxLDL contained 10-15 nmol thiobarbituric acid-reactive substances/mg protein. The oxLDL was further dialyzed against phosphate-buffered saline containing 200 μ M EDTA at 4°C, and used within 10 days.

Immunoblot Analysis

hCSMCs were treated and stimulated with various reagents, including oxLDL and 4-HNE, as described in the figure legends. The culture medium and cells, as samples, were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% gel. The separated proteins were transferred onto a nitrocellulose membrane. Antibodies against MMP-1, ERK1/2, and phosphorylated ERK1/2 were applied. The bound antibodies were visualized using a peroxidase-conjugated secondary antibody and enhanced

Fig. 1. Inhibitory effects of Ginkgolide-24 on the production of MMP-1 induced by oxLDL (A) and 4-HNE (B).

hCSMCs were treated with various concentrations of Ginkgolide-24 (G-24) for 30 min, and then stimulated with (+) or without (-) oxLDL (50 $\mu\text{g/mL}$) or 4-HNE (20 μM) for 24h. MMP-1 in the culture medium was analyzed by immunoblotting. The amount of MMP-1 was estimated by measuring the density of the MMP-1 band. Each value represents the mean $\pm$ SEM of three separate experiments. * $p < 0.01$, relative to the response of oxLDL or 4-HNE alone.

chemiluminescence Western blotting detection reagents (GE Healthcare Biosciences Corp., Piscataway, NJ, USA). Representative results of three separate experiments are shown. To estimate the amounts of MMP-1, the density of each band on a film was analyzed with a film scanner and NIH image analysis software. The results are expressed in arbitrary units (mean $\pm$ SEM, $n = 3$).

Immunoprecipitation of PDGFR- β

hCSMCs were incubated with Ginkgolide-24 for 30 min or AG1295 for 1h in the presence of 5 mM Na_3VO_4 , and stimulated with oxLDL or 4-HNE as described in the figure legends. After being washed, the cells were lysed and subjected to immunoprecipitation of PDGFR- β as described previously³³. The lysate was incubated with anti-PDGFR- β antibodies overnight at 4°C and further with protein A-agarose for 3h. After centrifugation, the pellet was subjected to immunoblotting (a 5% gel) with antibodies against PDGFR- β or the phosphorylated tyrosine residue of PDGFR- β . Representative results of three separate experiments are shown. To estimate the phosphorylation of PDGFR- β , the density of the bands for PDGFR- β and its phosphorylated form on a film was analyzed as above. Results are adjusted for PDGFR- β levels and expressed in arbitrary units (mean $\pm$ SEM, $n = 3$).

Statistical Analysis

Values are expressed as the mean $\pm$ SEM. Data were analyzed with one-way analysis of variance and Dunnett's test or with Student's t -test. $P < 0.05$ was considered statistically significant.

Results

Effects of Ginkgolide-24 on the Production of MMP-1 and the Activation of ERK1/2

Stimulation of hCSMCs with oxLDL (50 $\mu\text{g/mL}$) for 24h induced an increase in MMP-1 protein in the culture medium, as estimated by immunoblotting, as shown in **Fig. 1A**. The present study examined the effects of Ginkgolide-24, a leaf extract of *Ginkgo Biloba* L., on the production of MMP-1. The results in **Fig. 1A** demonstrated that pretreatment of hCSMCs with Ginkgolide-24 (5-20 $\mu\text{g/mL}$) for 30 min dose-dependently suppressed the oxLDL-induced increase in MMP-1 protein. At 20 $\mu\text{g/mL}$, Ginkgolide-24 almost completely inhibited the production of MMP-1. Furthermore, Ginkgolide-24 (2-10 $\mu\text{g/mL}$) also prevented the 4-HNE (20 μM)-induced production of MMP-1 in a dose-dependent manner with apparent inhibition at 10 $\mu\text{g/mL}$, as shown in **Fig. 1B**.

The induction of MMP-1 gene expression is suggested to be mediated by ERK1/2 in canine fibroblast-like synoviocytes stimulated with basic calcium phosphate crystals³⁴. We also reported that oxLDL and 4-HNE induces the activation of ERK1/2, inhibition of which by PD98059, an inhibitor of mitogen-activated protein kinase/ERK kinase, resulted in the suppression of MMP-1 production in hCSMCs¹⁵, suggesting the involvement of ERK1/2 in the production of MMP-1. The present study showed that pretreatment of hCSMCs with Ginkgolide-24 (2-10 $\mu\text{g/mL}$) inhibited the phosphorylation of ERK1/2 induced by stimulation with oxLDL (50 $\mu\text{g/mL}$) or 4-HNE (20 μM) for 10 min, as shown in **Fig. 2**. A protein kinase C activator, such as phorbol 12-myristate 13-acetate

Fig. 2. Effects of Ginkgolon-24 on the phosphorylation of ERK1/2 induced by oxLDL (A), 4-HNE (B), and PMA (C).

hCSMCs were treated with various concentrations of Ginkgolon-24 (G-24) for 30 min, and then stimulated with (+) or without (–) oxLDL (50 $\mu\text{g/mL}$), 4-HNE (20 μM), or PMA (200 nM) for 10 min. ERK1/2 and its phosphorylated form (p-ERK1/2) in the cells were analyzed by immunoblotting. The results are representative of three separate experiments.

Fig. 3. Effects of Ginkgolon-24 (A) and PD98059 (B) on the production of MMP-1 induced by PMA.

hCSMCs were treated with various concentrations of Ginkgolon-24 (G-24) or PD98059 (5 μM) for 30 min, and then stimulated with (+) or without (–) PMA (200 nM) for 24h. MMP-1 in the culture medium was analyzed by immunoblotting. The results are representative of three separate experiments.

(PMA), has been shown to activate ERK1/2 independently of the activation of receptors³⁵. In contrast to oxLDL and 4-HNE, Ginkgolon-24 (5–20 $\mu\text{g/mL}$) did not affect the phosphorylation of ERK1/2 (**Fig. 2C**) or the production of MMP-1 induced by PMA (200 nM) (**Fig. 3A**). We confirmed that PD98059 (5 μM) prevented the PMA-induced production of MMP-1 (**Fig. 3B**). These results suggest that the Ginkgolon-24-caused inhibition of ERK1/2 phosphorylation in response to oxLDL and 4-HNE is not ascribable to a direct suppressive effect on the activation of ERK1/2. It is possible, therefore, that Ginkgolon-24 inhibits the ERK1/2-dependent production of MMP-1 by suppressing a signaling pathway upstream of ERK1/2.

Effects of Ginkgolon-24 on the Activation of PDGFR- β

The activation of ERK1/2 is triggered by the stimulation of receptors for growth factors such as PDGFR- β in a variety of cell types including SMCs^{36, 37}. oxLDL and 4-HNE have been shown to induce the activation of PDGFR- β in hCSMCs and rabbit arterial SMCs^{15, 38, 39}. We further reported that inhibition of the oxLDL-induced activation of PDGFR- β resulted in the suppression of ERK1/2 phosphorylation and MMP-1 production¹⁵, suggesting that PDGFR- β mediates the ERK1/2-dependent production of MMP-1 induced by oxLDL; therefore, the effects of Ginkgolon-24 on the activation of PDGFR- β were examined.

As shown in **Fig. 4A**, stimulation with oxLDL (50 $\mu\text{g/mL}$) for 10 min induced the tyrosine phosphorylation of PDGFR- β , as estimated based on the immunoprecipitation of PDGFR- β and immunoblotting with antibodies against PDGFR- β bearing a phospho-tyrosine residue. Ginkgolon-24 (10 $\mu\text{g/mL}$) apparently suppressed the tyrosine phosphorylation of PDGFR- β induced by oxLDL (**Fig. 4A**) to a similar extent to AG1295 (100 μM), an inhibitor of tyrosine kinase of PDGFR (**Fig. 4C**). Furthermore, 4-HNE (40 μM) stimulated the tyrosine phosphorylation of PDGFR- β , which was also prevented by Ginkgolon-24 (5 or 10 $\mu\text{g/mL}$) (**Fig. 4B**).

Discussion

The formation of atherosclerotic plaques is associated with the development of a lipid core and the formation of fibrous caps to cover the lipid core, events which are mediated by oxLDL-induced biological responses, including the transformation of macrophages into lipid-laden foam cells and the migration of SMCs into the intima. In the advanced stages of atherosclerosis, the stability of atherosclerotic plaques decreases with the thinning of the fibrous caps, the resulting vulnerable plaques being susceptible to rupture. Disruption of the plaques triggers the formation of a thrombus, which is the cause of acute coronary syndromes. Among these events in the progression of atherosclerosis, the migration of SMCs and the rupture of plaques occur with the degradation of extracellular matrix proteins, such as collagen, catalyzed by MMPs including MMP-1^{1–4}. The expression of MMP-1 is increased in human atherosclerotic plaques, where SMCs^{6, 7} and macrophages^{6, 8} are colocalized. Our recent study with hCSMCs showed that the production of MMP-1 was accelerated by oxLDL and 4-HNE, which are atherogenic factors¹⁵. Since collagenases, including MMP-1, are the only MMPs that can degrade native collagen, an increase in MMP-1 is a criti-

Fig. 4. Inhibitory effects of Ginkgolide-24 and AG1295 on the phosphorylation of PDGFR- β .

hCSMCs were treated with various concentrations of Ginkgolide-24 (G-24) for 30 min (A, B) or AG1295 (100 μ M) for 1h (C), and then stimulated with (+) or without (-) oxLDL (50 μ g/mL) (A, C) or 4-HNE (40 μ M) (B) for 10 min. PDGFR- β (PDGFR) and its tyrosine-phosphorylated form (p-PDGFR) in the cells were detected by the immunoprecipitation of PDGFR- β followed by immunoblotting. The degree of PDGFR phosphorylation was estimated by measuring the density of PDGFR and p-PDGFR bands (mean $\pm$ SEM). Each value represents the mean $\pm$ SEM of three separate experiments. * p < 0.02, ** p < 0.01, relative to the response to oxLDL or 4-HNE alone.

cal cellular response in the progression of atherosclerosis. It is possible, therefore, that preventing the production of MMP-1 induced by atherogenic factors could be beneficial in cases of atherosclerosis. The current study showed that Ginkgolide-24, an extract of *Ginkgo Biloba L.*, suppressed the production of MMP-1 induced by oxLDL and 4-HNE in hCSMCs, suggesting that this inhibitory activity is also involved in the therapeutic effects of the extract on cardiovascular disorders²⁸⁾ and intimal hyperplasia in balloon-injured abdominal aorta in an animal model²⁹⁾.

With regard to the mechanisms responsible for the production of MMP-1, the induction of MMP-1 gene expression was mediated by ERK1/2 in canine fibroblast-like synoviocytes³⁴⁾. The activation of ERK1/2 was mediated by the activation of receptors for growth factors such as PDGFR- β in SMCs^{36, 37)}. We and other investigators showed that oxLDL stimulated the activation of PDGFR- β in hCSMCs and rabbit arterial SMCs^{15, 38, 39)}. Our recent study further demonstrated that the production of MMP-1 induced by oxLDL and 4-HNE was suppressed under conditions where the activation of PDGFR- β and ERK1/2 was inhibited by an inhibitor of tyrosine kinase (AG1295 and genistein) and by an inhibitor of mitogen-activated protein kinase/ERK kinase (PD98059), respectively¹⁵⁾. Moreover, suppression of the oxLDL-induced activation of PDGFR- β resulted in the inhibition of ERK1/2 phosphorylation¹⁵⁾. Considering these findings, it is conceivable that oxLDL accelerates the production of MMP-1 through the phosphorylation of PDGFR- β followed by the activation of ERK1/2. The present study showed that Ginkgolide-24

inhibited the activation of PDGFR- β and ERK1/2 induced by oxLDL and 4-HNE. However, the extract had no effect on the activation of ERK1/2 or the production of MMP-1 induced by PMA, which activates ERK1/2 independently of the activation of receptors³⁵⁾, indicating that the Ginkgolide-24-caused inhibition of MMP-1 production is not ascribable to a direct suppressive effect on the activation of ERK1/2. Consequently, the present results suggest that Ginkgolide-24 inhibits the oxLDL- and 4-HNE-induced production of MMP-1 by suppressing the activation of PDGFR- β .

The leaf extract of *Ginkgo Biloba L.* has beneficial effects on the prevention and treatment of cardiovascular, cerebrovascular, and neurological disorders. These therapeutic effects are thought to result, in part, from antioxidative actions²⁸⁾. In fact, the extract has been shown to act as a scavenger of ROS^{18, 19, 22)}. Our previous study with rat mesangial cells demonstrated that Ginkgolide-24 suppresses the oxLDL-induced production of fibronectin by preventing the generation of ROS⁴⁰⁾. In contrast, oxLDL-stimulated hCSMCs produce MMP-1 independently of the generation of ROS, because *N*-acetylcysteine, an antioxidant, had no effect on the oxLDL-induced production of MMP-1 or activation of ERK1/2 despite inhibiting the generation of ROS¹⁵⁾. It is possible, therefore, that the antioxidative effects of Ginkgolide-24 are not involved in its inhibitory effect on the oxLDL-induced production of MMP-1 in hCSMCs. Major constituents of *Ginkgo Biloba* extracts are quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside, which are flavonoid glycosides. Some flavonoids (quercetin, kaempferol) and

isoflavonoids (genistein) are used as inhibitors of tyrosine kinase as well as antioxidants. In our recent report, genistein inhibited the tyrosine phosphorylation of PDGFR- β induced by oxLDL and 4-HNE in hCSMCs¹⁵⁾. Quercetin and kaempferol were shown to prevent the tyrosine phosphorylation of epidermal growth factor receptor in A431 tumor cells³⁰⁾ and of PDGFR- β in rat aortic vascular SMCs³¹⁾, respectively. Considering these findings and their structures, we speculate that constituents of Ginkgo Biloba extracts, probably quercetin and/or kaempferol derivatives, act as inhibitors of tyrosine kinase of PDGFR- β rather than antioxidants, thus exhibiting inhibitory effects on the oxLDL- and 4-HNE-induced tyrosine phosphorylation of PDGFR- β and subsequent production of MMP-1.

In summary, the present study showed that Ginkgolon-24, an extract of *Ginkgo Biloba L.*, suppressed the production of MMP-1 and activation of PDGFR- β and ERK1/2 in response to oxLDL and 4-HNE in hCSMCs. The inhibition of MMP-1 production probably results from an impairment of PDGFR- β activation. In addition to the antioxidative actions of Ginkgo Biloba extracts, the suppressive effects of Ginkgolon-24 shown here may be involved in their beneficial effects.

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References

- Falk E, Shah PK, and Fuster V: Coronary plaque disruption. *Circulation*, 1995; 92:657-671
- Galis Z and Khatir JJ: Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res*, 2002; 90:251-262
- Heeneman S, Cleutjens JP, Faber BC, Creemers EE, van Suylen RJ, Lutgens E, Cleutjens KB, and Daemen MJ: The dynamic extracellular matrix: intervention strategies during heart failure and atherosclerosis. *J Pathol*, 2003; 200:516-525
- Katsuda S and Kaji T: Atherosclerosis and extracellular matrix. *J Atheroscler Thromb*, 2003; 10:267-274
- Visse R and Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. *Circ Res*, 2003; 92:827-839
- Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE, and Clowes AW: Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. *Circulation*, 1995; 92:1393-1398
- Major TC, Liang L, Lu X, Rosebury W, and Bocan TMA: Extracellular matrix metalloproteinase inducer (EMMPRIN) is induced upon monocyte differentiation and is expressed in human atheroma. *Arterioscler Thromb Vasc Biol*, 2002; 22:1200-1207
- Sukhova GK, Schönbeck U, Rabkin E, Schoen FJ, Poole AR, Billingham RC, and Libby P: Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation*, 1999; 99:2503-2509
- Herman MP, Sukhova GK, Libby P, Gerdes N, Tang N, Horton DB, Kilbride M, Breitbart RE, Chun M, and Schönbeck U: Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: A novel collagenolytic pathway suggested by transcriptional profiling. *Circulation*, 2001; 104:1899-1904
- Kuzuya M and Iguchi A: Role of matrix metalloproteinases in vascular remodeling. *J Atheroscler Thromb*, 2003; 10:275-282
- Watari M, Watari H, DiSanto ME, Chacko S, Shi GP, and Strauss JF III: Pro-inflammatory cytokines induce expression of matrix-metabolizing enzymes in human cervical smooth muscle cells. *Am J Pathol*, 1999; 154:1755-1762
- Chase AJ, Bond M, Crook MF, and Newby AC: Role of nuclear factor- κ B activation in metalloproteinase-1, -3, and -9 secretion by human macrophages in vitro and rabbit foam cells produced in vivo. *Arterioscler Thromb Vasc Biol*, 2002; 22:765-771
- Lee WH, Kim SH, Lee Y, Lee BB, Kwon B, Song H, Kwon BS, and Park JE: Tumor necrosis factor receptor superfamily 14 is involved in atherogenesis by inducing proinflammatory cytokines and matrix metalloproteinases. *Arterioscler Thromb Vasc Biol*, 2001; 21:2004-2010
- Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, and Schönbeck U: Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for atherogenesis. *J Exp Med*, 2002; 195:245-257
- Akiba S, Kumazawa S, Yamaguchi H, Hontani N, Matsumoto T, Ikeda T, Oka M, and Sato T: Acceleration of matrix metalloproteinase-1 production and activation of platelet-derived growth factor receptor β in human coronary smooth muscle cells by oxidized LDL and 4-hydroxynonenal. *Biochim Biophys Acta*, 2006; 1763:797-804
- Lusis AJ: Atherosclerosis. *Nature*, 2000; 407:233-241
- de Winther MPJ and Hofker MH: Scavenging new insights into atherogenesis. *J Clin Invest*, 2000; 105:1039-1041
- Pincemail J, Dupuis M, Nasr C, Hans P, Haag-Berrurier M, Anton R, and Deby C: Superoxide anion scavenging effect and superoxide dismutase activity of Ginkgo biloba extract. *Experientia*, 1989; 45:708-712
- Maitra I, Marcocci L, Droy-Lefaix MT, and Packer L: Peroxyl radical scavenging activity of Ginkgo biloba extract EGb 761. *Biochem Pharmacol*, 1995; 49:1649-1655
- Marcocci L, Maguire JJ, Droy-Lefaix MT, and Packer L: The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochem Biophys Res Commun*, 1994; 201:748-755
- Chen JW, Chen YH, Lin FY, Chen YL, and Lin SJ: Gink-

- go biloba extract inhibits tumor necrosis factor- α -induced reactive oxygen species generation, transcription factor activation, and cell adhesion molecule expression in human aortic endothelial cells. *Arterioscler Thromb Vasc Biol*, 2003; 23:1559-1566
- 22) Yan LJ, Droy-Lefaix MT, and Packer L: Ginkgo biloba extract (EGb 761) protects human low density lipoproteins against oxidative modification mediated by copper. *Biochem Biophys Res Commun*, 1995; 212:360-366
 - 23) Lamant V, Mauco G, Braquet P, Chap H, and Douste-Blazy L: Inhibition of the metabolism of platelet activating factor (PAF-acether) by three specific antagonists from Ginkgo biloba. *Biochem Pharmacol*, 1987; 36:2749-2752
 - 24) Shen J, Wang J, Zhao B, Hou J, Gao T, and Xin W: Effects of EGb 761 on nitric oxide and oxygen free radicals, myocardial damage and arrhythmia in ischemia-reperfusion injury in vivo. *Biochim Biophys Acta*, 1998; 1406:228-236
 - 25) Zhang WR, Hayashi T, Kitagawa H, Sasaki C, Sakai K, Warita H, Wang JM, Shiro Y, Uchida M, and Abe K: Protective effect of ginkgo extract on rat brain with transient middle cerebral artery occlusion. *Neurol Res*, 2000; 22:517-521
 - 26) Chandrasekaran K, Mehrabian Z, Spinnewyn B, Drieu K, and Fiskum G: Neuroprotective effects of bilobalide, a component of the Ginkgo biloba extract (EGb 761), in gerbil global brain ischemia. *Brain Res*, 2001; 922:282-292
 - 27) DeFeudis FV and Drieu K: Ginkgo biloba extract (EGb 761) and CNS functions: basic studies and clinical applications. *Curr Drug Targets*, 2000; 1:25-58
 - 28) Yoshikawa T, Naito Y, and Kondo M: Ginkgo biloba leaf extract: review of biological actions and clinical applications. *Antioxid Redox Signal*, 1999; 1:469-480
 - 29) Lin SJ, Yang TH, Chen YH, Chen JW, Kwok CF, Shiao MS, and Chen YL: Effects of Ginkgo biloba extract on the proliferation of vascular smooth muscle cells in vitro and on intimal thickening and interleukin-1 β expression after balloon injury in cholesterol-fed rabbits in vivo. *J Cell Biochem*, 2002; 85:572-582
 - 30) Huang YT, Hwang JJ, Lee PP, Ke FC, Huang JH, Huang CJ, Kandaswami C, Middleton E Jr., and Lee MT: Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. *Br J Pharmacol*, 1999; 128:999-1010
 - 31) Kim SY, Jin YR, Lim Y, Kim JH, Cho MR, Hong JT, Yoo HS, and Yun YP: Inhibition of PDGF beta-receptor tyrosine phosphorylation and its downstream intracellular signal transduction in rat aortic vascular smooth muscle cells by kaempferol. *Planta Med*, 2005; 71:599-603
 - 32) Akiba S, Yoneda Y, Ohno S, Nemoto M, and Sato T: Oxidized LDL activates phospholipase A₂ to supply fatty acids required for cholesterol esterification. *J Lipid Res*, 2003; 44:1676-1685
 - 33) Hayama M, Inoue R, Akiba S, and Sato T: ERK and p38 MAP kinase are involved in arachidonic acid release induced by H₂O₂ and PDGF in mesangial cells. *Am J Physiol*, 2002; 282:F485-F491
 - 34) Sun Y, Wenger L, Brinckerhoff CE, Misra RR, and Cheung HS: Basic calcium phosphate crystals induce matrix metalloproteinase-1 through the Ras/mitogen-activated protein kinase/c-Fos/AP-1/metalloproteinase 1 pathway. Involvement of transcription factor binding sites AP-1 and PEA-3. *J Biol Chem*, 2002; 277:1544-1552
 - 35) Luo H and Reidy MA: Activation of big mitogen-activated protein kinase-1 regulates smooth muscle cell replication. *Arterioscler Thromb Vasc Biol*, 2002; 22:394-399
 - 36) Conway AM, Rakhit S, Pyne S, and Pyne NJ: Platelet-derived-growth-factor stimulation of the p42/p44 mitogen-activated protein kinase pathway in airway smooth muscle: Role of pertussis-toxin-sensitive G-proteins, c-Src tyrosine kinases and phosphoinositide 3-kinase. *Biochem J*, 1999; 337:171-177
 - 37) Hayashi K, Takahashi M, Kimura K, Nishida W, Saga H, and Sobue K: Changes in the balance of phosphoinositide 3-kinase/protein kinase B (Akt) and the mitogen-activated protein kinases (ERK/p38MAPK) determine a phenotype of visceral and vascular smooth muscle cells. *J Cell Biol*, 1999; 145:727-740
 - 38) Escargueil-Blanc I, Salvayre R, Vacaresse N, Jürgens G, Darblade B, Arnal JF, Parthasarathy S, and Nègre-Salvayre A: Mildly oxidized LDL induces activation of platelet-derived growth factor β -receptor pathway. *Circulation*, 2001; 104:1814-1821
 - 39) Vindis C, Escargueil-Blanc I, Elbaz M, Marcheix B, Grazide MH, Uchida K, Salvayre R, and Nègre-Salvayre A: Desensitization of platelet-derived growth factor receptor- β by oxidized lipids in vascular cells and atherosclerotic lesions: prevention by aldehyde scavengers. *Circ Res*, 2006; 98:785-792
 - 40) Akiba S, Chiba M, Mukaida Y, Tamura A, and Sato T: The leaf extract of Ginkgo Biloba L. suppresses oxidized LDL-stimulated fibronectin production through an anti-oxidant action in rat mesangial cells. *Br J Pharmacol*, 2004; 142:419-424