LDL Stimulates Mitogen-Activated Protein Kinase Phosphatase-1 Expression, Independent of LDL Receptors, in Vascular Smooth Muscle Cells

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Abstract—Low density lipoprotein (LDL) is a well-established risk factor for atherosclerosis, stimulating vascular smooth muscle cell (SMC) differentiation and proliferation, but the signal transduction pathways between LDL stimulation and cell proliferation are poorly understood. Because mitogen-activated protein kinases (MAPKs) play a crucial role in mediating cell growth, we studied the effect of LDL on the induction of MAPK phosphatase-1 (MKP-1) in human SMCs and found that LDL stimulated induction of MKP-1 mRNA and proteins in a time- and dose-dependent manner. Heparin, inhibiting LDL-receptor binding, did not influence LDL-stimulated MKP-1 mRNA expression, and human LDL also induced MKP-1 expression in rat SMCs and fibroblasts derived from LDL receptor–deficient mice, indicating an LDL receptor–independent process. Pretreatment of SMCs with pertussis toxin markedly inhibited LDL-induced MKP-1 expression. Depletion of protein kinase C (PKC) by phorbol 12-myristate 13 acetate or inhibition of PKC by calphostin C blocked MKP-1 induction, but the phospholipase C inhibitor U73122 had no effect. Pretreatment of SMCs with genistein or herbimycin A abrogated LDL-stimulated MKP-1 induction. The MAPK kinase inhibitor PD98059 abolished LDL-stimulated activation of extracellular signal–regulated protein kinases (ERKs) but not MKP-1 induction. Furthermore, constitutive expression of MKP-1 in vivo reduced LDL-induced expression of Elk-1–dependent reporter genes, and SMC lines overexpressing recombinant MKP-1 exhibited decreased ERK activities and retarded proliferation in response to LDL. Our findings demonstrate that LDL induces MKP-1 expression in SMCs via activation of PKC and tyrosine kinases, independent of LDL receptors and ERK-MAPKs, and that MKP-1 plays an important role in the regulation of LDL-initiated signal transductions leading to SMC proliferation. (Arterioscler Thromb Vasc Biol. 1999;19:1862-1871.)

Key Words: LDL • mitogen-activated protein kinase phosphatase-1 • signaling • mitogen-activated protein kinases • smooth muscle cells
lymphocyte-specific PAC-1 protein.\textsuperscript{25–30} In the arterial wall, it has been demonstrated that an elevation in blood pressure induces MKP-1 gene expression.\textsuperscript{31} MKP-1 has been shown to dephosphorylate phosphothreonine and phosphotyrosine residues of both ERKs and SAPKs, resulting in their inactivation, although MKP-1 exhibits cell-type specificity.\textsuperscript{25–30} It is believed that the balance between MAPK activation and MKP-1 induction is an important issue in determining the fate of cells stimulated by environmental insults.\textsuperscript{32} To investigate the potential effects of LDL on MKP-1 induction, vascular SMCs cultivated from human and rat arteries were stimulated with human LDL, and kinase assays and Western and Northern blot analyses were performed. We demonstrate herein that LDL stimulation results in MKP-1 mRNA expression, followed by increased protein induction. The mechanism appears to involve LDL-stimulated tyrosine kinase and protein kinase C (PKC) activation, which is independent of both classic LDL receptors and MAPK kinase (MEK1/2)-ERK1/2 signal pathways. Moreover, MKP-1 overexpression in SMCs inhibits ERK activation and Elk-1–mediated gene expression and cell proliferation stimulated by LDL.

### Methods

#### Materials

Rat MKP-1 cDNA was isolated from a rat lung cDNA library by Liu et al.\textsuperscript{29} Plasmids expressing sense and antisense recombinant (r) MKP-1 were propagated in \textit{Escherichia coli}, and cDNA was obtained by cutting the plasmids with EcorRI. The Elk-1 PathDetect trans-reporting systems were purchased from Stratagene. Plasmid pRL-TK expressing \textit{Renilla} luciferase and the dual-luciferase reporter assay system were purchased from Promega. SuperFect reagent for transfection was obtained from Qiagen. Polyclonal antibodies against MKP-1, mammalian ERK2, and JNK1/SAPK and mouse monoclonal antibodies against phosphorylated ERK1/2 were obtained from Santa Cruz Biochemicals. Heparin, pertussis toxin, genistein, herbimycin A, and N-acetylcysteine were from Calbiochem-Novabiochem International. Calphostin C, phorbol 12-myristate 13-acetate (PMA), C2-ceramide, sphingomyelinase, and calpain were from Calbiochem (PC-3), digested with collagenase and elastase, and cut into pieces (≈1 mm). The intima and inner layer of the media were dissected from the arteries and cut into pieces (≈1 mm), digested with collagenase and elastase, and cultured in RPMI 1640 (Gibco) supplemented with 20% FCS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were incubated at 37°C with 5% CO\textsubscript{2}. The medium was changed every 3 days, and cells were passaged by treatment with a 0.05% trypsin–0.02% EDTA solution. Experiments were conducted on SMCs that had just achieved confluence. The purity of SMCs was routinely confirmed by immunostaining with antibodies against α-actin.

All animal experiments were performed according to protocols approved by the Institutional Committee for the Use and Care of Laboratory Animals. All animals were housed in cages at 22°C with a relative humidity of 55%; drank water ad libitum, and were fed a normal standard chow diet. The rats were killed under anesthesia with pentobarbital sodium (50 mg/kg body weight IP).

Fibroblasts were isolated by enzymatic digestion of lung tissues from LDL receptor–deficient mice (see Reference 34; The Jackson Laboratory, Bar Harbor, Me) according to established procedures\textsuperscript{35} and cultured in RPMI 1640 supplemented with 10% FCS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Experiments were conducted on fibroblasts from the second passage after a 24-hour culture in serum-free medium. After exposure to LDL or reagents, the cells were harvested for protein extracts.

#### LDL Isolation

EDTA-plasma was pooled from normolipemic, fasting (12 to 14 hours) male and female donors, aged 20 to 35 years. Lipoproteins were prepared by differential centrifugation with the addition of solid KBr to adjust the density as described by Havel et al.\textsuperscript{36} LDLs were obtained in the fractions between 1.020 and 1.050 g/mL. During preparation, LDL was protected from oxidation by EDTA (1 mmol/L). The sample was dialyzed against 150 mmol/L NaCl with 0.1 mmol/L EDTA, sterilized on a 0.2-μm Millipore membrane, and stored at 4°C for up to 3 weeks. No oxidation of LDL was observed at least 3 weeks after LDL isolation, as determined by measurement of malondialdehyde by the thiobarbituric acid method.\textsuperscript{37} The endotoxin contents of freshly isolated LDL and of LDL after 3 weeks of storage at 4°C were both below the detection limit (1 ng/mL; endotoxin kit, Sigma). Concentrations of LDL were determined gravimetrically by aliquot weight after drying, and quantities of lipoproteins were expressed as total weights.

#### LDL Lipid Extracts

The procedure for lipid extraction was similar to that described elsewhere.\textsuperscript{16} In brief, LDL lipids were extracted by chloroform and methanol. Chloroform extracts were dried under N\textsubscript{2} gas, dissolved in dimethyl sulfoxide, and added to the culture.

#### RNA Isolation and Northern Blot Analysis

Total RNA was isolated by following a standard protocol.\textsuperscript{38} RNA (10 μg per lane) was denatured with formaldehyde (Merck), electrohoresed in a 1% agarose gel, transferred onto a nylon membrane (Zeta Probe, Bio-Rad Laboratories), and UV–cross-linked in a UV Stratalinker (Stratagene Inc). Hybridizations were performed with a fluorescein-labeled (Amersham Co) cDNA probe for MKP-1. The membranes were then washed, detected with anti-fluorescein alkaline phosphatase conjugate (1:5000, Amersham), and exposed to enhanced chemiluminescence films (Amersham). Graphs of the blots were obtained in the linear range of detection. Accuracy of loading and transfer as well RNA integrity was confirmed by quantitative analysis of the 28S and 18S RNAs.

#### Protein Extractions and Western Blot Analysis

SMCs and fibroblasts were serum-starved for 2 (human and mouse) or 3 (rat) days and incubated with LDL with or without inhibitors at 37°C for the times indicated in the figure legends. After 2 washes with cold (4°C) PBS (pH 7.4), the cells were harvested on ice in buffer A, containing 20 mmol/L HEPES (pH 7.4), 2 mmol/L EDTA, 50 mmol/L β-glycerophosphate, 1 mmol/L DTT, 1 mmol/L Na\textsubscript{3}VO\textsubscript{4}, 1% Triton X-100, 10% glycerol, 1 μg/mL leupeptin, 1 μg/mL aprotinin, and 100 μmol/L PMSF. The suspension was incubated on ice for 20 minutes with vortexing every 5 minutes. Cellular debris was then pelleted by centrifugation for 30 minutes at 13,000 rpm (Eppendorf centrifuge) at 4°C, supernatants were collected, and protein concentrations were measured by the Bio-Rad assay (Bio-Rad Laboratories). The procedure used for Western blot analysis was similar to that described previously.\textsuperscript{39} In brief, 50 or 100 μg of total cell proteins was separated by electrophoresis through a 10% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes. The blots were probed with affinity-purified polyclonal antibodies against MKP-1 or phosphorylated-ERK1/2, and specific antibody-antigen complexes were detected with the enhanced chemiluminescence Western blot detection kit. Graphs of the blots were obtained in the linear range of detection and quantified for specific induction or inhibition by scanning laser densitometry (Power-Look II, UMAX Data System Inc) of the graphs.

#### Kinase Assays

For kinase assays, 0.5 mL of the supernatant containing 0.5 mg protein was incubated with 10 μL of antibodies against mammalian ERK2 or JNK1/SAPK for 2 hours at 4°C with rotation.
quently, 40 μL of protein G-agarose suspension (Santa Cruz Biochemicals) was added, and rotation was continued for 1 hour at 4°C. Immunocomplexes were precipitated by centrifugation and washed 2 times with buffers A, B (500 mmol/L LiCl, 100 mmol/L Tris, 1 mmol/L DTT, and 0.1% Triton X-100; pH 7.6), and C (20 mmol/L MOPS, 2 mmol/L EGTA, 10 mmol/L MgCl2, 1 mmol/L DTT, and 0.1% Triton X-100; pH 7.2), respectively.

ERK2 activities in the immunocomplexes were measured as described previously. In brief, immunocomplexes were incubated with 35 μL of buffer C supplemented with 6 μg of myelin basic protein (Upstate Biotechnology Inc), [γ-32P]ATP (5 μCi), MgCl2 (50 mmol/L), and ATP (30 μmol/L) for 20 minutes at 37°C with vortexing every 3 minutes. To stop the reaction, 15 μL of 4X Laemmlli buffer was added and the mixture was boiled for 5 minutes. Proteins in the kinase reaction were resolved by SDS–polyacrylamide gel electrophoresis (PAGE, 15% gel) and subjected to autoradiography.

The JNK/SAPK assay was performed as described above by using glutathione S-transferase–c-Jun as the substrate (the plasmid was provided by Dr. J. Woodgett, Ontario Cancer Center, Toronto, Canada) produced in E.coli and isolated with glutathione-Sepharose 4B RediPack Columns (Pharmacia Biotech Inc) as per the manufacturer’s protocol. Proteins in the kinase reaction were resolved by SDS-PAGE (12% gel) and subjected to autoradiography.

**Cotransfection and Luciferase Activity Assays**

Human SMCs were seeded at a concentration of 8×104 per 60-mm dish 1 day before transfection. Plasmids pRL-TK–Renilla luciferase (1 μg) and Elk-1–luciferase (1 μg), along with 5 μg of either pSG5 (vector) or constructs expressing sense (pSG5–rMKP-1) or antisense (pSG5–rMKP-1) were mixed with SuperFect reagent in a 1:2 ratio (w/w/v). The mixture was added to SMCs in the medium (3 mL per dish) containing 10% FCS and incubated for 20 hours. The cells were serum-starved for 12 hours and then treated with LDL for 16 hours. Protein extracts were prepared from treated and untreated SMCs and assayed for luciferase activities by using a dual-luciferase reporter assay kit according to the manufacturer’s instructions. Luciferase activities were measured with the Beta-Jet-Luminometer (Wallac). Elk-1 luciferase activity was normalized with respect to Renilla luciferase activity.

**SMC Lines Stably Transfected With MKP-1**

Rat vascular SMCs were transfected stably with MKP-1 plasmid by using the SuperFect reagent as described above. Transfected cells were selected in the presence of G418 (500 μg/mL, Sigma) for 5 weeks, and individual cell colonies were expanded and maintained in culture medium supplemented with 200 μg/mL G418. MKP-1–transfected SMC lines were identified by Western blotting analysis.

**SMC Proliferation Assays**

Transfected SMCs (104), cultured in 96-well plates in medium containing 20% FCS at 37°C for 24 hours, were serum-starved for 4 days. LDL in 2% serum was added, and incubation was continued at 37°C for 24 hours. [H]Thymidine was added 6 hours before cell harvest. Radiation activities were measured with a radiation counter and recorded as counts per minute.

**Statistical Analysis**

ANOVA was performed when >2 groups were compared. An unpaired Student’s t test was used to assess differences between 2 groups. A value of P<0.05 was considered significant.

**Results**

**LDL-Stimulated MKP-1 Induction**

MKP-1 mRNA levels in LDL-treated SMCs were analyzed by Northern blots. As shown in Figure 1A, LDL (100 μg/mL) treatment resulted in significantly increased MKP-1 mRNA in SMCs; kinetic analysis indicated that this response occurred as early as 10 minutes (Figure 1A), with maximum levels (10-fold greater than untreated controls) being achieved 30 minutes after treatment and returning to basal levels by 3 hours. The lower panel of Figure 1A shows the amounts of 28S and 18S RNAs from the corresponding blot. Likewise, growth-arrested SMCs were exposed to 100 μg/mL LDL for various times, and equal amounts of proteins from control and experimental samples were used in Western blots to test MKP-1 induction. As shown in Figure 1B, exposure of cells to LDL resulted in a time-dependent induction of MKP-1 proteins, being evident at 15 minutes, peaking at 30 minutes, and declining thereafter. Figure 1C summarizes MKP-1 protein induction as determined by quantification of optical densities from autoradiograms of 3 experiments. Exposure of cells to LDL produced 5- to 8-fold changes in MKP-1 proteins in protein extracts of SMCs. MKP-1 proteins did not return to baseline by 6 hours after treatment.

To further establish the relationship between LDL treatment and MKP-1 expression, we performed a dose-response analysis.
MKP-1 induction by LDL. These results suggest a similar signal pathway as described by Sachinidis et al., mediated by pertussis toxin–sensitive G proteins that may be involved during MKP-1 gene expression.

**LDL-Induced MKP-1 via Tyrosine Kinase Activation**

There is evidence that LDL stimulation results in activation of inositol phospholipid catabolism and calcium mobilization, in which tyrosine kinases have been shown to be involved. Therefore, we pretreated SMCs with genistein or herbimycin A to inhibit different types of tyrosine kinases. As shown in Figure 5, genistein treatment blocked LDL-stimulated *MKP-1* mRNA induction in human SMCs, and herbimycin A significantly inhibited this induction. Our data support the involvement of genistein- and herbimycin-sensitive tyrosine kinases in LDL-induced MKP-1 expression. Because it has been demonstrated that LDL stimulates superoxide generation in a concentration-dependent manner in neutrophils, we assessed whether LDL-induced MKP-1 expression was mediated by released free radicals via activation of NADPH oxidase. The antioxidant *N*-acetylcysteine, shown to be a superoxide anion scavenger, blocked H₂O₂-induced ERK activation in HeLa cells. Addition of *N*-acetylcysteine to the culture with the pH adjusted did not influence LDL-stimulated MKP-1 production (Figure 6).

**MKP-1 Induction Is Dependent on PKC**

LDL and oxidized-LDL have been shown to activate PKC, and the PKC agonist PMA induces MKP-1 gene expression in several cell types. We confirmed MKP-1 expression induced by low concentrations of PMA in human vascular SMCs (Figure 7A). To determine whether PKC mediates LDL-stimulated MKP-1 induction in SMCs, PKC was either depleted by exposing SMCs to 1 μmol/L PMA for 24 hours or inhibited by incubating the cells with calphostin C before LDL stimulation. Whereas PMA pretreatment for 24 hours significantly reduced *MKP-1* mRNA levels, PMA alone slightly stimulated *MKP-1* mRNA expression (Figure 7B). Similar findings were obtained with the PKC-specific inhibitor calphostin C, ie, significant (80%) inhibition of LDL-stimulated expression (Figure 7C). These results indicate that LDL-stimulated MKP-1 expression is dependent on PKC. We also determined the effects of the phospholipase C inhibitor U73122 on MKP-1 expression, because phospholipase C has been shown to be involved in LDL-stimulated signaling. The data shown in Figure 7D indicate no marked influence on MKP-1 induction by this inhibitor, and Figure 7E summarizes data from 2 or 3 independent experiments, demonstrating a PKC-dependent and phospholipase C–independent MKP-1 induction.

**ERK-Independent MKP-1 Induction**

It has been shown that ERK-MAPKs can be activated by LDL and oxidized-LDL. To investigate whether ERK kinases are involved in LDL-induced MKP-1 expression, activities of both ERK and JNK/ SAPK kinases and MKP-1 induction were simultaneously determined in SMCs pretreated with PD98059, a specific MEK1/2 inhibitor. We confirmed LDL-stimulated ERK activation and demonstrated that LDL-activated ERK1/2 was inhibited by PD98059 in a
concentration-dependent manner (Figure 8A). Kinase assays indicated that 50 μmol/L PD98059 completely blocked ERK2 activation induced by LDL (Figure 8A and 8B) but not JNK/SAPK activation (Figure 8C). MKP-1 induction was not influenced by this inhibitor (Figure 8D). These results support the concept that LDL-induced MKP-1 production is independent of ERK activation.

MKP-1–Inhibited Elk-Mediated Gene Expression During LDL Stimulation

Elk-1, an essential transcription factor for cell growth, has been shown to be activated by both MAPKs, ERK and JNK/SAPK. It has been demonstrated that MKP-1 inactivates ERK in several cell types, including SMCs, but whether MKP-1 inhibits Elk-1–mediated gene expression in SMCs induced by LDL stimulation remains unclear. To address this question, human SMCs were cotransfected with Elk-1 luciferase plasmids and constructs expressing rMKP-1 in the sense or antisense orientation. Transfected SMCs were subsequently treated with LDL, and Elk-1–mediated gene expression was determined on the basis of luciferase activity. Figure 9 indicates that MKP-1 expression inhibited Elk-1 luciferase activity, LDL stimulation increased Elk-1 luciferase activity 5-fold in the absence of rMKP-1, and rMKP-1 had little effect on the basal levels of Elk-1 luciferase but significantly (>60%) inhibited induction in response to LDL stimulation. In contrast, the construct expressing antisense rMKP-1 enhanced Elk-1 luciferase expression (Figure 9). These results suggest that MKP-1 expression blocks Elk-1–
mediated gene transcription, possibly via inactivation of both ERK and JNK/SAPK in SMCs stimulated by LDL.

MKP-1–Inhibited ERK Activation and SMC Proliferation

To directly determine the influence of MKP-1 on MAPK inactivation and the effects of MKP-1 on SMC proliferation, we established stably transfected-SMC lines overexpressing MKP-1 and determined ERK phosphorylation and DNA synthesis in the transfected cell lines in response to LDL. Because the antibody recognizes both endogenous and overexpressed rMKP-1, MKP-1 proteins of transfected SMCs were detected with Western blot analysis after stimulation with a low concentration of serum (2%). Figure 10A data indicate that MKP-1 is overexpressed in MKP-1–transfected cells and that such treatment did not significantly induce endogenous MKP-1 expression implicated in the pSG5 vector–transfected cells. In comparison with cells transfected with vector, rMKP-1–transfected SMCs showed a 40% to 60% reduction in ERK phosphorylation when stimulated with LDL or serum (Figure 10B). To determine the effects of MKP-1 on DNA synthesis induced by LDL, rMKP-1– or vector-transfected cell lines were treated with LDL. [3H]Thymidine incorporation in rMKP-1–transfected SMCs was significantly lower than in vector-transfected cells (Figure 10C), indicating the role of MKP-1 in the inhibition of SMC growth.

Discussion

Proliferation of vascular SMCs is a hallmark in the pathogenesis of atherosclerotic lesions. LDLs are mitogenic to cultured SMCs and have been demonstrated to activate MAPK-ERK signal pathways.15–18 In the present study, we have provided the first evidence that LDLs can stimulate MKP-1 expression, which is crucial in the regulation of MAPK activities in SMCs. MKP-1 serves as a negative regulator, controlling cell growth via inactivation of both MAPKs, because LDL-stimulated ERK and JNK/SAPK activation appears to be a component common to signaling pathways initiated by a wide range of growth-stimulating factors, including mitogens and hormones.50–52 Thus, our findings could significantly advance our understanding of the possible role of MKP-1 under physiological conditions or during pathological changes, ie, inhibition of SMC proliferation stimulated by LDL.

It is well known that LDL specifically binds to apoB/E receptors to deliver cholesterol to SMCs,1 but LDL-initiated signal transduction pathways leading to SMC proliferation are not fully understood. In the present study, we have demonstrated that LDL-induced MKP-1 expression is mediated by tyrosine kinases and PKC, independent of LDL receptors and ERK-MAPKs. LDL-stimulated MKP-1 expres-
Activation was observed in either human or rat SMCs or in LDL receptor–deficient fibroblasts, and such induction was not influenced by heparin, an inhibitor of LDL-receptor binding. The mechanism by which LDL initiates signaling in LDL-stimulated MKP-1 induction is presently unknown, and we hypothesize that 2 pathways may be responsible for the induction: (1) An atypical LDL binding site on human SMCs, characterized as being independent of the classic receptors, may mediate LDL (lipid)-induced tyrosine kinase activation and MKP-1 expression. (2) Neutral sphingomyelinase present on cell membranes might directly catabolize the sphingomyelin of LDL to generate ceramide, which serves as a second messenger between LDL stimulation and tyrosine kinase activation. This concept is supported by the fact that sphingomyelinase-treated LDL markedly enhanced MKP-1 induction and that ceramide treatment mimicked MKP-1 induction in SMCs (Figure 3D and 3E). In addition, it has been shown that HDL stimulates MAPK activation in human skin fibroblasts. We have also observed that HDL moderately induces MKP-1 expression in SMCs (data not shown) and that arachidonic acid stimulation results in MKP-1 induction. Our findings together with other reports further...
support the role of the lipid moiety of lipoproteins in the regulation of expression of this gene.

Recently, Suc et al.\(^\text{57}\) reported that oxidized LDL and native LDL (to a lesser extent) directly stimulated endothelial growth factor receptor phosphorylation or activation and subsequently activated phospholipase C and inositol trisphosphate kinases. In the present experiment, we did not find any involvement of phospholipase C in LDL-induced MKP-1 expression (Figure 7). Interestingly, pertussis toxin significantly inhibited MKP-1 induction, indicating that pertussis toxin–sensitive G-protein signal pathways may be involved. In agreement with our findings, Sachinidis et al.\(^\text{17}\) demonstrated that LDL stimulates Ca\(^{2+}\) elevation and ERK activation via a pertussis toxin–sensitive G-protein pathways. LDL might also elicit G protein–coupled receptor conformation or activation, by which LDL initiates signals leading to cell growth. It would be interesting to clarify whether the atypical LDL binding site described by Tkachuk et al.\(^\text{54}\) is a G protein–coupled receptor.

PKC, a large and diverse family of protein kinases, plays important signaling roles in cell growth, differentiation, and homeostasis\(^\text{58,59}\) and can be activated by LDL.\(^\text{42,45,46}\) Tyrosine kinases, such as Pyk2 and Src-related kinases, have been shown to be essential intermediates linking upstream kinases and MKP-1 gene expression. In our system, PKC and tyrosine kinase inhibitors significantly blocked LDL stimulation of MKP-1, indicating that both PKC and tyrosine kinases play important roles during signaling. Both PKC and tyrosine kinases activate MAPK pathways via phosphorylation and activation of Ras, or c-Raf kinases and MEK.\(^\text{6–11,60}\)

These pathways may be important in LDL-induced signaling, because LDL simultaneously activates both ERK\(^\text{15–18}\) and JNK/SAPK (Figure 8), which share a common point during activation via Ras.\(^\text{5–11}\) Interestingly, Bokemeyer et al.\(^\text{61}\) recently demonstrated that JNK/SAPK activation is responsible for MKP-1 gene expression, at least in fibroblasts. Our data, together with the other noted observations, support the role of JNK/SAPK in LDL-stimulated MKP-1 induction.

As described above, atherosclerosis and its complications, including myocardial infarction and stroke, are the most prevalent cause of morbidity and mortality in Western countries. During atherogenesis, early lesions spread progressively and form atherosclerotic plaques. In this process, SMC proliferation plays a key role.\(^\text{4}\) Although multiple factors, including growth factors, cytokines, mechanical stress, neurotransmitters, and hormones, are believed to contribute to the process leading to SMC growth,\(^\text{4,62}\) LDL levels in the blood, strongly predictive of coronary heart disease, play an important role in atherogenesis. In addition to cholesterol transport, LDLs are mitogenic to vascular SMCs.\(^\text{2–4}\) LDL
stimulates SMCs to generate both positive (ERK–Elk-1 pathway) and negative (MKP-1) signals. MKP-1 may inactivate both MAPKs, ERK and JNK/SAPK, and block Elk-1 transcription factor activation during LDL stimulation. The balance between MKP-1 and MAPK levels/activities stimulated by LDL in SMCs is critical for maintaining homeostasis of the arterial wall. If LDL-initiated signals leading to MAPK activation and MKP-1 induction can be arbitrarily dissected, ie, switched on for MKP-1 or switched off for ERK in vivo, new strategies could be developed for the prevention or treatment of atherosclerosis.

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References


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