

A Mutant High-Density Lipoprotein Receptor Inhibits Proliferation of Human Breast Cancer Cells

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ABSTRACT

High-density lipoprotein (HDL) stimulates the growth of many types of cells, including those of breast cancer. High levels of HDL are associated with an increased risk of breast cancer development. A scavenger receptor of the B class (SR-BI)/human homolog of SR-BI, CD36, and LIMPII analogous-1 (CLA-1) facilitates the cellular uptake of cholesterol from HDL and thus augments cell growth. Furthermore, HDL is also believed to have antiapoptotic effects on various cell types, and this feature adds to its ability to promote cell growth. These collaborative roles of HDL and CLA-1 prompted us to assess the function of these components on human breast cancer cells. In this study, we created a mutant CLA-1 (mCLA) that lacked the COOH-terminal tail to determine its potential role in breast cancer cell growth. Expression of mCLA inhibited the proliferation of breast cancer cell line MCF-7. This inhibitory action of mCLA required the transcriptional factor activator protein-1 (AP-1), and the mutant receptor also affected the antiapoptotic features of HDL. The effect of HDL on AP-1 activation and [³H]thymidine incorporation was abrogated by wortmannin, a specific inhibitor of phosphoinositide 3-kinase. Furthermore, the dominant negative mutant of Akt abolished the ability of HDL to activate AP-1. These findings raise the possibility that the inhibitors of the effects of HDL may be of therapeutic value for breast cancer.

INTRODUCTION

Breast cancer is one of the most common malignancies in females and is the leading cause of premature death for women in Western societies (1). In rapidly dividing cancer cells, the availability of cholesterol is essential for proliferation and progression of the cancer (2, 3). High-density lipoprotein (HDL) is well known as a growth stimulator for many types of cells, including those of breast cancer (2, 4). In breast cancers, the effect of HDL is more pronounced in estrogen receptor-positive cells than in estrogen-negative cell lines (4). Cellular uptake of cholesterol from HDL is facilitated by a scavenger receptor of the B class (SR-BI) that was recently identified as a HDL receptor in rodents (5). Human CD36 and LIMPII analogous-1 (CLA-1) shares 81% sequence homology with hamster SR-BI (6).

We reported recently that CLA-1 is also functionally related to SR-BI (7–9). Like SR-BI, CLA-1 is expressed primarily in liver and steroidogenic tissues including mammary gland cells (10–13). Pussinen *et al.* (13) showed that the breast cancer cell line HBL-100 acquires cholesterol from HDL. This process appears to be mediated by CLA-1. HDL stimulation of cellular uptake of cholesterol is not the only mechanism for augmenting cell growth because other studies suggest that HDL has antiapoptotic effects on various cell types (14). These additive roles of HDL and CLA-1 in cancer cell proliferation prompted us to assess the function of these components on human breast cancer cells. For these studies, we created a mutant CLA-1 (mCLA)-1 and used this clone to establish a stably transfected cell

line. The studies arising from the use of these tools suggest that CLA-1 mediates the proliferative and antiapoptotic features of HDL, and this effect involves, in part, the activity of a transcription factor, activator protein-1 (AP-1).

MATERIALS AND METHODS

Material. Wortmannin, PD98059, c-Jun NH₂-terminal kinase (JNK) inhibitor 1, and SB203580 were purchased from Calbiochem. Tumor necrosis factor (TNF)- α was obtained from Research Biochemicals International (Natick, MA). All other reagents were of analytical grade.

Tissue Samples. The tissues of interest were from patients affected by breast cancer. The diagnosis was confirmed on the basis of on histopathological findings and clinical outcome. Informed consent was obtained from all patients for the use of the tissue samples in our studies.

RNA Isolation and Reverse Transcription-PCR Analysis. CLA-1 expression was determined by PCR analysis of the reverse transcribed RNA as described previously (7, 9). A primer pair matching the published sequence (6) of CLA-1 (sense, 5'-ATGATCGTGATGGTGCCGTC-3'; antisense, 5'-ACTGAACCTGCAGGTGCTGA-3') was used to amplify a 930-bp fragment.

Immunohistochemical Localization. Tissue specimens were fixed in formalin and embedded in paraffin. After deparaffinization and rehydration, sections were blocked for 60 min in 10% normal goat serum in PBS and incubated for 2 h with a guinea pig antibody directed against CLA-1 (7) in 4% normal goat serum in PBS or with preimmune antibody under identical conditions. Antibody binding was visualized with the diaminobenzidine reaction.

Plasmid Construction. CLA-1 expression vectors were constructed to express chimeric CLA-1 mutants fused to FLAG in full-length CLA-1 (amino acids 1–509) or mCLA-1 (amino acids 1–464) fused to FLAG in its NH₂ terminus. The cDNA coding the full length of CLA-1 (fCLA-1) or mCLA-1 fused to FLAG was obtained by PCR using the CLA-1 expression vector as described previously. The PCR product was digested with *HindIII/XbaI* and subcloned into pcDNA3 mammalian expression vector (Invitrogen, San Diego, CA), and the sequence of these expression vectors was verified by dideoxysequencing. An expression vector encoding a dominant-negative mutant of Akt (Akt-DN) was described previously (15).

HDL Cell Association and Selective Cholesterol Ester (CE) Uptake. Human HDL (density = 1.070–1.20 g/ml) was isolated by preparative ultracentrifugation from fresh plasma collected in EDTA (1 mg/ml) as described previously (7). The transfected MCF-7 cells were washed once with serum-free medium/0.5% BSA. [¹²⁵I]-Dilactitol tyramine-[³H]cholesterol oleyl ether HDL₃ particles were added at a concentration of 10 μ g protein/ml in serum-free medium/0.5% BSA. After incubation for 1.5 h at 37°C, the medium was removed, and the cells were washed three times with PBS/0.1% BSA and one time with PBS. The cells were lysed with 1.1 ml of 0.1 N NaOH, and the lysate was processed to determine trichloroacetic acid soluble and insoluble [¹²⁵I] radioactivity and organic solvent-extractable [³H] radioactivity (7, 16, 17). The values for cell-associated HDL apolipoprotein (expressed as HDL CE), endocytosed and degraded HDL apolipoprotein, total cell-associated HDL CE, and the selective uptake of HDL CE were obtained as described previously (16, 17).

Western Blot Analysis. The cytoplasmic proteins were lysed in radioimmunoprecipitation assay buffer as described previously (7). The proteins were separated on a 7.5% SDS-polyacrylamide gel and then transferred to polyvinylidene difluoride membrane (7). The membranes were incubated with the anti-CLA-1 antibody (diluted 1:3000 from whole antiserum; Ref. 7) or anti-cyclophilin A antibody (Biomol Research, Plymouth Meeting, PA; diluted

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1:1000). The antibody binding was visualized by chemiluminescence detection (ECL; Amersham Corp., Arlington Heights, IL).

Proliferation Assay. MCF-7 cells (10^4) were cultured in 96-well plates in 200 μ l of DMEM with 10% lipoprotein deficiency serum. The medium was replaced with DMEM plus lipoprotein deficiency serum with or without 100–500 μ g/ml HDL, and the cells were incubated for an additional 24 h at 37°C. Four h before harvesting, cells were pulsed with 1 μ Ci/well [3 H]thymidine (specific activity = 6.7 Ci/mmol; DuPont Company, Wilmington, DE), harvested onto glass fiber filters using an automated cell harvester, and counted in a Packard liquid scintillation counter (Hewlett-Packard Co., Palo Alto, CA).

Transfection of MCF-7 Cells and Luciferase Reporter Gene Assay. The AP-1 reporter gene used in our studies was purchased from Stratagene (La Jolla, CA). Purified reporter plasmid was transfected into MCF-7 cells (at 60% confluence) using a conventional cationic liposome transfection method (LipofectAMINE; Life Technologies, Inc., Gaithersburg, MD). All assays were corrected for β -galactosidase activity, and the total amount of protein in each reaction was identical. Twenty- μ l aliquots were taken for the luciferase assay, which was performed according to the manufacturer's instructions (ToyoInk, Tokyo, Japan).

Electrophoretic Mobility Shift Analysis. MCF-7 cells were treated with or without 500 μ g/ml HDL for 12 h, and nuclear extracts were prepared according to a technique described previously (18). A synthetic DNA duplex spanning AP-1 (5'-TTCCGGCTGACTCAAGCG-3'), nuclear factor κ B (5'-AGTTGAGGGGACTTCCAGGC-3'), and nuclear factor for interleukin-6 [5'-GGACGTCACATTGCACAATCTTAATAA-3' (Nihon Bioservice, Asagiri, Japan)] used in these studies was radiolabeled as described previously (18). All reactions were incubated at room temperature for 20 min and then separated on a native 6% polyacrylamide gel. The gel was dried, and the radioactive signals were detected using a Bioimaging Analyzer (BAS 1000 system, Fuji Photo Film, Tokyo, Japan).

Detection of Apoptotic Cells. Phosphatidyl-serine externalization of apoptotic cells in the early stage was visualized using annexin-V-FITC stain and a flow cytometer (EPICScan; Coulter, Hialeah, FL) according to the manufacturer's instructions (MBL, Nagoya, Japan). We also measured the percentage of apoptotic cells by propidium iodide method. In brief, after induction of apoptosis with anti-Fas monoclonal antibodies, cells were fixed in 70% ethanol at -20°C for 30 min and then incubated with 500 μ l of 100 μ g/ml RNase solution at 37°C for 20 min. The cells were washed and then stained with 100

μ l of 25 μ g/ml propidium iodide (Sigma) solution for 20 min on ice. The propidium iodide fluorescence of individual nuclei was measured by flow cytometry. The percentage of cells containing subdiploid DNA was determined as the apoptotic cell rate, as described previously (19).

Statistical Analysis. Statistical comparisons were made by one-way ANOVA and Student's *t* test, and *P* < 0.01 was considered significant.

RESULTS

CLA-1 Expression in Breast Cancer Cells. We have examined eight samples of human breast cancers and adjacent mammary tissue for the presence of CLA-1 mRNA and protein using reverse transcription-PCR and Western blot analysis, respectively. Results (Fig. 1A) showed that expression of CLA-1 mRNA was most abundant in the breast cancer cells (Lanes 2, 4, 6, and 8). In contrast, the CLA-1 expression was exceedingly low or not detected in the adjacent noncancerous tissue (Fig. 1A, Lanes 1, 3, 5, and 7). Western blot analysis (Fig. 1B) showed that the abundance of CLA-1 protein in breast cancer tissue (Lanes 2, 4, 6, and 8) was much higher than that in the adjacent normal tissue (Lanes 1, 3, 5, and 7).

Next, immunohistochemical analysis using an anti-CLA-1 antibody allowed us to localize CLA-1 in the tissue samples. Results (Fig. 1C) showed strong immunostaining for CLA-1 in the breast cancer cells (right panel). The use of nonspecific IgG failed to show staining in the cells (Fig. 1C, left panel). Together, these findings show that CLA-1 is abundantly expressed in human breast cancer but not in adjacent normal tissue.

Mutant CLA-1 Inhibits Cell Growth and Blocks the Stimulatory Response of HDL. To determine the role of CLA-1 in the breast cancer, we created a mCLA-1 that retained an intact extracellular domain (amino acids 1–464), which facilitated binding of the receptor to HDL. The mCLA-1 lacked the COOH-terminal tail, the intracellular domain of the receptor that is believed to regulate HDL signaling. The mutant was used to establish a stably transfected MCF-7 cell line. The ability of these cells to proliferate was measured by assess-

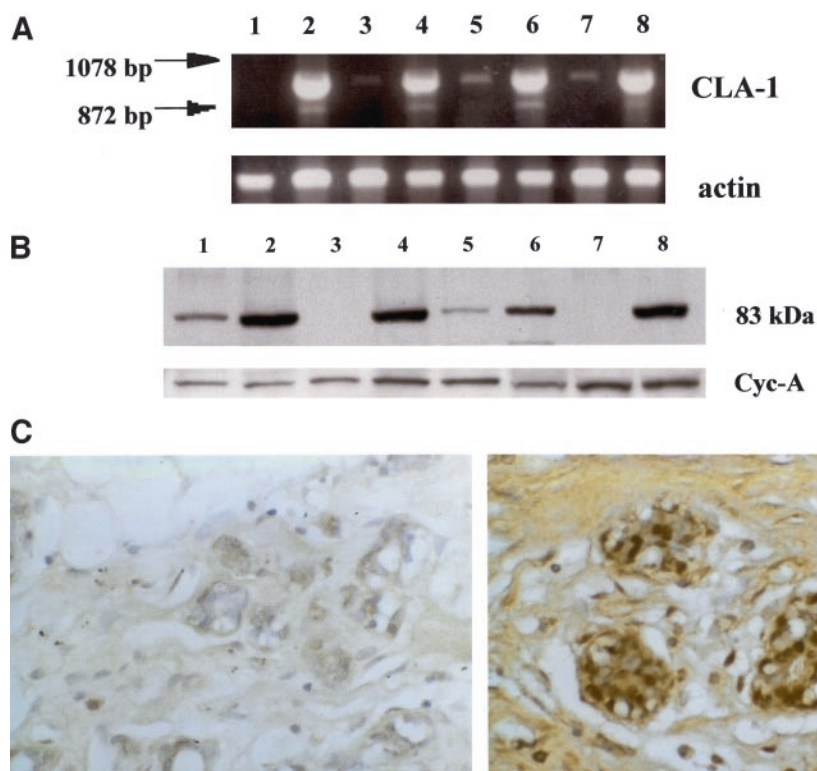


Fig. 1. hSR-B1/CLA-1 expression in breast cancer and adjacent noncancerous cells. A, reverse transcription-PCR analysis of CLA-1 mRNA expression in breast cancers. Top panel, CLA-1; bottom panel, β -actin. Lanes 1, 3, 5, and 7 and Lanes 2, 4, 6, and 8 show results from noncancerous mammary tissue and breast cancers, respectively. B, CLA-1 protein expression in breast cancers. Lanes 1, 3, 5, and 7 and Lanes 2, 4, 6, and 8 show results from noncancerous mammary tissue and breast cancers, respectively. C, immunohistochemical localization of CLA-1 in breast cancer. Immunoperoxidase staining of CLA-1 is present in the cytoplasm of the breast cancer (right panel), and tissue stained with control IgG was negative (left panel).

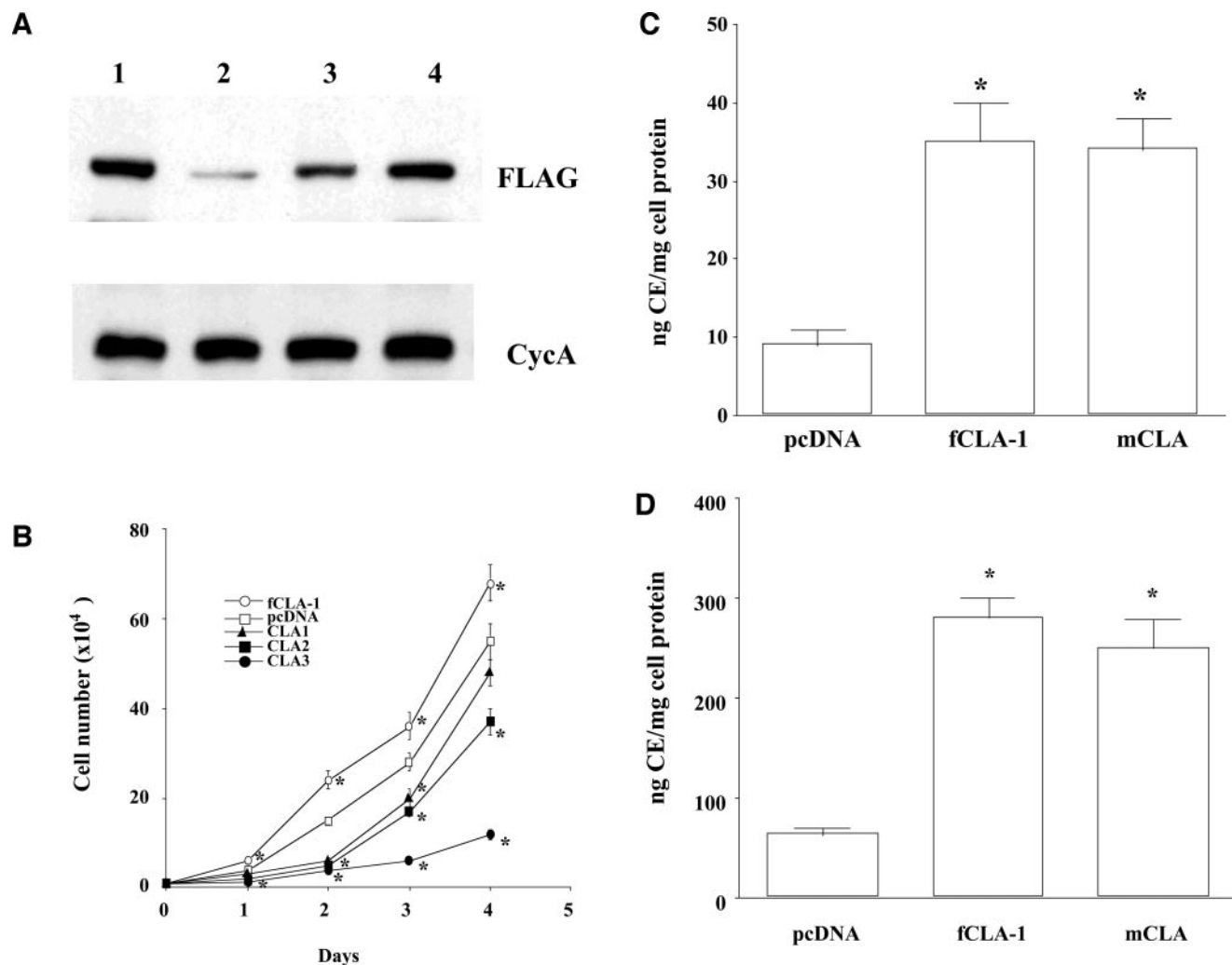


Fig. 2. Establishment of MCF-7 cells stably transfected with mutant CLA (mCLA). *A*, expression of tag protein in mCLA-transfected clones. *Lane 1*, full-length CLA-1-transfected cells; *Lanes 2–4*, CLA-1, -2, and -3 (mCLA-transfected cell clones), respectively. *Top panel*, FLAG; *bottom panel*, cyclophilin A. *B*, proliferation rate of mCLA-overexpressing MCF-7 cells. Ten thousand cells were seeded in 6-well plates on day 0 and counted at 24-h intervals for the duration of the experiment. The data show the mean \pm SE ($n = 5$) of separate transfections. There was a significant difference [$*$, $P < 0.01$ versus controls (pcDNA)] in the number of cells/well from days 1–4 of the experiment. *fCLA-1*, full-length CLA-1-transfected cells; *CLA1*, *CLA2*, and *CLA3*, mCLA-transfected cell clones; *pcDNA*, vector pcDNA3-transfected cells. *C* and *D*, cell-associated high-density lipoprotein (HDL) cholesterol ester (CE) and selective uptake of HDL CE. Cells transfected with vector pcDNA3 (*pcDNA*), *fCLA-1*, or *mCLA* were incubated at 37°C for 1.5 h with 10 μ g/ml [125 I, 3 H]HDL, after which cells were processed to determine cell-associated HDL CE (*C*) and HDL CE selective uptake (*D*). Values represent the mean of triplicate determinations. The asterisk denotes a significant difference ($P < 0.01$).

ing cell number after various days of growth. Several stably transfected clones were isolated (Fig. 2A), and their rates of growth were compared with that of cells transfected with an intact CLA-1, *fCLA-1* (Fig. 2B). Results showed that after 4 days of growth, the number of cells in wells seeded with the mCLA-expressing clones (CLA1–3) was significantly less than that in wells containing cells transfected with *fCLA-1*. The growth of all mCLA-expressing clones was lower compared with that of the control. Clone CLA3 was selected for additional studies because of its high level of tag protein expression (Fig. 2A).

Previous reports, including that from our laboratory, showed that cells take up CE from HDL by a selective, nonendocytotic pathway for the delivery of HDL-associated CE (7, 20). To test the function of mCLA-1, we measured the kinetics of CE uptake using doubly labeled HDL. Results showed that HDL cell association (Fig. 2C) and HDL CE selective uptake (Fig. 2D) by cells carrying the mCLA-1 receptor were fully active and equivalent to those transfected with *fCLA-1*. These results are consistent with the previous observations of Connelly *et al.* (16).

Next, we measured [3 H]thymidine uptake as a reflection of cell

growth in mCLA-expressing cells and control cells. Not surprisingly, [3 H]thymidine uptake was significantly lower in the mCLA-expressing cells than in the *fCLA-1*-transfected MCF-7 cells (Fig. 3A). Cells were exposed to 0, 100, or 500 μ g/ml HDL added to the culture media followed by measurement of [3 H]thymidine uptake into the cells. Results (Fig. 3A) showed that [3 H]thymidine uptake into the *fCLA-1*-transfected clone (Fig. 3A, \square) increased significantly in a dose-dependent manner. In contrast, the exposure of the mCLA-expressing cells (Fig. 3A, \boxtimes) to HDL had no effect on [3 H]thymidine uptake.

To test whether protein kinases are involved in HDL-mediated cell proliferation, we studied the effect of pharmacological inhibitors on HDL-mediated [3 H]thymidine uptake. In this study, HDL stimulation (100 μ g/ml) was combined with cotreatment of MCF-7 cells using inhibitors of phosphatidylinositol 3'-kinase [PI3K (10 μ M wortmannin)], a mitogen-activated extracellular signal-regulated kinase (10 μ M PD98059), a JNK (1 μ M JNK inhibitor I), or a p38 mitogen-activated protein kinase (1 μ M SB203580). Results (Fig. 3B) showed that HDL-mediated 3 H uptake was not sensitive to inhibitors of extracellular signal-regulated kinase, JNK, and p38 mitogen-activated

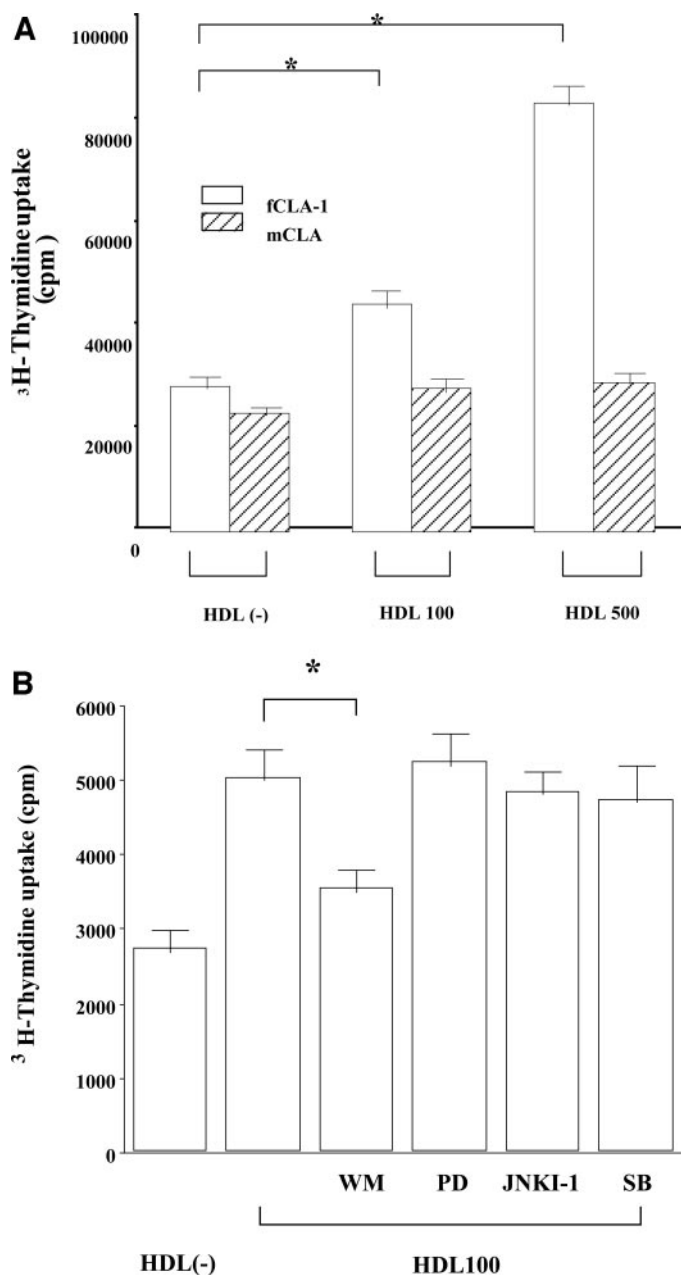


Fig. 3. Proliferation assay in MCF-7 cells stably transfected with mutant CLA. **A**, cell proliferation was measured by exposure to [³H]thymidine for 6 h before harvest. Each column represents cellular uptake of [³H]thymidine (mean \pm SE; $n = 5$). *fCLA-1*, full-length CLA-1-transfected cells; *mCLA*, mutant CLA-1-transfected cells; *CLA3*, mutant CLA-1 transfected clone. The asterisk denotes a significant difference ($P < 0.01$). **B**, effects of phosphatidylinositol 3'-kinase inhibitor wortmannin (*WM*), MAPK or ERK kinase 1 (MEK1) inhibitor PD98059 (*PD*), c-Jun NH₂-terminal kinase inhibitor I (*JNKI-1*), or p38 mitogen-activated protein kinase inhibitor SB203580 (*SB*) on high-density lipoprotein-stimulated [³H]thymidine incorporation into MCF-7 cell DNA. Inhibitors were preloaded for 1.5 h before high-density lipoprotein (100 μ g/ml) stimulation. Values represent the mean of triplicate determinations. The asterisk denotes a significant difference ($P < 0.01$).

protein kinase, but it was sensitive to wortmannin, an inhibitor of PI3K.

Actions of CLA-1 in the Presence of HDL Affect AP-1 Activity. Recently, Liu *et al.* (21) reported that AP-1 blockade inhibited the growth of MCF-7 cells both *in vitro* and *in vivo*. To determine whether mCLA affected AP-1 DNA binding and function, we measured these parameters using electrophoretic mobility shift analysis and reporter gene analysis, respectively. Results (Fig. 4) showed that the addition of HDL (500 μ g/ml) to cells stimulated the DNA binding activity of

AP-1 (Fig. 4A) in the fCLA-1-containing cells, but not in mCLA-1-containing cells. The increase in AP-1 activity was specific because the DNA binding activity of other transcription factors (Fig. 4B; nuclear factor κ B and NF-IL6) in the same extracts was not affected by HDL treatment.

It was not known whether the increase in AP-1 DNA binding activity matched the transcriptional activity of the protein. Therefore, we measured the activity of pAP1-LUC in cells stably transfected with fCLA-1 or mCLA-1. Results (Fig. 4C) showed that luciferase activity was increased 2.5-fold in the fCLA-1-containing cells after treatment with HDL (Fig. 4C). In contrast, HDL had no effect on either DNA binding activity of AP-1 or transcription of the reporter gene in the mCLA-expressing cells.

To analyze the signal transduction pathways that are involved in HDL-stimulated AP-1 transcriptional activity, we studied the effect of various pharmacological inhibitors used in the above studies. In agreement with the data on HDL-stimulated AP-1 activity, HDL-stimulated AP-1 activity was not sensitive to inhibition of extracellular signal-regulated kinase, JNK, and p38 mitogen-activated protein kinase, but it was sensitive to inhibition of PI3K by wortmannin.

Although PI3K has many potential downstream targets, we focused our attention on Akt because of several reports that showed the importance of this kinase on the effects of HDL. We assessed the actions of a dominant negative mutant of Akt (Akt-DN) on AP-1 promoter activity in MCF-7 cells cotransfected with pAP-LUC plus the Akt-DN or empty vector (Fig. 4E). Consistent with preceding studies, there was a 2.4-fold rise in luciferase activity after HDL stimulation. As expected, expression of Akt-DN inhibited the actions of HDL induction of pAP-LUC activity. Together, these findings support the idea that the PI3K/Akt pathway is required for HDL induction of AP-1 promoter activity in MCF-7 cells.

CLA-1 Protects MCF-7 Cells against Apoptosis. Next, we examined whether mCLA affected the rate of apoptosis in the transfected MCF-7 cells. Results of a standard apoptosis assay (Fig. 5) showed the expected effect of TNF- α on fCLA-1-transfected cells giving rise to typical features of apoptosis. This effect of TNF- α was attenuated in cells exposed to HDL, thus documenting the protective role of HDL in MCF-7 cells against the apoptotic actions of TNF- α .

Next, we tested the effect of mCLA in the stably transfected MCF-7 cells. As predicted, HDL did not attenuate the apoptosis of mCLA-expressing MCF-7 cells after exposure to TNF- α . When annexin-V was used to detect apoptosis at the early stage, the results were compatible with those obtained by the propidium iodide method (data not shown).

DISCUSSION

In this study, we have examined the role of an mCLA-1, a mutant HDL receptor, on the growth and cell signaling in a breast cancer cell line, MCF-7. Our data show that expression of mCLA-1 inhibited the proliferation of the estrogen receptor-positive cells, and HDL activation of the transcription factor AP-1 was suppressed.

Previous studies of breast cancer cells suggested that growth factors and hormones such as insulin-like growth factor I, epidermal growth factor, estrogens, and retinoids may modulate the activity of AP-1, which, in turn, affected gene transcription (4, 21). AP-1 blockade suppressed mitogenic signals from multiple different peptide growth factors as well as estrogen and inhibited the growth of MCF-7 cells (21). Additionally, the activation of AP-1 may also contribute to tumor cell invasive capacity and tamoxifen resistance (22). These previous studies provide indirect evidence that seems to suggest that the AP-1 transcription factor plays an important role in breast cancer cell growth, invasion, and resistance to antiestrogens. Our finding that

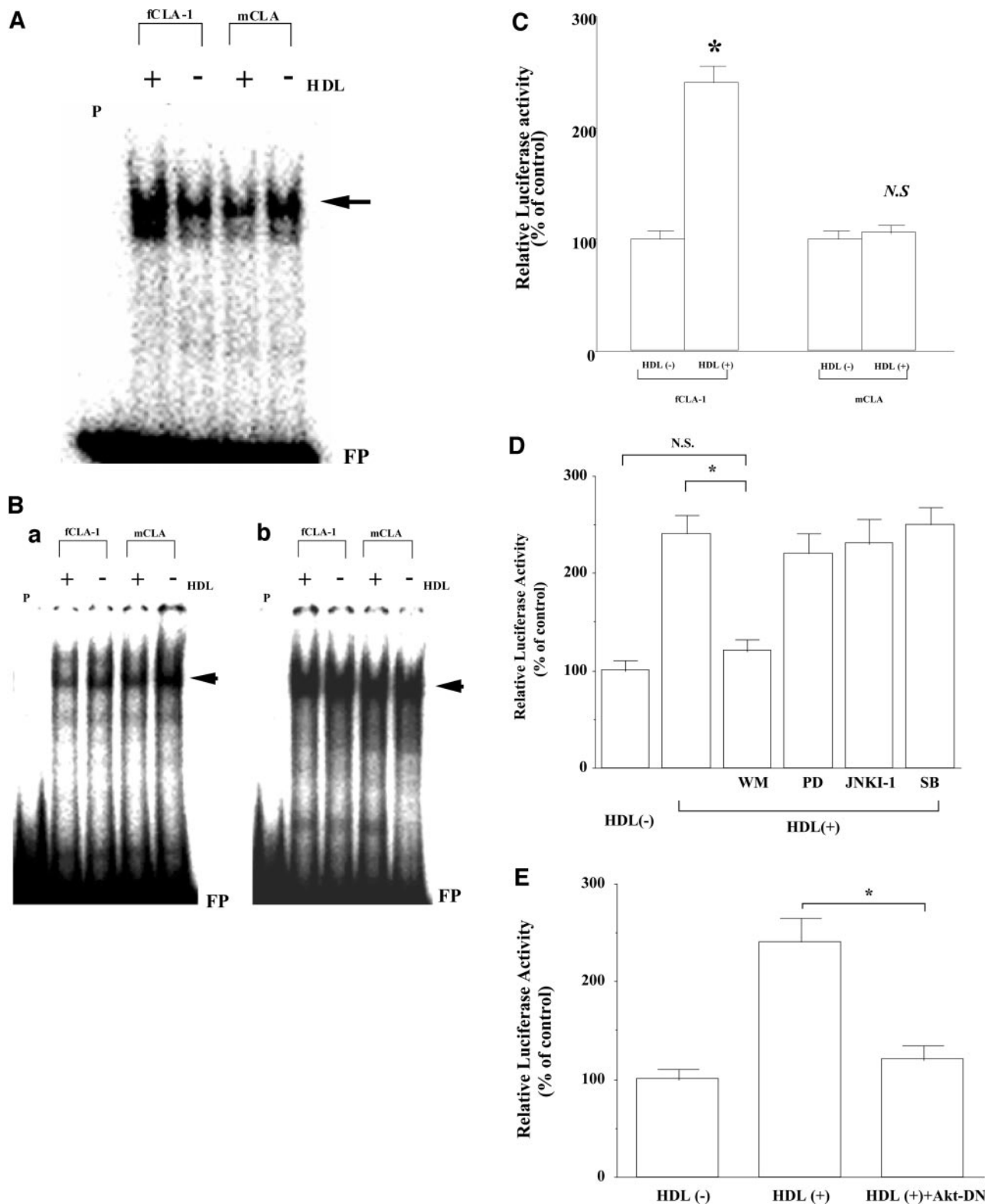


Fig. 4. Effect of high-density lipoprotein (HDL) on activator protein-1 (AP-1) binding activity and transcriptional activity in mutant CLA-expressing cells. *A* and *B*, cells were exposed to 500 $\mu\text{g/ml}$ HDL for 6 h before preparing the nuclear extracts, and binding activity of AP-1 (*A*), nuclear factor κB (*B*, *a*), and NF-IL6 (*B*, *b*) was examined using electrophoretic mobility shift analysis. *fCLA-1*, full-length CLA-1-transfected cells; *mCLA*, mutant CLA-1-transfected clone (CLA3). *P*, probe only; *HDL*, 500 $\mu\text{g/ml}$ HDL. Arrow indicates the DNA-protein complex. *FP*, free probe. An identical experiment performed independently gave similar results. *C*, MCF-7 cells were transfected with 1 μg of pAP1-LUC and treated with 500 $\mu\text{g/ml}$ HDL for 24 h before cell harvest. All assays were corrected for β -galactosidase activity, and total amount of protein/reaction was identical. The results were expressed as relative luciferase activity compared with control cells arbitrarily set at 100. *fCLA-1*, full-length CLA-1-transfected cells; *mCLA*, mutant CLA-1 transfected clone (CLA3). Each data point shows the mean \pm SE ($n = 4$) of separate transfections. The asterisk and N.S. denote a significant difference ($P < 0.01$) and a nonsignificant difference, respectively. *D*, effects of PI3K inhibitor wortmannin (*WM*), MAPK or ERK kinase 1 (MEK1) inhibitor PD98059 (*PD*), c-Jun NH₂-terminal kinase inhibitor I (*JNKI-1*), or p38 mitogen-activated protein kinase inhibitor SB203580 (*SB*) on HDL (500 $\mu\text{g/ml}$)-stimulated AP-1 transcriptional activity in MCF-7 cells. Values represent the mean of triplicate determinations. The asterisk denotes a significant difference ($P < 0.01$). *E*, dominant negative Akt inhibits HDL-mediated AP-1 transcriptional activity in MCF-7 cells. MCF-7 cells were transfected with pAP-LUC and empty vector or Akt-DN and then treated with HDL for 24 h before cell harvest. The results were expressed as relative luciferase activity compared with control cells arbitrarily set at 100. Each data point shows the mean \pm SE ($n = 4$) of separate transfections. The asterisk denotes a significant difference ($P < 0.01$).

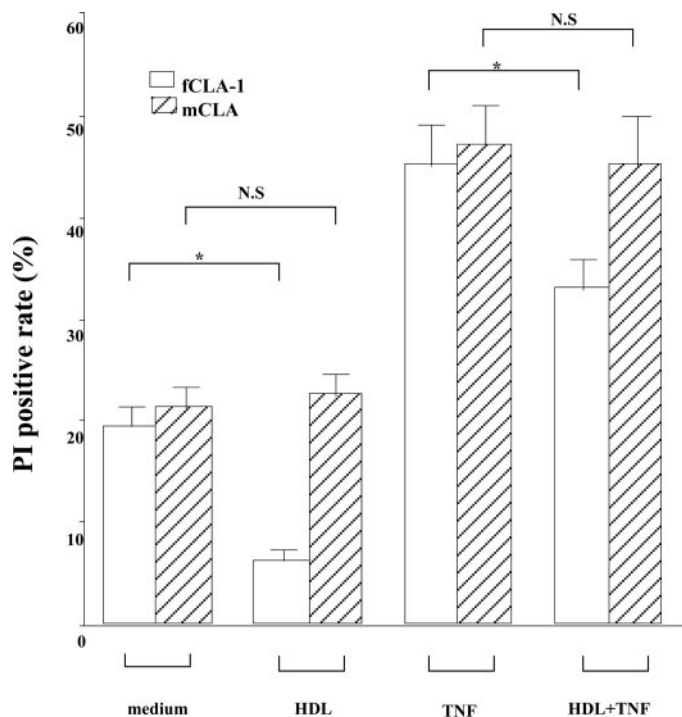


Fig. 5. Effect of high-density lipoprotein (HDL) on apoptosis in mCLA-expressing cells. Induction of apoptosis of mutant CLA-1-transfected cells. The percentage of cells undergoing apoptosis was detected by the propidium iodide method. *, $P < 0.01$ (versus control). fCLA-1, full-length CLA-1-transfected cells; mCLA, mutant CLA-1-transfected clone (CLA3). HDL, 500 $\mu\text{g/ml}$ HDL; TNF, 10 ng/ml TNF- α .

the activity of AP-1 was affected by the expression of mCLA points to the possibility that the mitogenic effect(s) of HDL might involve the activation of AP-1. This finding is consistent with the idea that the AP-1 family is an important family of transcriptional factors that function as transducers of multiple signaling pathways, including those related to mitogenesis and stress (23).

Many studies suggest that an active interplay between PI3K and Akt signaling plays important roles in breast cancer growth. For example, estrogen receptor has been shown to bind the regulatory subunit of PI3K, p85, in a ligand-dependent manner and activate PI3K and Akt (24, 25). Furthermore, PI3K and Akt can mediate estradiol-induced S-phase entry and cyclin D1 transcription in MCF-7 cells (26). Along a similar line, the constitutive and inducible Akt activity promotes resistance to chemotherapy in breast cancer cells (27). Activation of Akt in breast cancer predicts a worse outcome among tamoxifen- and/or goserelin-treated patients (28). These findings prompted us to examine whether PI3K/Akt signaling was involved in HDL-induced AP-1 activity in MCF-7 cells. Indeed, our results showed that the PI3K/Akt signaling pathway was involved in HDL-CLA-1-mediated breast cancer growth.

The expression of mCLA inhibited MCF-7 cell growth, and the mutant promoted apoptosis in the transfected cells. These actions of the mCLA effectively canceled the antiapoptotic effect of HDL in response to TNF- α treatment of MCF-7 cells (Fig. 4). Another example of HDL having similar actions is evident in the pathogenesis of atherosclerosis. The antiatherogenic actions of HDL on vascular endothelial cells arise from the antiapoptotic effects of HDL on these cells in response to exposure to TNF- α and oxidized low-density lipoprotein-induced (14). Both TNF- α and oxidized low-density lipoprotein induce apoptosis of endothelial cells by activation of CPP32-like protease. HDL suppresses the CPP32-like protease activity in endothelial cells (29). Further investigations will be needed to

clarify the inhibitory mechanism by which mCLA protects MCF-7 cells from HDL-induced apoptosis.

Previous studies showed that the extracellular domain of SR-BI (the rodent homolog of CLA-1), in addition to its role in binding HDL particles, is required for the efficient and selective uptake of HDL CE (30). Structure-function studies using selected SR-BI domains in swapping studies with CD36 and point mutations of SR-BI have shown that the extracellular portion of SR-BI contains the HDL binding domain. This region of the protein is essential for selective cellular uptake of HDL (30, 31). In support of this idea, Connelly *et al.* (16) reported that deletion of the 45-amino acid COOH terminus of SR-BI did not reduce selective uptake. Together, these data show that the extracellular domain of the receptor mediates selective uptake of HDL, but the COOH terminus of the protein is dispensable for SR-BI function. In addition, our recent studies indicate that the ability of the SR-BI to alter endothelial nitric oxide synthase activity is specific to HDL. The cytoplasmic COOH-terminal tail of the receptor might have a role in coupling to processes that ultimately cause eNOS activation (32). The results of the current study show that CLA-1 minus the COOH-terminus inhibited cell growth (Fig. 2). We expect that the coupling might involve intermediary proteins such as CLAMP, a protein containing four PDZ domains that associates with the extreme COOH terminus of SR-BI (33). The role of COOH-terminal tail on cell proliferation and antiapoptosis, as well as its function as a scavenger or signaling receptor in breast cancer cells, deserves further investigation.

In summary, we examined the role of HDL on cell proliferation and apoptosis using expression of mCLA in the breast cancer cell line MCF-7. The results show that mCLA inhibits cell proliferation and also attenuates the antiapoptotic effect of HDL in MCF-7 cells. These findings raise the possibility that inhibitor for HDL signaling may be of therapeutic value for the treatment of breast cancer.

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