Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 344 (2006) 226–232
BBRC
www.elsevier.com/locate/ybbrc

High-density lipoprotein is a potential growth factor for adrenocortical cells

Koji Murao [a,*], Hitomi Imachi [a], Wenming Cao [a], Xiao Yu [a], Junhua Li [a], Kazuya Yoshida [a], Rania A.M. Ahmed [a], Kensuke Matsumoto [a], Takamasa Nishiuchi [a], Norman C.W. Wong [b], Toshihiko Ishida [a]

[a] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe Miki-cho, Kita-gun, Kagawa, Japan*

[b] *Departments of Medicine and Biochemistry and Molecular Biology, Faculty of Medicine, University of Calgary, Health Sciences Center, 3330 Hospital Drive NW, Calgary, Alta., Canada T2N 4N1*

Received 9 March 2006
Available online 29 March 2006

Abstract

The entry of cholesterol contained within high-density lipoprotein (HDL) into adrenocortical cells is mediated by a human homologue of SR-BI, CD36, and LIMPII Analogous-1 (CLA-1) and thus augmenting their growth. To address the role of CLA-1, we created a mutant mCLA that lacked the C-terminal tail. HDL CE selective uptake by cells carrying the mCLA-1 receptor was fully active and equivalent to those transfected with full-length CLA-1 (fCLA-1). Expression of mCLA inhibited the proliferation of an adrenocortical cell line and the incorporation of [^{3}H]thymidine into the cells. This effect was sensitive to wortmannin, an inhibitor of phosphoinositide 3-kinase (PI3K). Our transcriptional studies revealed that the inhibitory action of mCLA required the transcriptional factor AP-1 and the effect of HDL on AP-1 activation was also abrogated by wortmannin. These findings raise the possibility that the inhibitors of the effects of HDL may be of therapeutic value for adrenocortical tumor.

Keywords: Adrenocortical cell; HDL; CLA-1; AP-1; PI3K; Akt

Cholesterol is a precursor for the cellular synthesis of steroid hormones. Extracellular lipoprotein cholesterol taken up from plasma is an important source of substrate in most steroidogenic tissues [1]. One source of cellular cholesterol is HDL and this lipoprotein particle triggers a physiologic response leading to increased steroidogenesis [2]. Consistent with this idea, lipoprotein cholesterol uptake by tumors originating from steroidogenic tissues may actually diminish circulating cholesterol levels [3]. The cellular uptake of cholesterol in HDL is facilitated by a scavenger receptor of the B class (SR-BI). This protein was identified as an HDL receptor in rodents [4]. The human CD36 and LIMPII Analogous-1 (CLA-1) share 81% sequence homology with hamster SR-BI [5]. Our previous reports show that human SR-BI (hSR-BI/CLA-1), like mouse SR-BI, functions as a receptor for HDL [6–13]. Human SR-BI/CLA-1 is also similar to the mouse homologue because it can mediate selective uptake of cholesterol ester and it is expressed in liver plus steroidogenic tissues including adrenal gland. These features suggest that hSR-BI/CLA-1 is functionally related to mouse SR-BI.

The highest expression of SR-BI per gram tissue is found in rodent adrenal glands [4,14]. Immunostaining showed that SR-BI is expressed primarily on the surface of steroidogenic parenchymal cells, such as in the zona fasciculata and zona reticularis cells of the adrenal cortex [14,15]. ACTH treatment of mice induces adrenal steroidogenesis, enhances selective uptake of HDL cholesterol [16], and increases SR-BI protein expression in adrenocortical

* Corresponding author. Fax: +81 878 91 2147.
E-mail address: mkoji@kms.ac.jp (K. Murao).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.03.131

cells [15]. The action of ACTH on SR-BI expression is likely mediated by the second messenger cAMP [17–19]. These findings support the hypothesis that in adrenocortical tissue SR-BI mediates physiologically relevant selective uptake of cholesteryl esters to supply substrate for steroid hormone synthesis. Consistent with this idea, anti-SR-BI antibodies block HDL binding and inhibit HDL-dependent steroidogenesis in cultured murine adrenocortical cells [20]. Furthermore, SR-BI facilitates the entry of HDL cholesteryl esters into cultured human adrenocortical cells for steroidogenesis, thus suggesting a potential role for SR-BI in human adrenals [7]. Analysis of mice with targeted null mutations in the SR-BI gene shows that SR-BI plays a role in murine adrenocortical cholesterol metabolism. These mice exhibit depleted adrenal cholesterol stores (cytoplasmic cholesteryl ester storage droplets) in a gene-dose-dependent manner with reductions of 42% in heterozygotes and 72% in homozygote null SR-BI mutants [15]. Our recent studies show that HDL induces AP-1 activity via the PI3K/Akt signaling pathway and participates in HDL-CLA-1-mediated cell growth [13].

In this study, we have investigated the potential role of CLA-1 in adrenocortical cell function by creating a mutant CLA-1 (mCLA). This clone was used to establish a stably transfected cell line and data arising from the use of these tools suggested that CLA-1 mediated the proliferative features of HDL. This effect involves, in part, the activity of the PI3K/Akt pathway.

Materials and methods

Materials. Wortmannin, PD98059, JNKI-1, and SB203580 were purchased from Calbiochem. TNF-α was obtained from Research Biochemicals International (Natick, MA). All other reagents were of analytical grade.

Cell culture. Y-1, mouse adrenocortical tumor cell line was provided by NIHS (Osaka, Japan). These cells were cultured in Ham's F-10 medium supplemented with 15% horse serum, 2.5% fetal calf serum, 100 μg/ml streptomycin, and 100 U/ml penicillin in a humidified atmosphere containing 5% CO_2.

RNA isolation and RT-PCR analysis. SR-BI/CLA-1 expression was determined by PCR analysis of the reverse transcribed RNA as described previously [6,7]. A primer pair matching the published sequence [5] of CLA-1 (sense; 5′-ATGATCGTGATGGTGCCGTC-3′ and antisense; 5′-ACTGAACCTGCAGGTGCTGA-3′) was used to amplify a 930 bp fragment.

Plasmid construction. CLA-1 expression vectors were constructed to express chimeric CLA-1 mutants fused to FLAG in full-length CLA-1 (aa 1–509) or mutant CLA-1 (aa 1–464) fused to FLAG in its N-terminus. The cDNA containing full-length CLA-1 (fCLA-1) or a mutant CLA-1 (mCLA-1) fused to FLAG was obtained by PCR using the CLA-1 expression vector as previously described [13]. The PCR product was digested with *Hin*dIII/*Xba*I and subcloned into pcDNA3 mammalian expression vector (Invitrogen, San Diego, CA), and the sequence of these expression vectors was verified by dideoxy-sequencing. An expression vector encoding a dominant-negative mutant of Akt (Akt-DN) was described previously [21].

HDL selective CE uptake. Human HDL ($d = 1.070$–1.20 g/ml) was isolated by preparative ultracentrifugation from fresh plasma collected in EDTA (1 mg/ml) as described [6]. The transfected Y-1 cells were washed once with serum-free medium/0.5% BSA. [^{125}I]Dilactitol tyramine-[^{3}H]cholesterol oleyl ether HDL_3 particles were added at a concentration of 10 μg of 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 ^{125}I radioactivity and organic solvent-extractable ^{3}H radioactivity [6,22,23]. 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 previously described [22,23].

Western blot analysis. The cytoplasmic proteins were lysed in RIPA buffer as described previously [6]. The proteins were separated on a 7.5% SDS–polyacrylamide gel and then transferred to PVDF membrane. The membranes were incubated with the anti-CLA-1 antibody (diluted 1/3000 from whole antiserum) [6] or anti-cyclophilin A antibody (Biomol Research, Plymouth Meeting, PA; diluted 1/1000). The antibody binding was visualized by chemiluminescence detection (ECL, Amersham Corp., Arlington Heights, IL).

Proliferation assay. Y-1 cells (10^4) were cultured in 96-well plates in 200 μl of Ham's F-10 medium with 10% lipoprotein-deficiency serum, LPDS. The medium was replaced with DMEM plus LPDS with or without 500 μg/ml HDL and the cells were incubated for an additional 24 h at 37 °C. Four hours before harvesting, cells were pulsed with 1 μCi/well [^{3}H]TdR (sp. act. = 6.7 Ci/mmol, Du Pont Company, Wilmington, DE) and then 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 Y-1 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 Y-1 cells (at 60% confluence) using a conventional cationic liposome transfection method (Lipofectamine, Life Technologies, Gaithersburg, MD). All assays were corrected for β-galactosidase activity and total amount of protein in each reaction was identical. Twenty microliter of aliquots were taken for the luciferase assay, which was performed according to the manufacturer's instructions (ToyoInk, Tokyo, Japan).

Electrophoretic mobility shift analysis (EMSA). Y-1 cells were treated with or without 500 μg/ml HDL for 12 h and nuclear extracts were prepared according to a technique described previously [24]. A synthetic DNA duplex spanning AP-1 (5′-TTCCGGCTGACTCAAGCG-3′) (Nihon Bioservice, Asagiri, Japan) used in these studies was radiolabeled as described previously [13]. 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 detected using a Bioimaging Analyzer (BAS 1000 system, Fuji Photo Film, Tokyo, Japan).

Statistical analysis. Statistical comparisons were made by one-way analysis of variance and Student's t test, with $P < 0.01$ considered significant.

Results

Expression of SR-BI/CLA-1 in mouse adrenocortical cell line, Y-1

The expression of CLA-1 has been demonstrated in various cell types including adrenal gland and cell lines [4,6] but its presence in the mouse adrenocortical cell line Y-1 is not known. To address this question, we examined the mRNA expression of SR-BI/CLA-1 in Y-1 cells using RT-PCR. Results (Fig. 1A) showed that SR-BI/CLA-1 was present in Y-1 and control mouse adrenocortical cells. Consistent with this finding SR-BI/CLA-1 protein was detected (Fig. 1B) in lysate from the Y-1 cells. Together these findings show that the mRNA encoding and the corresponding protein of SR-BI/CLA-1 were present in Y-1 cells and are consistent with the previous report [25].

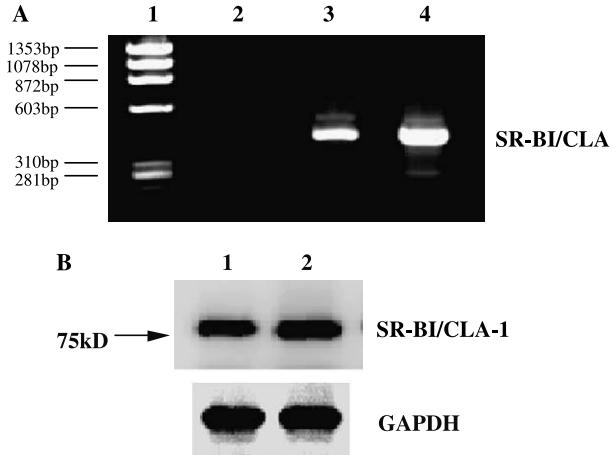

Fig. 1. Expression of SR-BI/CLA-1 in mouse adrenocortical cell line, Y-1. (A) RT-PCR analysis of SR-BI/CLA-1 mRNA expression. Total RNA was isolated from Y-1 and mouse adrenal gland, and RT-PCR was performed as described in Materials and methods. Lane 1, molecular marker; lane 2, without DNA (negative control); lane 3, Y-1 cell; lane 4, mouse adrenal gland (positive control). (B) Western blot analysis. Total cell protein extracted with 1% NP40 from Y-1 cells and mouse adrenal gland was separated by SDS–PAGE. Each lane contained 10 μg of protein. Lane 1, Y-1 cells; lane 2, mouse adrenal gland.

Mutant CLA-1 inhibits cell growth and blocks the stimulatory response of HDL

To determine the role of SR-BI/CLA-1 in the adrenal cancer, we created a mutant CLA-1 (mCLA). This mutant retained an intact extracellular domain (1–464 amino acid residues) that facilitated the binding of the receptor to HDL but lacked the C-terminal tail, the intracellular domain of CLA-1 believed to regulate HDL signaling. Mutant CLA-1 was used to establish a stably transfected Y-1 cell line. The ability of these cells to proliferate was measured by assessing cell number after varying days of growth. Several stably transfected clones were isolated (Fig. 2A) and their rates of growth compared with that of cells transfected with a full-length CLA-1, fCLA-1 (Fig. 2B). Clone CLA-3 was selected for further studies because of its high level of tag protein expression (Fig. 2A). Results showed that after 4 days of growth, the numbers of cells in wells seeded with CLA-3, the mCLA-1 containing clone, were significantly less than that containing fCLA-1. The growth of mCLA expressing clone was also lower compared to that of the control (mock-transfected cell).

Previous findings of others added to those from our laboratory showed that cells take up cholesterol ester from HDL by a selective, non-endocytotic pathway for the delivery of HDL-associated CE [1,6]. To test the function of mCLA-1, we measured abundance of CE uptake using doubly labeled HDL. Results (Fig. 3) showed that HDL CE selective uptake by cells carrying the mCLA-1 receptor was fully active and no different from those carrying fCLA-1. These results are consistent with the previous observations of Connelly et al. [22].

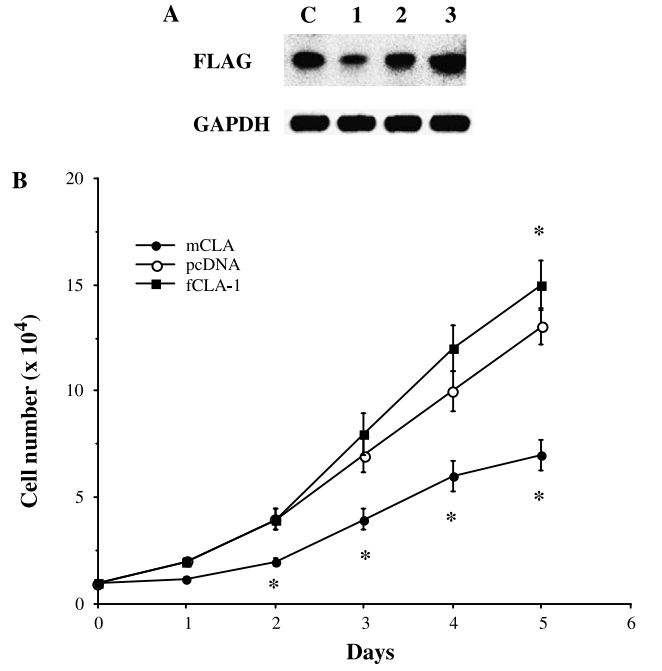

Fig. 2. Establishment of Y-1 cells stably transfected with mCLA. (A) Expression of tag protein in mCLA transfected clones. Lane C, full-length CLA-1-transfected cells; lanes 1–3, CLA1, 2, 3 (mCLA-transfected cell clones), respectively. Top, FLAG; bottom, GAPDH. (B) Proliferation rate of mCLA-overexpressing Y-1 cells. 1×10^3 cells were seeded in 6-well plates on day 0 and counted at 24-h intervals for the duration of the experiment. Each data point shows the means ± SEM ($n = 5$) of separate experiments. There was a significant difference {$*P < 0.01$ versus controls (pcDNA)} in the number of cells per well from days 1–5 of the experiment. fCLA-1, full-length CLA-1-transfected cells; mCLA (mCLA-transfected cell clone, CLA-3); and pcDNA, vector pcDNA3-transfected cells.

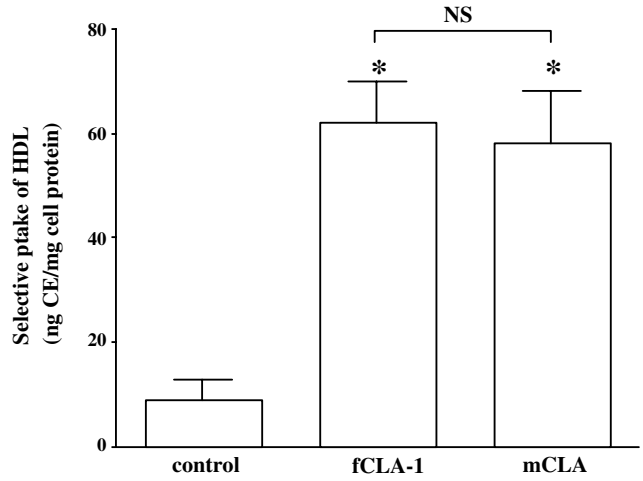

Fig. 3. Selective uptake of HDL CE in the transfected Y-1 cells. Cells transfected with vector pcDNA3 (control), full-length CLA-1 (fCLA-1) or mutant CLA-1 (mCLA) were incubated at 37 °C for 1.5 h with 10 μg/ml [^{125}I, ^{3}H]HDL, after which the cells were processed in order to determine HDL CE selective uptake. Values represent means of triplicate determinations. The asterisk and NS denote a significant ($P < 0.01$) and a non-significant difference, respectively.

Next, we measured [^{3}H]thymidine uptake to detect cell growth in mCLA expressing and control cells. Not surprisingly, [^{3}H]thymidine uptake was significantly lower in the CLA-3 cells versus that in the fCLA-1-transfected Y-1 cells (Fig. 4A). Cells were exposed to 0, 100, or 500 μg/ml of HDL added to the culture media followed by measurement of [^{3}H]thymidine uptake into the cells. Results (Fig. 4A) showed that [^{3}H]thymidine uptake into the fCLA-1-transfected clone (open bars) increased significantly in a dose-dependent manner. In contrast, the exposure of the mCLA expressing cells (shaded bars) to HDL had no effect on [^{3}H]thymidine uptake.

To test whether protein kinases were 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 (500 μg/ml) was combined with cotreatment of Y-1 cells using inhibitors of a PI3K (10 μM wortmannin), a mitogen-activated ERK (10 μM PD98059), a JNK (1μM JNK Inhibitor-I) or a p38 MAP-kinase (1μM SB203580). Results (Fig. 4B) showed that HDL-mediated [^{3}H] uptake was not sensitive to inhibitors of ERK, JNK, and p38 MAP-kinase but it was sensitive to wortmannin, an inhibitor of phosphoinositide 3-kinase (PI3K). Together the above findings show the need for an intact CLA-1 in Y-1 cell proliferation. This proliferation is stimulated by HDL and is inhibited by wortmannin.

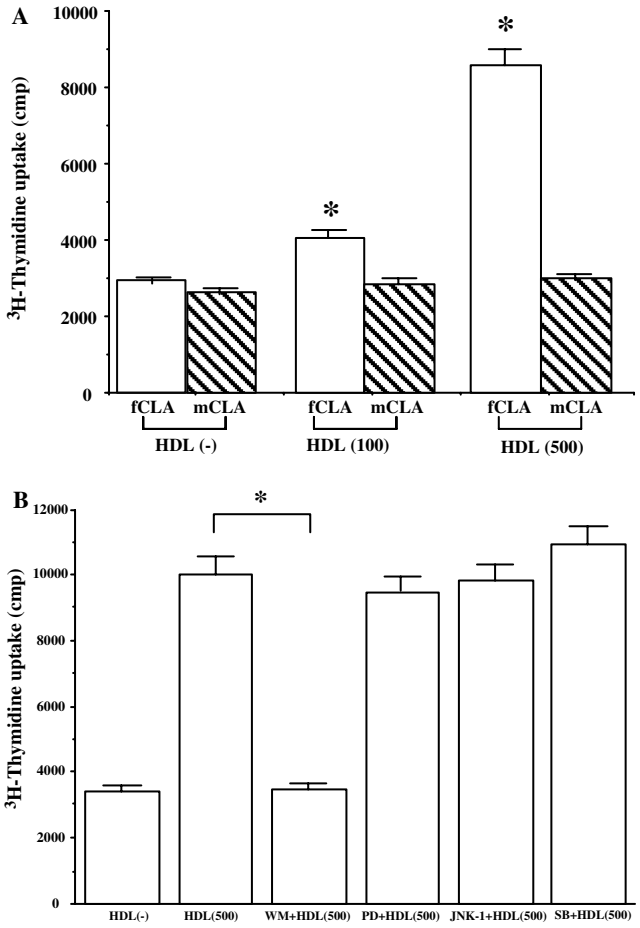

Fig. 4. Proliferation assay in Y-1 cells stably transfected with mCLA. (A) Cell proliferation was assessed by treating cells with [^{3}H]thymidine for 6 h prior to harvest. Each column represents cellular uptake of [^{3}H]thymidine (means ± SEM, $n = 5$). fCLA-1, full-length CLA-1-transfected cells; mCLA, mutant CLA-1 transfected clone (CLA-3). The asterisk denotes a significant difference ($P < 0.01$). (B) Effects of a PI3K inhibitor Wortmannin (WM), a MEK1 inhibitor PD98059 (PD), a JNK inhibitor JNK inhibitor-I (JNKI-I) or a p38 MAP-kinase inhibitor SB203580 (SB) on HDL-stimulated [^{3}H]thymidine incorporation into Y-1 cell DNA. Inhibitors were added for 1.5 h prior to HDL (500 μg/ml) stimulation. Values represent means of triplicate determinations. The asterisk denotes a significant difference ($P < 0.01$).

Actions of CLA-1 in the presence of HDL affect AP-1 activity

We recently reported that the expression of mCLA inhibited the proliferation of a breast cancer cell line [13]. This inhibitory action of mCLA required the transcriptional factor AP-1. To determine whether mCLA affected AP-1 DNA binding and function in Y-1 cells, we measured these parameters using EMSA and reporter gene analysis, respectively. Results (Fig. 5) showed that the addition of HDL 500 μg/ml to cells stimulated the DNA binding activity of AP-1 (Fig. 5A) in the fCLA-1 containing cells but not in those carrying mCLA-1. The increase in AP-1 activity was specific because the DNA binding activity of other transcription factors; NF-κB and NF-IL6 in the same extracts were not affected by HDL treatment (data not shown).

Whether the increase in AP-1 DNA binding activity matched transcriptional activity of the protein was not known. Therefore, we measured the activity of pAP1-LUC in cells stably transfected with fCLA-1 or mCLA-1. Results (Fig. 5B) showed that luciferase activity was increased 1.5-fold in the fCLA-1 containing cells following treatment with HDL (Fig. 5B), but HDL had no effect on either the DNA binding activity of AP-1 or on transcription of the reporter gene in the mCLA expressing cells.

To test whether the same protein kinases that are involved in HDL-stimulated AP-1 transcriptional activity, we studied the effect of various pharmacological inhibitors used in the above studies (Fig. 6A). In agreement with the inhibitor data above, HDL-stimulated AP-1 activity was not sensitive to inhibition of ERK, JNK, and p38 MAP-kinase but wortmannin inhibition of PI3K blocked HDL induction of AP-1 mediated transcriptional activity.

Although PI3K has many potential downstream targets, we focused our attention on Akt because of several reports [21,12] showing the importance of this kinase in connection with HDL. We assessed the actions of a dominant negative mutant of Akt (Akt-DN) on AP-1 promoter activity in Y-1 cells co-transfected with pAP-LUC plus the Akt-DN or empty vector. Consistent with proceeding studies; results (Fig. 6B) showed a 2.4-fold rise in luciferase activity following HDL stimulation. As expected, expression of Akt-DN inhibited the actions of HDL-induction of pAP-LUC

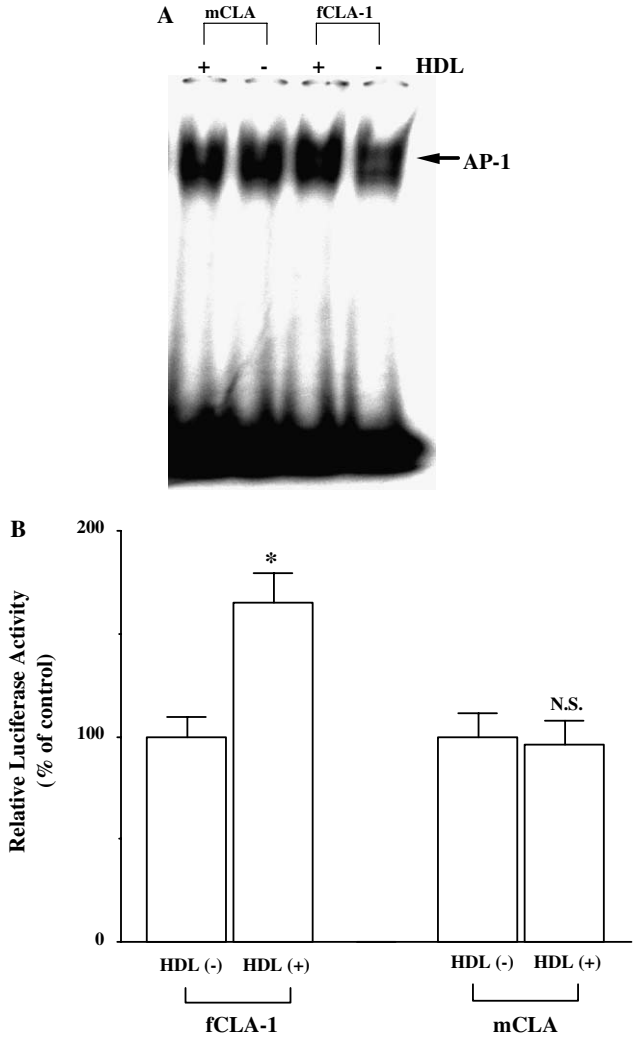

Fig. 5. Effect of HDL on AP-1 binding activity and transcriptional activity and in mCLA expressing cells. (A) Cells were exposed to 500 μg/ml HDL for 6 h before preparing the nuclear extracts and binding activity of the AP-1 was examined using EMSA. fCLA-1, full-length CLA-1-transfected cells; mCLA, mutant CLA-1 transfected clone (CLA-3). HDL, HDL 500 μg/ml. Arrow indicates the DNA-protein complex. An identical experiment independently performed gave similar results. (B) Y-1 cells were transfected with 1 μg of pAP1-LUC and treated with 500 μg/ml HDL for 24 h prior to cell harvest. All assays were corrected for β-galactosidase activity and total amount of protein per reaction was identical. The results were expressed as relative luciferase activity compared to control cells arbitrarily set at 100. fCLA-1, full-length CLA-1-transfected cells; mCLA, mutant CLA-1 transfected clone (CLA-3). Each data point shows the mean ± SEM ($n = 4$) of separate transfections. The asterisk and NS denote a significant ($P < 0.01$) and a non-significant difference, respectively.

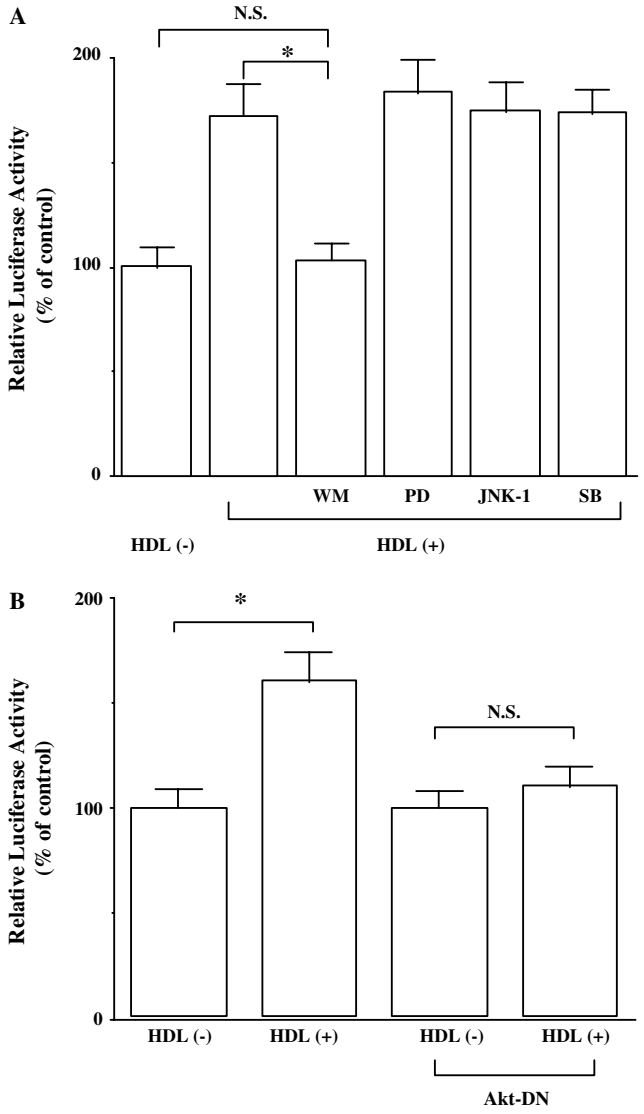

Fig. 6. A PI3K/Akt pathway mediates the activation of AP-1 by HDL. (A) Effects of a PI3K inhibitor Wortmannin (WM), a MEK1 inhibitor PD98059 (PD), a JNK inhibitor JNK Inhibitor-I (JNKI-I) or a p38 MAP kinase inhibitor SB203580 (SB) on HDL(500 μg/ml)-stimulated AP-1 transcriptional activity in Y-1 cells. Values represent means of triplicate determinations. The asterisk denotes a significant difference ($P < 0.01$). (B) Dominant negative Akt inhibits HDL mediated AP-1 transcriptional activity in Y-1 cells. Y-1 cells were transfected with pAP-LUC and empty vector or Akt-DN and then treated with HDL for 24 h prior to cell harvest. The results were expressed as relative luciferase activity compared to control cells arbitrarily set at 100. Each data point shows the means ± SEM ($n = 4$) of separate transfections. The asterisk and NS denote a significant ($P < 0.01$) and a non-significant difference, respectively.

activity. Together these findings support the idea that PI3K/Akt pathway is required for HDL induction of AP-1 promoter activity in Y-1 cells.

Discussion

In this study, we have examined the adrenocortical cell line Y-1 to further understand the roles of HDL, its receptor CLA-1, PI3K/Akt, and AP-1 in the function of this cell type. The roles of these components in cell function are important because of their potential connection to mitogenic signaling in mammalian cell-type-specific function.

The signaling pathway that controls cell cycle and growth in adrenocortical cells remains unclear. Amongst the many signaling pathways that are active in cells, the PI3K/Akt pathway is considered highly relevant [26]. In a normal cell, this pathway plays a pivotal role in essential cellular functions including survival, proliferation, migration, and

differentiation that underlie the biology of human cancer [26]. Aberrant activation of the PI3K/Akt pathway contributes to tumorigenesis, tumor metastasis, and resistance to standard cancer therapy [27]. Multiple components of this pathway are involved in oncogenesis [27,28]. For example, growth factor receptor protein tyrosine kinases, integrin-dependent cell adhesion, and G-protein-coupled receptors may activate PI3K either directly or indirectly through activation of Ras [28]. The loss of PTEN, PI3K amplification, and Akt overexpression are described in many malignancies including adrenocortical tumors [28].

Since the early observations of Masui and Garren [29], the anti-mitogenic effects of ACTH are believed to be exerted via the cAMP/PKA pathway [30], but its mechanisms remain unknown. Recent reports that ACTH promotes dephosphorylation of the Akt/PKB enzymes and degradation of c-Myc protein support the idea that the anti-mitogenic effects are mediated by the cAMP/PKA pathway [31]. The use of adrenocortical Y-1 cells that respond to ACTH to understand the potential role of mitogenic signaling is supported by our recent findings of high constitutive levels of phosphorylated Akt/PKB, the maintenance of which is completely dependent on PI3K activity [32]. The rationale for using HDL to stimulate the signaling pathway comes from our recent report that expression of the mutant receptor for HDL inhibits the proliferation of breast cancer cell line, MCF-7 cells. This effect involves, in part, a transcription factor, activator protein-1 (AP-1) that is activated by PI3K/Akt pathway [13]. Together the findings of others added to our data provide the essential components for examining whether PI3K/Akt signaling is involved in HDL-induced cell growth of Y-1 cells. Indeed, our results showed that PI3K/Akt signaling pathway is involved in HDL-CLA-1 mediated adrenocortical tumor growth.

AP-1 factors are known to participate in a variety of cellular processes such as proliferation and differentiation by regulating gene transcription [33]. They are protein complexes comprised of dimers of Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, Fos-B, Fra-1, and Fra-2) family members. Some of the AP-1 transcription factors are homodimers of Jun, while others are heterodimers from Jun and Fos family members. Previous studies suggest that growth factors and hormones may modulate the activity of AP-1 which in turn affects gene transcription [34]. These findings provide indirect evidence that suggests AP-1 transcription factor may play an important role in the cell growth, invasion, and resistance to anti-cancer drugs. Our previous finding that activity of AP-1 is affected by 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 members of the AP-1 transcription factor family function as transducers of multiple signaling pathways including those related to mitogenesis and stress [35].

Previous studies show that the extracellular domain of SR-BI (the rodent homologue of CLA-1) is not only important in binding HDL particles it is also required for the efficient and selective uptake of HDL cholesterol ester [36,22]. Recent findings of others including those from our laboratory indicate that SR-BI/CLA-1 supplies cholesterol, the substrate for steroid hormone synthesis in the adrenocortical cells [20,37]. Structure-function studies using selected SR-B1 domains in swapping studies with CD36 added to point mutation studies 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 [36,38]. In support of this idea, Connelly et al. [22] reported that deletion of the 45-amino acid carboxyl terminus of SR-BI did not reduce selective uptake. Thus, the extracellular domain of the receptor appears to mediate selective uptake of HDL but the C-terminus of the protein is dispensable for SR-BI function. Our recent report showed that the C-terminus of CLA-1 might play an important role on cell growth and anti-apoptotic effects [13]. We predict that the coupling might involve intermediary proteins such as CLAMP, a protein containing four PDZ domains that associates with the extreme C-terminus of SR-BI [39]. The role of C-terminal tail on cell proliferation, as well as its function as a potential signaling receptor in adrenocortical cells, deserves further investigation.

In summary, we examined the role of HDL on cell proliferation using expression of mCLA in the adrenocortical cell line Y-1. Our results show that a mCLA inhibits cell proliferation via the PI3K/Akt pathway and involves the AP-1 transcription factor in these cells. These findings make it tempting to think that an inhibitor for HDL signaling may be of therapeutic value for the treatment of adrenocortical tumors.

References

[1] J.T. Gwynne, J.F. Srauss 3rd, The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic gland, Endocr. Rev. 3 (1982) 299–328.
[2] J.T. Gwynne, B. Hess, The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis, J. Biol. Chem. 255 (1980) 10875–10883.
[3] S.B. Leichter, W.H. Daughaday, Massive steroid excretion and hypocholesterolemia with an adrenal adenoma. Report of a case, Ann. Intern. Med. 81 (1974) 638–640.
[4] S. Acton, A. Rigotti, K.T. Landschulz, S. Xu, H.H. Hobbs, M. Krieger, Identification of scavenger receptor SR-BI as a high density lipoprotein receptor, Science 271 (1996) 518–520.
[5] D. Calvo, M.A. Vega, Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family, J. Biol. Chem. 268 (1993) 18929–18935.
[6] K. Murao, V. Terpstra, S.R. Green, N. Kondratenko, D. Steinberg, O. Quehenberger, Characterization of CLA-1, a human homologue of rodent scavenger receptor BI, as a receptor for high density lipoprotein and apoptotic thymocytes, J. Biol. Chem. 272 (1997) 17551–17557.
[7] H. Imachi, K. Murao, M. Sato, H. Hosokawa, T. Ishida, J. Takahara, (a) CD36 LIMPII analogous-1, a human homolog of the rodent scavenger receptor B1, provides the cholesterol ester for steroidogenesis in adrenocortical cells, Metabolism 48 (1999) 627–630.

[8] H. Imachi, K. Murao, Y. Sayo, H. Hosokawa, M. Sato, M. Niimi, S. Kobayashi, A. Miyauchi, T. Ishida, J. Takahara, (b) Evidence for a potential role for HDL as an important source of cholesterol in human adrenocortical tumors via the CLA-1 pathway, Endocr. J. 46 (1999) 27–34.
[9] H. Imachi, K. Murao, C. Hiramine, Y. Sayo, M. Sato, H. Hosokawa, T. Ishida, T. Kodama, O. Quehenberger, D. Steinberg, J. Takahara, Human scavenger receptor B1 is involved in recognition of apoptotic thymocytes by thymic nurse cells, Lab. Invest. 80 (2000) 263–270.
[10] H. Imachi, K. Murao, W.M. Cao, T. Ohyama, M. Sato, Y. Sasaguri, T. Ishida, J. Takahara, Expression of HDL receptor, CLA-1 in human smooth-muscle cells and effect of interferon-gamma on its regulation, Horm. Metab. Res. 33 (2001) 389–393.
[11] H. Imachi, K. Murao, W.M. Cao, S. Tada, T. Taminato, N.C. Wong, J. Takahara, T. Ishida, Expression of human scavenger receptor B1 on and in human platelets, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 898–904.
[12] W.M. Cao, K. Murao, H. Imachi, X. Yu, H. Dobashi, K. Yoshida, T. Muraoka, N. Kotsuna, S. Nagao, N.C. Wong, T. Ishida, (a) Insulin like growth factor-I regulation of hepatic scavenger receptor class BI, Endocrinology 145 (2004) 5540–5547.
[13] W.M. Cao, K. Murao, H. Imachi, X. Yu, H. Abe, A. Yamauchi, M. Niimi, A. Miyauchi, N.C. Wong, T. Ishida, (b) A mutant hdl receptor inhibits proliferation of human breast cancer cells, Cancer Res. 64 (2004) 1515–1521.
[14] K. Landschulz, R. Pathak, A. Rigotti, M. Krieger, H. Hobbs, Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat, J. Clin. Invest. 98 (1996) 984–995.
[15] A. Rigotti, E.R. Edelman, P. Seifert, S.N. Iqbal, R.B. DeMattos, R.E. Temel, M. Krieger, D.L. Williams, Regulation by adrenocorticotropic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland, J. Biol. Chem. 271 (1996) 33545–33549.
[16] J.L. Smith, S.R. Lear, T.M. Forte, W. Ko, M. Massimi, S.K. Erickson, Effect of pregnancy and lactation on lipoprotein and cholesterol metabolism in the rat, J. Lipid. Res. 39 (1998) 2237–2249.
[17] G. Cao, C.K. Garcia, K.L. Wyne, R.A. Schultz, K.L. Parker, H.H. Hobbs, Structure and localization of the human gene encoding SRBI/CLA-1. Evidence for transcriptional control by steroidogenic factor 1, J. Biol. Chem. 272 (1997) 33068–33076.
[18] J. Liu, R. Voutilainen, P. Heikkila, A.I. Kahri, Ribonucleic acid expression of the CLA-1 gene, a human homolog to mouse high density lipoprotein receptor SR-BI, in human adrenal tumors and cultured adrenal cells, J. Clin. Endocrinol. Metab. 82 (1997) 2522–2527.
[19] G. Martin, A. Pilon, C. Albert, M. Valle, D.W. Hum, J.C. Fruchart, J. Najib, V. Clavey, B. Staels, Comparison of expression and regulation of the high-density lipoprotein receptor SR-BI and the low-density lipoprotein receptor in human adrenocortical carcinoma NCI-H295 cells, Eur. J. Biochem. 261 (1999) 481–491.
[20] R.E. Temel, B. Trigatti, R.B. DeMattos, S. Azhar, M. Krieger, D.L. Williams, Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein cholesterol to the steroidogenic pathway in cultured mouse adrenocortical cells, Proc. Natl. Acad. Sci. USA 94 (1997) 13600–13605.
[21] W.M. Cao, K. Murao, H. Imachi, M. Sato, T. Nakano, T. Kodama, Y. Sasaguri, N.C. Wong, J. Takahara, T. Ishida, Phosphatidylinositol 3-OH kinase-Akt/protein kinase B pathway mediates Gas6 induction of scavenger receptor a in immortalized human vascular smooth muscle cell line, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1592–1597.
[22] M.A. Connelly, M. de la Llera-Moya, P. Monzo, P.G. Yancey, D. Drazul, G. Stoudt, N. Fournier, S.M. Klein, G.H. Rothblat, D.L. Williams, Analysis of chimeric receptors shows that multiple distinct functional activities of scavenger receptor, class B, type I (SR-BI), are localized to the extracellular receptor domain, Biochemistry 40 (2001) 5249–5259.
[23] M.A. Connelly, G. Kellner-Weibel, G.H. Rothblat, D.L. Williams, SR-BI-directed HDLcholesteryl ester hydrolysis, J. Lipid. Res. 40 (2003) 331–341.
[24] K. Murao, Y. Wada, T. Nakamura, A.H. Taylor, A.D. Mooradian, N.C. Wong, Effects of glucose and insulin on rat apolipoprotein A-I gene expression, J. Biol. Chem. 273 (1998) 18959–18965.
[25] G. Cao, L. Zhao, H. Stangl, T. Hasegawa, J.A. Richardson, K.L. Parker, H.H. Hobbs, Developmental and hormonal regulation of murine scavenger receptor, class B, type 1, Mol. Endocrinol. 13 (1999) 1460–1473.
[26] J.E. Dancey, Molecular targeting: PI3 kinase pathway, Ann. Oncol. 15 (2004) 233239.
[27] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655–1657.
[28] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501.
[29] H. Masui, L.D. Garren, Inhibition of replication in functional mouse adrenal tumor cells by adrenocorticotropic hormone mediated by adenosine 3′:5′-cyclic monophosphate, Proc. Natl. Acad. Sci. USA 68 (1971) 3206–3210.
[30] C.F. Lotfi, Z. Todorovic, H.A. Armelin, B.P. Schimmer, Unmasking a growth-promoting effect of the adrenocorticotropic hormone in Y1 mouse adrenocortical tumor cells, J. Biol. Chem. 272 (1997) 29886–29891.
[31] F.L. Forti, H.A. Armelin, ACTH inhibits A Ras-dependent anti-apoptotic and mitogenic pathway in mouse Y1 adrenocortical cells, Endocr. Res. 26 (2000) 911–914.
[32] F.L. Forti, T.T. Schwindt, M.S. Moraes, C.B. Eichler, H.A. Armelin, ACTH promotion of p27(Kip1) induction in mouse Y1 adrenocortical tumor cells is dependent on both PKA activation and Akt/PKB inactivation, Biochemistry 41 (2002) 10133–10140.
[33] M. Karin, T. Smeal, Control of transcription factors by signal transduction pathways: the beginning of the end, Trends Biochem. Sci. 17 (1992) 418–422.
[34] Y. Liu, J. Ludes-Meyers, Y. Zhang, D. Munoz-Medellin, H.T. Kim, C. Lu, G. Ge, R. Schiff, C.K. Osborne, P.H. Brown, Inhibition of AP-1 transcription factor causes blockade of multiple signal transduction pathways and inhibits breast cancer growth, Oncogene 21 (2002) 7680–7689.
[35] R.J. Imbra, M. Karin, Metallothionein gene expression is regulated by serum factors and activators of protein kinase C, Mol. Cell. Biol. 7 (1987) 1358–1363.
[36] X. Gu, B. Trigatti, S. Xu, S. Acton, J. Babitt, M. Krieger, The efficient cellular uptake of high density lipoprotein lipids via scavenger receptor class B type I requires not only receptor-mediated surface binding but also receptor-specific lipid transfer mediated by its extracellular domain, J. Biol. Chem. 273 (1998) 26338–26348.
[37] M.A. Connelly, D.L. Williams, SR-BI and cholesterol uptake into steroidogenic cells, Trends Endocrinol. Metab. 14 (2003) 467–472.
[38] X. Gu, R. Lawrence, M. Krieger, Dissociation of the high density lipoprotein and low density lipoprotein binding activities of murine scavenger receptor class B type I (mSR-BI) using retrovirus library-based activity dissection, J. Biol. Chem. 275 (2000) 120–9130.
[39] D.L. Silver, A carboxyl-terminal PDZ-interacting domain of scavenger receptor B, type I is essential for cell surface expression in liver, J. Biol. Chem. 277 (2002) 34042–34047.