β-Migrating Very Low Density Lipoprotein (βVLDL) Activates Smooth Muscle Cell Mitogen-activated Protein (MAP) Kinase via G Protein-coupled Receptor-mediated Transactivation of the Epidermal Growth Factor (EGF) Receptor

EFFECT OF MAP KINASE ACTIVATION ON βVLDL PLUS EGF-INDUCED CELL PROLIFERATION

Dezheng Zhao, Jennifer Letterman, and Barbara M. Schreiber‡

From the Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

This study examined the premise that the atherogenic lipoprotein, β-migrating very low density lipoprotein (βVLDL), might activate the mitogen-activated protein (MAP) kinases ERK1/ERK2, thereby contributing to the induction of smooth muscle cell proliferation in atherosclerosis. The data show that βVLDL activates rabbit smooth muscle cell ERK1/ERK2. Interestingly, ERK1/ERK2 activation is mediated by G protein-coupled receptors that transactivate the epidermal growth factor (EGF) receptor. βVLDL-induced MAP kinase activation depends on Ras and Src activity as well as protein kinase C. The inhibition of lysosomal degradation of βVLDL has no effect on ERK1/ERK2 activation. The contribution of βVLDL-induced activation of ERK1/ERK2 to smooth muscle cell proliferation was also explored. βVLDL induces expression of egr-1 and c-fos mRNA. Despite its ability to stimulate early gene expression, βVLDL alone is unable to induce quiescent cells into S phase. When added in conjunction with EGF, however, stimulation of [3H]thymidine incorporation into DNA as well as in histone gene expression are observed. Moreover, βVLDL plus EGF synergistically induce cyclin D1 expression and down-regulate p27KIP1 expression. The addition of either βVLDL or EGF stimulates a robust activation of ERK1/ERK2, but the addition of both agents simultaneously sustains the activation for a longer time period. Inhibition of MAP kinase kinase, pertussis toxin-sensitive G proteins, the EGF receptor, or protein kinase C blocks βVLDL-induced proliferation, demonstrating that activation of the βVLDL-induced signaling pathway results in smooth muscle cell proliferation.

Elevated plasma lipids are associated with a risk for the development of atherosclerosis, a disease now believed to be an inflammatory one characterized by vascular lesions containing lipid, macrophages, T cells, and fibrous components. Smooth muscle cell migration from the medial layer of the vessel to the intima accompanied by proliferation is critical to the development of the plaque (1). A potential relationship between hyperlipidemia and disease progression has been suggested in studies showing that atherogenic lipoproteins such as low density lipoprotein (LDL)1 and oxidized LDL induce smooth muscle cell proliferation (2–11). β-migrating very low density lipoprotein (βVLDL) is a cholesteryl ester-rich atherogenic lipoprotein that accumulates in the plasma of cholesterol-fed animals and humans with type III hypercholesterolemia (12). Earlier we showed that βVLDL enhances smooth muscle cell growth potentiating activity of monocytes/macrophages (13). Moreover, βVLDL added directly to cultured smooth muscle cells stimulates the rate of cell proliferation (14, 15).

It has been shown that smooth muscle cell proliferation can be achieved via the activation of mitogen-activated protein (MAP) kinases (16). The importance of MAP kinase activation to smooth muscle cell proliferation in vivo has been shown in balloon injury models (17–20). LDL (21, 22) as well as modified and oxidized LDL (11, 22–24) have been shown to stimulate MAP kinases in smooth muscle cells, suggesting a potential link to their growth-promoting ability. Treatment with LDL results in the activation of protein kinase C and MAP kinase as well as the induction of the cell cycle-related genes c-fos, c-myc (5), and early growth response gene-1 (egr-1; Ref. 25). Interestingly, it has been shown that the activation of MAP kinase can induce not only cell proliferation but differentiation as well, highlighting the importance of measuring biological responses downstream of MAP kinase activation (26–30).

The MAP kinases ERK1/ERK2 (p44MAPK and p42MAPK, respectively) are stimulated in pathways initiated by extracellular stimuli that activate receptor tyrosine kinases such as the epidermal growth factor (EGF) receptor or nontyrosine kinase receptors including G protein-coupled receptors. Phosphorylation of the EGF receptor can activate Ras, commencing the Raf-1→MAP kinase kinase (MEK)→MAP kinase cascade. Pertussis toxin-sensitive G protein-coupled receptors such as those for lysophosphatidic acid (LPA), thrombin, and α2-adrenergic agonists stimulate the MAP kinase pathway by Gi-mediated Ras activation as shown in Rat-1 cells (31). LPA-mediated activation of MAP kinase via a pertussis toxin-sensitive transactivation of the EGF receptor has also been described (32, 33), and Ras-independent activation of MAP kinase through pro-

* This work was supported by National Institutes of Health Grants AG-9006 and HL-13262. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed: Dept. of Biochemistry, 715 Albany St., Boston, MA 02118. Tel.: 617-638-5094; Fax: 617-638-5339; E-mail: schreibe@biochem.bumc.bu.edu.

1 The abbreviations used are: LDL, low density lipoprotein; βVLDL, β-migrating very low density lipoprotein; MAP, mitogen-activated protein; EGF, epidermal growth factor; MEK, MAP kinase kinase; LPA, lysophosphatidic acid; DMEM, Dulbecco's modified Eagle's Medium supplemented with 3.7 g/liter NaHCO3, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.1 mM minimum Eagle's medium nonessential amino acids and 1 mM minimum Eagle's medium sodium pyruvate solution; FBS, fetal bovine serum; bFGF, basic fibroblast growth factor; PMA, phorbol-12-myristate-13-acetate; tet, tetracycline.
tein kinase C has been noted (34).

Although lipopolysaccharides have been shown to activate MAP kinases, the molecular events upstream of MAP kinases leading to their activation remain uncertain. Moreover, the possibility that the atherogenic lipoprotein βVLDL activates MAP kinases has not been explored. We sought to determine whether βVLDL activates MAP kinase and, if so, what the targets upstream of MAP kinase might be. Interestingly, the data show that βVLDL activates the MAP kinases ERK1/ERK2 via a G protein-coupled receptor that transactivates the EGF receptor, is Ras- and Src-dependent, and involves protein kinase C. Additional experiments examining the effect of βVLDL on the proliferation of quiescent aortic smooth muscle cells showed that despite its ability to activate MAP kinase, βVLDL alone does not stimulate quiescent cells to enter the S phase of the cell cycle. In combination with EGF, however, serum-deprived aortic smooth muscle cells enter S phase. Moreover, the stimulation of proliferation is mediated through a sustained activation of MAP kinase. To our knowledge, this is the first report demonstrating that a lipoprotein can transactivate the EGF receptor via activation of a pertussis toxin-sensitive G protein-coupled receptor.

**EXPERIMENTAL PROCEDURES**

Isolation of Aortic Smooth Muscle Cells—Neonatal rabbit aortic smooth muscle cells were isolated as described previously (14, 15). Experiments were performed by plating into first or second passage at a density of 2 × 10⁴/cm² (except for Ras expression studies in which cells were seeded at 0.52 × 10⁴/cm² in Dulbecco's modified Eagle's Medium (J.R.H. Biosciences, Lenexa, KS) supplemented with 3.7 g/liter NaHCO₃, 100 units/ml penicillin, 100 μg/ml streptomycin, 1.0 μM sodium pyruvate, and 1 μM EDTA, 0.2 mM sodium vanadate, 1 μM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 1 μg/ml leupeptin, and 2 μg/ml pepstatin). Cells were washed twice with cold PBS and incubated on ice for 10 min with 1 μl of radiolabeled precipitation buffer (20 μM Tris, pH 7.5, 0.15 μM NaCl, 1% Triton X-100, 1 mM Na₃VO₃, 1 mM EGTA, 1 mM phenylmethylsulfonil fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 2 μg/ml pepstatin). Lysates were centrifuged at 13,000 rpm (Eppendorf centrifuge, 4°C for 10 min) to remove insoluble material. The protein concentration was determined by the BCA method. The BCA method. The data show that expression levels in transient transfections were so high that experiments were performed on transiently transfected cultures, as shown previously by Paulus et al. (38), and the puromycin resistance gene (puro) that was precut with HinIII and ClaI and then blunt-ended with the Klenow enzyme. The puro fragment was ligated into pBPST-4 (see below) that was digested with EcoRI and BamHI and ligated into pBPST-4 (see below) that was digested with EcoRI and BamHI and ligated into pBPST-4. The ras-17N- and β-galactosidase-expressing vectors were referred to as pBPST-Ras-17N and pBPST-βgal, respectively.

The pBPST-4 expression plasmid was generated originally to make ras-17N as shown previously by Paulus et al. (38), and the puromycin resistance gene (puro) that was precut with HinIII and ClaI and then blunt-ended with the Klenow enzyme. The puro fragment was ligated into pBPST-4 (see below) that was digested with EcoRI and BamHI and ligated into pBPST-4. The ras-17N- and β-galactosidase-expression vectors were referred to as pBPST-Ras-17N and pBPST-βgal, respectively.

Formation of a Dominant Negative Ras Expression Vector—DNA restriction endonucleases, DNA polymerase I large fragment (Klenow), and T4 DNA ligase were purchased from New England Biolabs. The dominant negative ras mutant (ras-17N) prepared by Feig and Cooper (36) was kindly provided by Dr. Debabrata Mukhopadhyay, Beth Israel Hospital, Boston, MA) was pretreated with EcoRI and BamHI and ligated into pBPST-4 (see below) that was digested with EcoRI and BamHI and ligated into pBPST-4 (see below) that was digested with EcoRI and BamHI and ligated into pBPST-4. The ras-17N- and β-galactosidase-expression vectors were referred to as pBPST-Ras-17N and pBPST-βgal, respectively.

The pBPST-4 expression plasmid was generated originally to make stable transfectants and incorporates a tetracycline (tet)-inducible system as shown previously by Paulus et al. (37). Preliminary studies showed that expression levels in transient transfections were so high that experiments were performed on transiently transfected cultures, and thus the inducible nature of the vector was not taken advantage of in these studies. A tet-inducible retroviral vector was constructed from the tet-inducible expression system containing two plasmids, pBPST-Ras-17N and pUHD15, in which the puro fragment was ligated into pBPST-4. The ras-17N- and β-galactosidase-expression vectors were referred to as pBPST-Ras-17N and pBPST-βgal, respectively.

The pBPST-4 expression plasmid was generated originally to make stable transfectants and incorporates a tetracycline (tet)-inducible system as shown previously by Paulus et al. (37). Preliminary studies showed that expression levels in transient transfections were so high that experiments were performed on transiently transfected cultures, and thus the inducible nature of the vector was not taken advantage of in these studies. A tet-inducible retroviral vector was constructed from the tet-inducible expression system containing two plasmids, pBPST-Ras-17N and pUHD15, in which the puro fragment was ligated into pBPST-4. The ras-17N- and β-galactosidase-expression vectors were referred to as pBPST-Ras-17N and pBPST-βgal, respectively.
Sn7/2Bln 5'AGCTTAAAGTAT-3' and 3'AATGATTACG-5'.

The pBPS-Sn7/2Bln was cut with Sn7/2Bln, dephosphorylated with calf intestinal phosphatase, and then ligated with the tet-regulated transcription activator (tTA) gene, which was isolated from pUHD15-1 with EcoRI and BamHI and blunt-ended with Klenow enzyme. The new plasmid was named pBPS1. To generate pBPS1-4, a 0.88-kb fragment containing the tet-inducible promoter tetO-CMV was extracted from pUHD10-3 with HindIII and inserted in reverse orientation to the internal SV40 promoterinto pBPS1, which was digested with ClaI.

Transient Transfection—Transfection was performed using Effectene transfection reagent according to manufacturer instructions (Qiagen). Briefly, smooth muscle cells were seeded at a density of 5.2 × 10^4 cells/well. After an overnight incubation, the cells were washed and incubated in 0.9 ml of DMEM with 10% FBS for 2-4 h. For each transfection, 0.8 μg of DNA (pBPS-Ras-17N or pBPS-Ras-lacZ) were added and incubated at 37 °C for 16-24 h, at which time the medium was removed, and the cells were washed with Fuc's saline and incubated in serum-free DMEM for 48 h. The cells were then control-treated or treated with pFGF, EGF, LPA, or βVLDL for 5 min. Total cell protein was isolated and subjected to Western blot analysis using antibodies against Ras and the active forms of ERK1/ERK2. To examine the transfection efficiency, the cells were fixed in 0.5% glutaraldehyde and incubated in 50 mM ferrocyanide, 2 mM MgCl2, and 25 mM 5-bromo-4-chloro-3-indoly-β-n-galactoside (Sigma) in PBS at 37 °C overnight. The percentage of stained (β-galactosidase-positive) cells was evaluated.

Effect of βVLDL and EGF on Cell Proliferation—Quiescent smooth muscle cells were treated with βVLDL, EGF (Sigma), βVLDL plus EGF, or FBS for the indicated time. Total RNA was isolated and subjected to Northern blot analysis to measure the steady-state levels of expression of egr-1, c-fos, and histone H2B (see below). DNA synthesis was measured by labeling cells with [3H]thymidine and evaluating its incorporation into DNA and the percentage of radiolabeled nuclei (see below). In some cases, cells were pretreated in the absence or presence of the following inhibitors prior to the addition of reagents: PD98059, bisindolymaleimide I, AG1478, or pertussis toxin.

Northern Blot Analysis—Total cellular RNA was extracted, and Northern blot analysis was performed essentially as described previously; once rendered quiescent, the cells were incubated with reagents for 20 h, at which time the medium was removed, and the cells were washed with serum-free DMEM. The cells were radiolabeled for 4 h with 100 μl of [methyl-3H]thymidine (20 μCi/ml, 5 Ci/mmol; PerkinElmer Life Sciences) in serum-free DMEM. Cells were harvested, and [3H]thymidine incorporation into DNA was assessed as described previously (15). Alternatively, cells similarly treated in 6-well trays were evaluated for the percentage of radiolabeled nuclei as follows: the [3H]thymidine-containing medium was removed, and the cells were washed three times with cold PBS and then fixed for 2 min with 100% methanol. The cells were exposed to a photographic emulsion (1:1 Kodak NTB-2/H2O; Eastman Kodak Co.) for 3 days at room temperature in the dark, at which time the radiolabeled nuclei were visualized after counterstaining of the cells with hematoxylin. The percentage of radiolabeled nuclei was determined by counting both positive and negative nuclei and expressing the number of positives as a percentage of the total.

RESULTS

βVLDL Activates the MAP Kinase Pathway—To determine whether βVLDL activates MAP kinase, the phosphorylation of MAP kinase isoforms ERK1/ERK2 was monitored in neonatal rabbit aortic smooth muscle cells. Fig. 1a shows that βVLDL-activated ERK1/ERK2 in a dose-responsive fashion at concentrations ranging from 3-100 μg/ml βVLDL. Total levels of ERK1/ERK2 were similar in control as well as βVLDL-treated samples (Fig. 1b). At a concentration of 30 μg/ml, βVLDL induced a time-dependent activation of ERK1/ERK2 with a maximal response noted after 5 min in the presence of the lipoprotein (Fig. 1c). Phosphorylated ERK1/ERK2 was measured as described for a, d, smooth muscle cells were preincubated in the absence (−) or presence (+) of PD98059 (20 μM) for 30 min and then incubated in the absence (−) or presence (+) of βVLDL (30 μg/ml) for 5 min. Phosphorylated ERK1/ERK2 was measured as described for a.

VLDL Activates Smooth Muscle Cell MAP Kinase

VLDL Activates Smooth Muscle Cell MAP Kinase

Fig. 1a. VLDL activates MAP kinase. a, smooth muscle cells were treated with increasing concentrations of βVLDL (3-100 μg/ml) for 5 min. Total cell protein was extracted and subjected to Western blot analysis using an antibody against phosphorylated ERK1/ERK2. b, smooth muscle cells were treated as described for a. The samples were subjected to Western blot analysis using an antibody against total ERK1/ERK2. c, smooth muscle cells were treated with βVLDL (30 μg/ml) for various times (5 min-15 h). Phosphorylated ERK1/ERK2 was measured as described for a. d, smooth muscle cells were preincubated in the absence (−) or presence (+) of PD98059 (20 μM) for 30 min and then incubated in the absence (−) or presence (+) of βVLDL (30 μg/ml) for 5 min. Phosphorylated ERK1/ERK2 was measured as described for a.

βVLDL Activates a Pertussis Toxin-sensitive G Protein-coupled Receptor That Transactivates the EGF Receptor—To analyze the contribution of pertussis toxin-sensitive G proteins to the βVLDL-induced MAP kinase activation, the effect of pertussis toxin on the phosphorylation of ERK1/ERK2 by βVLDL was examined. The data in Fig. 2a show that pretreatment with pertussis toxin (100 ng/ml) for 24 h inactivated pertussis toxin-sensitive G protein-coupled receptors as evidenced by a loss of LPA-induced MAP kinase activation. As expected, the activation by EGF was unaffected by pertussis toxin treatment. Interestingly, the data clearly show that the βVLDL-induced activation of MAP kinase is mediated by G protein-coupled receptors, because its effect was diminished by the addition by pertussis toxin.

In a preliminary study, Western blot analysis of βVLDL-treated cells was performed using an antibody against phosphotyrosine residues. The data (not shown) suggested that a 170-kDa protein was phosphorylated in response to βVLDL. The EGF receptor is 170 kDa and has been shown to be transactivated by LPA (32). To determine directly whether βVLDL...
activated the EGF receptor, AG1478, a specific EGF receptor tyrosine kinase inhibitor, was used. The cells were pretreated with the inhibitor, and then bFGF, EGF, LPA or βVLDL, was added for 5 min, at which time MAP kinase activity was assessed. The data in Fig. 2b show that low levels of MAP kinase activity in control cultures were inhibited by AG1478, suggesting that baseline activity results from activation of the EGF receptor. As expected, bFGF-induced activation of MAP kinase was unaffected by AG1478. The inhibitor was shown to be effective in that MAP kinase activation by EGF was completely abolished in its presence. LPA-induced activation of smooth muscle cell MAP kinase was partially mediated by EGF receptor tyrosine kinase, as shown previously in Rat-1 fibroblasts (31). Finally, the data show that AG1478 partially inhibited the ability of βVLDL to activate MAP kinase, furthering the hypothesis that it transactivates the EGF receptor.

To pursue the possibility that pertussis toxin-sensitive G proteins mediate the βVLDL-induced EGF receptor transactivation, smooth muscle cells were treated with pertussis toxin for 24 h and then with EGF or βVLDL for 5 min. The EGF receptor was immunoprecipitated, and then Western blot analysis was performed with an antibody against phosphotyrosine residues to visualize activated receptors. The data in Fig. 2c show that βVLDL induced tyrosine phosphorylation of the EGF receptor. As expected, phosphorylation of the EGF receptor by EGF was unaffected by pertussis toxin. Confirmation of the activation of the EGF receptor was achieved by the demonstration that pertussis toxin completely blocked the EGF receptor tyrosine phosphorylation that was induced by βVLDL.

To determine whether the MAP kinase activation by a pertussis toxin-sensitive G protein-coupled receptor is entirely mediated through transactivation of the EGF receptor, the cells were pretreated either with pertussis toxin, AG1478, or pertussis toxin plus AG1478, and then βVLDL was added and MAP kinase activation was measured. The data (Fig. 2d) show that pertussis toxin had no additional effect on the partial inhibition by AG1478, suggesting that the pathway to βVLDL-mediated MAP kinase activation by a G protein-coupled receptor was entirely through transactivation of the EGF receptor.

βVLDL Activates Src Kinase—To determine whether Src is involved in βVLDL-induced MAP kinase activation, smooth muscle cells were pretreated with the specific Src family tyrosine kinase inhibitor PP2. The data in Fig. 3 show that PP2 inhibited MAP kinase activation by LPA but had no effect on EGF-induced activation of MAP kinase at low doses, as expected. Furthermore, the data show that pretreatment with the inhibitor significantly decreased the activation of MAP kinase by βVLDL.

Protein Kinase C Mediates βVLDL-induced MAP Kinase Activation—Protein kinase C was shown to be activated by LDL (5). To determine whether protein kinase C is involved in βVLDL-induced MAP kinase activation, smooth muscle cells were pretreated with a protein kinase C inhibitor, bisindolylmaleimide I, for 30 min and then treated with EGF, PMA, or βVLDL. The data in Fig. 4a show that as expected, the inhibitor did not affect EGF-induced MAP kinase activation but completely blocked PMA-induced MAP kinase activation (42, 43). Interestingly, βVLDL-induced MAP kinase activation was substantially blocked by pretreatment with the inhibitor. To further substantiate the role of protein kinase C and to see if the activation of MAP kinase partially or fully depended on protein kinase C, the cells were pretreated with either increasing doses of bisindolylmaleimide I, with the protein kinase C inhibitor calphostin C, or with PMA and then treated with βVLDL. The data in Fig. 4b show that although bisindolylmaleimide I only partially inhibited the βVLDL-induced activation of MAP kinase (except with very high doses of the inhibitor), pretreatment with calphostin C at doses known to specifically inactivate protein kinase C completely blocked the βVLDL-induced activation of MAP kinase. Likewise, cells that were depleted of protein kinase C by overnight treatment with PMA were vir-
bFGF-, EGF-, LPA-, or ion (31, 44). Finally, the data clearly show that the function of the Ras mutant was displayed by the inhibition of baseline MAP kinase activity and that the expression of ras-17N was equivalent in untreated and bFGF (20 ng/ml), EGF (20 ng/ml), or LPA (25 μM), or bVLDL (30 μg/ml) for 5 min. Total cell protein was extracted and subjected to Western blot analysis using an antibody against Ras. 

**Fig. 4.** Protein kinase C mediates bVLDL-induced MAP kinase activation. a, smooth muscle cells were preincubated in the absence (−) or presence (+) of bisindolylmaleimide I (1 μM) for 30 min and then control-treated or treated with EGF (20 ng/ml), PMA (100 ng/ml), or bVLDL (30 μg/ml) for 5 min. Phosphorylated ERK1/ERK2 was measured as described in the Fig. 1a legend. b, smooth muscle cells were preincubated with various concentrations of bisindolylmaleimide I (0.5, 10, and 20 μM) for 30 min or with calphostin C (0.5 and 2.5 μM) or PMA (100 and 500 ng/ml) overnight and then control-treated or treated with bVLDL (30 μg/ml) for 5 min. Phosphorylated ERK1/ERK2 was measured as described in the Fig. 1a legend.

Dominant Negative Ras Mutant Blocks bVLDL-induced MAP Kinase Activation—To determine whether Ras is required for bVLDL-induced MAP kinase activation, a dominant negative mutant form of Ras with an asparagine substituted for serine at position 17 (36) was expressed as described under “Experimental Procedures.” Ras expression was determined by Western blotting. Controls were provided by cells transfected with the same plasmid expressing β-galactosidase (pBPST-lacZ) instead of ras-17N. Initial experiments were performed with pBPST-lacZ to determine transfection efficiency; although it did vary somewhat from experiment to experiment, it was never lower than 20% and was sometimes as high as 60%. Most importantly, in cultures transfected with pBPST-Ras-17N, the levels of Ras were much higher than those of endogenous Ras (Fig. 5a), suggesting that this approach could be used to determine whether bVLDL activates Ras (the additional lanes show that the expression of ras-17N was equivalent in untreated and bFGF-, EGF-, LPA-, or bVLDL-treated cultures). To determine the effect of loss of Ras activity on bVLDL-induced MAP kinase activation, the levels of phosphorylated ERK1/ERK2 were evaluated in cultures transiently transfected with pBPST-Ras-17N and pBPST-lacZ. The data in Fig. 5b show that the dominant negative mutant inhibited baseline MAP kinase activity and that the function of the Ras mutant was displayed by the inhibition of MAP kinase activation by bFGF, EGF, and LPA, all previously shown to activate MAP kinase in a Ras-dependent fashion (31, 44). Finally, the data clearly show that bVLDL-induced stimulation of MAP kinase was inhibited by expression of the Ras mutant, demonstrating that it is a Ras-dependent activation.

**Fig. 5.** Dominant negative mutant ras-17N blocks bVLDL-induced MAP kinase activation. a, smooth muscle cells seeded at low density were transfected with pBPST-Ras-17N or pBPST-lacZ and then incubated in serum-free medium for 2 days. The cells were then control-treated or treated with bFGF (20 ng/ml), EGF (20 ng/ml), LPA (25 μM), or bVLDL (30 μg/ml) for 5 min. Total cell protein was extracted and subjected to Western blot analysis using an antibody against Ras. 

**βVLDL Activates Smooth Muscle Cell MAP Kinase**

To determine whether Ras is required for bVLDL-induced MAP kinase activation, a dominant negative mutant form of Ras with an asparagine substituted for serine at position 17 (36) was expressed as described under “Experimental Procedures.” Ras expression was determined by Western blotting. Controls were provided by cells transfected with the same plasmid expressing β-galactosidase (pBPST-lacZ) instead of ras-17N. Initial experiments were performed with pBPST-lacZ to determine transfection efficiency; although it did vary somewhat from experiment to experiment, it was never lower than 20% and was sometimes as high as 60%. Most importantly, in cultures transfected with pBPST-Ras-17N, the levels of Ras were much higher than those of endogenous Ras (Fig. 5a), suggesting that this approach could be used to determine whether bVLDL activates Ras (the additional lanes show that the expression of ras-17N was equivalent in untreated and bFGF-, EGF-, LPA-, or bVLDL-treated cultures). To determine the effect of loss of Ras activity on bVLDL-induced MAP kinase activation, the levels of phosphorylated ERK1/ERK2 were evaluated in cultures transiently transfected with pBPST-Ras-17N and pBPST-lacZ. The data in Fig. 5b show that the dominant negative mutant inhibited baseline MAP kinase activity and that the function of the Ras mutant was displayed by the inhibition of MAP kinase activation by bFGF, EGF, and LPA, all previously shown to activate MAP kinase in a Ras-dependent fashion (31, 44). Finally, the data clearly show that bVLDL-induced stimulation of MAP kinase was inhibited by expression of the Ras mutant, demonstrating that it is a Ras-dependent activation.

**βVLDL-induced MAP Kinase Activation Is Independent of its Degradation and Internalization**—βVLDL binds to LDL receptors and/or LDL receptor-related protein (LRP) and undergoes receptor-mediated endocytosis, subsequently being degraded in lysosomes. Free cholesterol released from lysosomes is esterified into cholesteryl ester in the endoplasmic reticulum (45, 46). To determine whether degradation of βVLDL is critical to its ability to activate MAP kinase, chloroquine was used to inhibit lysosomal function. Chloroquine (10 μM) inhibited the βVLDL-induced esterification of cholesterol (Fig. 6a). It had no effect on βVLDL-induced MAP kinase activation, however, suggesting that lysosomal degradation of the lipoprotein is not required (Fig. 6b).

**Induction of egr-1 and c-fos by βVLDL Is Sensitive to Inhibition of the MAP Kinase Cascade**—Because the activation of MAP kinase has been associated with cell proliferation and differentiation, it is important to measure biological responses downstream of MAP kinase activation (26–30). To determine the potential role of the signaling cascade induced by βVLDL, its contribution to smooth muscle cell proliferation was assessed. The induction of immediate early genes such as egr-1 and c-fos is crucial for growth factor-stimulated cell proliferation, and we first determined whether βVLDL can induce egr-1 and c-fos expression. Cultures were treated with 30 μg/ml βVLDL, and the expression of egr-1 and c-fos was examined by Northern blot analysis. As shown in Fig. 7a, βVLDL induced a rapid increase in both egr-1 and c-fos mRNA, with a maximal level achieved at 30 min. Fig. 7b shows that the induction of egr-1 and c-fos was dose-dependent, with maximal expression occurring at a lipoprotein concentration of 50–100 μg/ml βVLDL. The role of the MAP kinase cascade in the induction of egr-1 and c-fos by βVLDL was examined by determining the effect of PD98059 on expression of the genes. Pretreatment of smooth muscle cells with PD98059 (20 μM) for 30 min dramatically decreased the induction of both genes by βVLDL (Fig. 7c).

**βVLDL Plus EGF Synergistically Promote Smooth Muscle Cell Proliferation**—The effect of βVLDL on the proliferation of quiescent smooth muscle cells was examined. Quiescent cells were treated with either βVLDL or EGF (20 ng/ml), βVLDL plus EGF, or increasing concentrations of FBS (v/v). The cells were incubated for 20 h, at which time [3H]thymidine was added as described under “Experimental Procedures.” Four hours later, the cells were harvested, and the level of [3H]thymidine incorporation into DNA was determined. The data in Fig. 8 show that [3H]thymidine incorporation in the control cultures was very low and that the cells responded with a proliferative burst to the addition of FBS. (Additional experimentation demonstrated that EGF was effective in the 5–25 ng/ml range (data not shown). Moreover, Fig. 8 shows that the addition of βVLDL or EGF alone elicited only small increases in [3H]thymidine incorporation. The stimulation of [3H]thymidine incorporation into DNA induced by treatment of the cells with both 100 μg/ml βVLDL and EGF was robust. To further verify the proliferative capacity of this regimen, its effect on histone expression was determined by Northern blot analysis
was harvested, and steady-state levels of egr-1 and c-fos mRNA were assessed by Northern blot analysis. a, quiescent smooth muscle cells were treated with βVLDL (30 μg/ml) for the time indicated. Total RNA was harvested, and steady-state levels of egr-1 and c-fos mRNA were assessed by Northern blot analysis. b, quiescent smooth muscle cells were treated with the indicated concentration of βVLDL for 30 min, at which time total RNA was harvested, and steady-state levels of egr-1 and c-fos mRNA were assessed by Northern blot analysis. c, quiescent smooth muscle cells were preincubated in the absence (–) or presence (+) of the MEK inhibitor PD98059 (20 μM) for 30 min and then incubated in the absence (–) or presence (+) of βVLDL (30 μg/ml) for 30 min. Total RNA was harvested, and steady-state levels of egr-1 and c-fos mRNA were assessed by Northern blot analysis (10 μg/lane).

Likewise, treatment with βVLDL plus EGF resulted in an increase in the expression of steady-state levels of histone mRNA, although either agent alone was not sufficient to alter its expression. Similar experiments in which the percentage of [3H]thymidine-labeled nuclei was examined substantiated these results as shown in Table I, i.e., initially very few of the nuclei were radiolabeled (1.3%) confirming that the cells were quiescent, and the addition of 10% FBS or the simultaneous addition of βVLDL plus EGF stimulated a substantial increase in that number (31.0 and 24.1%, respectively).

βVLDL Plus EGF Synergistically Induce Cyclin D1 Expression and Down-regulate p27KIP1 Expression—To determine whether βVLDL plus EGF induce cyclin D1 expression, quiescent cells were treated, and the expression of cyclin D1 was examined by Western blot analysis. The data in Fig. 10a show that βVLDL plus EGF synergistically induced cyclin D1 expression; however, either agent alone was ineffective. The effect of βVLDL plus EGF on p27KIP1 expression was examined by Western blot analysis, which shows that similar to 10% FBS, βVLDL plus EGF down-regulated the expression of p27KIP1 protein; however, βVLDL alone or EGF alone had no effect on this parameter (Fig. 10b). The data on [3H]thymidine incorporation, histone mRNA, cyclin D1, and p27KIP1 expression show that neither βVLDL nor EGF alone are capable of
were control-treated or treated with \( \beta VLDL \)

\[
\text{Control} \\
\beta VLDL \\
\text{EGF} \\
\beta VLDL + \text{EGF} \\
10\% \text{FBS} \\
2.5\% \text{FBS}
\]

TABLE I

**Effect of \( \beta VLDL \) plus EGF on \([\text{H}]\text{thymidine labeling of nuclei}**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of radiolabeled nuclei*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>( \beta VLDL )</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>\text{EGF}</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>( \beta VLDL + \text{EGF} )</td>
<td>24.1 ± 1.4</td>
</tr>
<tr>
<td>10% \text{FBS}</td>
<td>31.0 ± 3.7</td>
</tr>
<tr>
<td>2.5% \text{FBS}</td>
<td>14.7 ± 0.7</td>
</tr>
</tbody>
</table>

*Cells were treated with \( \beta VLDL \), EGF, or FBS and labeled with \([\text{H}]\text{thymidine for 4 h, at which time cultures were fixed and processed for determination of the percentage of [\text{H}]thymidine-labeled nuclei as described under “Experimental Procedures.” The data are expressed as the average percentage of positively labeled nuclei in eight fields ± S.E.*

inducing quiescent smooth muscle cells into S phase.

It has been suggested that cell proliferation is induced only when MAP kinase activation is sustained. Because \( \beta VLDL \) or EGF alone do activate MAP kinase but do not induce the cells to enter S phase, it was of interest to determine the levels of activation of MAP kinase by each agent alone as well as in combination. As shown in Fig. 10c, \( \beta VLDL \) strongly activated the MAP kinases ERK1/ERK2 after 5 min of treatment as reported above. MAP kinases were strongly activated after 5 min of treatment with EGF as well, but by 7 h after the addition of either agent alone, the levels of phosphorylated ERK1/ERK2 were dramatically decreased, and by 17 h they were close to baseline levels. Interestingly, the response to the addition of \( \beta VLDL \) plus EGF was also highest at early time points; however the activation of MAP kinase was still evident even at 17 h at levels equivalent to those induced by 10% FBS.

**Pertussis Toxin-sensitive G Proteins, the EGF Receptor, and Protein Kinase C-mediated MAP Kinase Activation Are Responsible for the Mitogenic Effect of \( \beta VLDL \) Plus EGF**—To determine whether MAP kinases and their upstream signaling molecules are critical for \( \beta VLDL \)-induced DNA synthesis,

![FIG. 10. Sustained activation of ERK1/ERK2 by \( \beta VLDL \) plus EGF is associated with an induction of cyclin D1 expression and a decrease of \( p27^{\text{KIP1}} \) expression. a, quiescent smooth muscle cells were control-treated or treated with \( \beta VLDL \) (100 \( \mu \text{g/ml} \)), EGF (20 ng/ml), \( \beta VLDL \) plus EGF, or 10% FBS for the indicated time. Total cell protein was extracted and subjected to Western blot analysis using an antibody against cyclin D1. b, smooth muscle cells were treated as described for a. Total cell protein was extracted and subjected to Western blot analysis using a mouse monoclonal antibody against \( p27^{\text{KIP1}} \). c, quiescent smooth muscle cells were control-treated or treated with \( \beta VLDL \) (100 \( \mu \text{g/ml} \)), EGF (20 ng/ml), \( \beta VLDL \) plus EGF, or 10% FBS for the indicated time. Phosphorylated ERK1/ERK2 was measured as described in the Fig. 1a legend.**

![FIG. 11. Inhibition of MAP kinase activation blocks DNA synthesis induced by EGF plus \( \beta VLDL \). Quiescent smooth muscle cells were preincubated in the absence (no inhibitor) or presence of PD98059 (50 \( \mu \text{M} \)), AG1478 (1 \( \mu \text{M} \)), or bisindolylmaleimide I (1 \( \mu \text{M} \)) for 30 min or pertussis toxin (100 \( \text{ng/ml} \)) for 24 h. The cells were then control-treated or treated with \( \beta VLDL \) (100 \( \mu \text{g/ml} \)), EGF (20 ng/ml), or \( \beta VLDL \) plus EGF. Inset, quiescent smooth muscle cells were treated with increasing concentrations of FBS. After incubation for 20 h, the cells were radio-labeled with \([\text{H}]\text{thymidine (20 \( \mu \text{Ci/ml} \)) for an additional 4 h and harvested for determination of the incorporation of \([\text{H}]\text{thymidine into DNA as described under “Experimental Procedures.” The data are expressed as mean cpm/well ± S.D. (n = 5).**

[Downloaded from http://www.jbc.org/ by guest on August 27, 2017]
induced activation of MAP kinase can be significantly inhibited by AG1478, a specific EGF receptor tyrosine kinase inhibitor. Moreover, pertussis toxin inhibited βVLDL-induced tyrosine phosphorylation of the EGF receptor, indicating that pertussis toxin-sensitive G proteins mediate EGF receptor transactivation, thereby leading to MAP kinase activation. To our knowledge, this is the only report of lipoprotein-induced transactivation of the EGF receptor via activation of a G protein-coupled receptor. Our data also show that LPA-induced MAP kinase activation required transactivation of the EGF receptor in smooth muscle cells as was shown in Rat-1 cells (32). Interestingly, EGF receptor transactivation by LPA in GD25 fibroblast cells (52) and PC12 cells (53) was not shown, suggesting that involvement of the EGF receptor in G protein-coupled receptor-induced MAP kinase activation is cell type-dependent.

Incomplete inhibition of βVLDL-induced MAP kinase activation by AG1478 and pertussis toxin indicates that the MAP kinase activation may not be exclusively mediated via the G protein-coupled receptor-mediated transactivation of the EGF receptor pathway and must involve other upstream transducers. Because the activation is completely inhibited by calphostin C, it is likely that another pathway that goes through protein kinase C is responsible.

A potential intermediate in the G protein-coupled receptor-mediated transactivation of the EGF receptor could be the Src family kinases. Src has been shown to be recruited to a complex consisting of G protein βγ subunit, β-arrestin, and a G protein-coupled receptor (54) where it is tyrosine-phosphorylated and activated after stimulation of a G protein-coupled receptor in Cos-7 cells (53, 55). A constitutively active Src mutant stimulated tyrosine phosphorylation of the EGF receptor (56); however, there is conflicting evidence that Src is not required for LPA-induced MAP kinase activation in Rat-1 or Cos-7 cells (57). Our data using the Src kinase inhibitor PP2 demonstrates that the activation of MAP kinase by βVLDL depends on the activity of Src.

Agonist-stimulated MAP kinase can be Ras-dependent or Ras-independent. For example, it was noted that activation of MAP kinase by angiotensin II differed in cardiac fibroblasts and cardiac myocytes such that the activation was pertussis toxin-sensitive and Ras-dependent in fibroblasts, whereas MAP kinase activation in myocytes was Ras-independent and insensitive to treatment with pertussis toxin but strongly repressed by inhibitors of protein kinase C activity (58). The present study demonstrates that Ras is at least partially responsible for βVLDL-induced MAP kinase activation in aortic smooth muscle cells, because a dominant negative Ras mutant blocked activation. Our results strongly suggest that βVLDL induces the Ras→Raf-1→MEK→MAP kinase cascade. Oxidized LDL stimulated smooth muscle cell Ras-GTP formation and MAP kinase (23); however, the relationship between the two remains to be investigated. Sachindis et al. (21) found LDL-stimulated MAP kinase activation to be independent of Raf-1 phosphorylation in smooth muscle cells, and our data provide no direct evidence for Raf-1 involvement. Angiotensin II was shown to stimulate MAP kinase by a Ras-independent pathway in rat smooth muscle cells (59), whereas other workers showed that angiotensin II-mediated activation of MAP kinase in rat smooth muscle cells was pertussis toxin-insensitive, Ras-dependent, and mediated by phospholipase C (60). Takahashi et al. (59) proffer the explanation that phenotypic differences between smooth muscle cell cultures may be responsible for the variations in results. This highlights the importance of characterizing the cells under study and the recognition that receptor expression as well as availability of downstream targets are likely to vary not only with cell type but with changes in phenotype. The cells examined in this report were deprived of serum prior to the addition of βVLDL, rendering the cells quiescent. It would be of interest to determine whether changes in the proliferative/differentiation state of the cells contribute to the ability of βVLDL to activate MAP kinase and/or the pathway mediating such activation.

Deciphering the contribution of atherogenic lipoproteins to smooth muscle cell proliferation is important to understanding the development of the atherosclerotic plaque as well as restenosis after balloon angioplasty. The effect of atherogenic lipoproteins on smooth muscle cell proliferation was the subject of a number of past investigations (4, 5, 61). Our laboratory showed that βVLDL increased the proliferative rate of neonatal aortic smooth muscle cells that were actively proliferating in media containing lipid-deficient serum (14, 15). Others studying high density lipoprotein (HDL)- and LDL-induced human smooth muscle cell proliferation noted synergy with other growth factors including platelet-derived growth factor (PDGF), EGF, insulin-like growth factor-I (IGF), and bFGF (5, 10), although Resink et al. (10) found that LDL and high density lipoprotein stimulated proliferation even in the absence of additional growth factors. Our data extend this work to show that although the atherogenic lipoprotein βVLDL does not stimulate proliferation of quiescent smooth muscle cells alone, when added in combination with EGF this lipoprotein increased both total [3H]thymidine incorporation into DNA as well as the percentage of [3H]thymidine-labeled nuclei. Moreover, the expression of histone mRNA confirmed that βVLDL plus EGF stimulated entry of the cells into the S phase of the cell cycle. The dependence of the induction of cell proliferation on the pathway activating MAP kinase was shown by inhibiting G protein-coupled receptors, the EGF receptor, protein kinase C, or MEK, which resulted in a dramatic decrease in βVLDL plus EGF-induced cell proliferation. [3H]thymidine incorporation into DNA was inhibited ~80% by pertussis toxin, making it likely that the synergistic action of βVLDL plus EGF on smooth muscle cell proliferation largely depends on activation of a G protein-coupled receptor by βVLDL.

Cell cycle progression results from processes leading to the binding of AP-1 transcription factors (consisting of dimers of the Fos and Jun families), leading to the activation of target genes such as cyclin D1, which is the regulatory subunit of cyclin-dependent kinases essential for G1 progression (62, 63). Chatterjee et al. (23) found that oxidized LDL but not native LDL stimulated Ras, MAP kinase, and c-fos expression in human aortic smooth muscle cells. Somewhat different results were noted by Kusuhara et al. (22), who found that native as well as oxidized LDL preparations stimulated MAP kinase in smooth muscle cells; however, neither LDL nor oxidized LDL induced c-fos expression. Our results with βVLDL suggest that the native lipoprotein stimulates MAP kinase and a transient expression of c-fos and egr-1; however, this was unable to sustain cell cycle progression in the absence of EGF. We showed that cyclin D1 expression and ultimately G1 progression depended on the synergistic action of βVLDL plus EGF on smooth muscle cell proliferation largely depends on activation of a G protein-coupled receptor by βVLDL.
tained (up to 24 h) activation of MAP kinase and cell proliferation (65). A sustained activation of MAP kinase by LPA in Rat-1 cells seems essential for the induction of cell proliferation (66, 67). These authors noted that nonmitogenic doses of LPA stimulated a transient activation of MAP kinase, which induced c-fos expression; however, mitogenic doses were necessary for sustained MAP kinase activation and robust expression of Fra-1, Fra-2, c-Jun and JunB, leading to cell proliferation. Although c-fos expression was induced in a MAP kinase-dependent manner by nonmitogenic stimuli in CCL39 cells, only agents that were able to sustain activation of MAP kinase induce late AP-1 genes and cyclin D1 expression, leading to cell cycle entry (64, 68). Our results support the notion of sustained MAP kinase activation leading to cell proliferation in our system in that the activation of MAP kinase by βVLDL plus EGF was sustained for a longer period of time than was evident when either agent was used alone. Sustained activation of MAP kinase correlated with an increase in cyclin D1 expression (69–73). One must be cautious, however, in assigning categoric significance to the time of activation of MAP kinase, because it represents a measure of the convergence of multiple signaling pathways and is also a point of divergence of additional, perhaps more temporal, components of downstream signals. It is important to measure cell functionality, e.g., cell proliferation rather than MAP kinase activation, to be certain of the role of a particular agonist in cell/organ functionality. Bornfeldt et al. (30) found that platelet-derived growth factor-induced MAP kinase activation stimulated cell proliferation in cycloxygenase-2-negative human smooth muscle cells; however, the opposite effect on proliferation was noted when cells expressing cycloxygenase-2 were treated with platelet-derived growth factor despite a similar increase in MAP kinase activation. These authors proffer the explanation that target availability at the time of stimulation dictates among varying functional responses potentially elicited by MAP kinase activation. In this manner, a signal pathway can serve multiple functions, and therefore it is important to study functional responses downstream of MAP kinase activation.

The contribution of oxidation to the proliferative capacity of LDL remains controversial. Heery et al. (8) found that only oxidized LDL stimulated smooth muscle cell proliferation. Resink et al. (10) found that native and oxidized LDL were equally effective in stimulating [³H]thymidine incorporation into DNA, and Kusuhara et al. (22) noted that both native as well as oxidized LDL preparations stimulated MAP kinase in a protein kinase C-dependent manner; however, oxidized LDL was more effective. Although it seemed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid moiety was responsible for the activation, interestingly, neither thiobarbituric acid-reacting substances nor lipid peroxide content was a major determinant. Moreover, it was observed that a lipoprotein-associated lipid mo
βVLDL Activates Smooth Muscle Cell MAP Kinase

β-Migrating Very Low Density Lipoprotein (βVLDL) Activates Smooth Muscle Cell Mitogen-activated Protein (MAP) Kinase via G Protein-coupled Receptor-mediated Transactivation of the Epidermal Growth Factor (EGF) Receptor: EFFECT OF MAP KINASE ACTIVATION ON βVLDL PLUS EGF-INDUCED CELL PROLIFERATION

Dezheng Zhao, Jennifer Letterman and Barbara M. Schreiber

doi: 10.1074/jbc.M103761200 originally published online May 25, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M103761200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 73 references, 34 of which can be accessed free at http://www.jbc.org/content/276/33/30579.full.html#ref-list-1