Apolipoprotein A-I activates Cdc42 signaling through the ABCA1 transporter

Jerzy-Roch Nofer,[1,*,†] Alan T. Remaley,[§] Renata Feuerborn,[†] Iza Wolińska,[*] Thomas Engel,[†] Arnold von Eckardstein,[**] and Gerd Assmann[*,†]

Institut für Klinische Chemie und Laboratoriumsmedizin,* Westfälische Wilhelms-Universität, Münster, Germany; Leibniz-Institut für Arteriosklerosеforschung an der Universität Münster,† Münster, Germany; Department of Laboratory Medicine,§ Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD; and Institut für Klinische Chemie,** Universitäts-Spital Zürich, Zürich, Switzerland

Abstract **It has been suggested that the signal transduction initiated by apolipoprotein A-I (apoA-I) activates key proteins involved in cholesterol efflux. ABCA1 serves as a binding partner for apoA-I, but its participation in apoA-I-induced signaling remains uncertain. We show that the exposure of human fibroblasts to ABCA1 ligands (apolipoproteins and amphipathic helical peptides) results in the generation of intracellular signals, including activation of the small G-protein Cdc42, protein kinases (PAK-1 and p54^{JNK}), and actin polymerization. ApoA-I-induced signaling was abrogated by glyburide, an inhibitor of the ABC transporter family, and in fibroblasts from patients with Tangier disease, which do not express ABCA1. Conversely, induction of ABCA1 expression with the liver X receptor agonist, T0901317, and the retinoid X receptor agonist, R0264456, potentiated apoA-I-induced signaling. Similar effects were observed in HEK293 cells overexpressing ABCA1-green fluorescent protein (GFP) fusion protein, but not ABCA1-GFP (K939M), which fails to hydrolyze ATP, or a nonfunctional ABCA1-GFP with a truncated C terminus. We further found that Cdc42 coimmunoprecipitates with ABCA1 in ABCA1-GFP-expressing HEK293 cells exposed to apoA-I but not in cells expressing ABCA1 mutants. We conclude that ABCA1 transduces signals from apoA-I by complexing and activating Cdc42 and downstream kinases and, therefore, acts as a full apoA-I receptor.**—Nofer, J-R., A. T. Remaley, R. Feuerborn, I. Wolińska, T. Engel, A. von Eckardstein, and G. Assmann. **Apolipoprotein A-I activates Cdc42 signaling through the ABCA1 transporter.** ***J. Lipid Res.*** **2006.** 47: **794–803.**

Supplementary key words ATP binding cassette transporter A1 • high density lipoproteins • cholesterol efflux

ABCA1 mediates the active transfer of excess cellular cholesterol and phospholipids from cells to exchangeable apolipoproteins, such as apolipoprotein A-I (apoA-I), apoC-III, and apoE (1–3). The nascent HDL particles formed in this process initiate reverse cholesterol transport, one of the major mechanisms by which HDL protects against the development of atherosclerosis. The importance of ABCA1 for reverse cholesterol transport has been underscored by the identification of ABCA1 defects in Tangier disease, a severe HDL deficiency syndrome characterized by cholesterol deposition in tissue macrophages and premature atherosclerosis (1–3).

The mechanism by which ABCA1 facilitates cholesterol egress from cells is still a matter of debate. There is emerging evidence, however, that both binding of apolipoproteins to ABCA1 and apolipoprotein-induced cellular signaling play important roles in initiating lipid efflux. Cross-linking studies documented that apoA-I and other exchangeable apolipoproteins interact directly with ABCA1 on the cell surface (4–7). In addition, apoA-I was shown to activate several serine/threonine protein kinases, including protein kinase A (PKA), protein kinase C (PKC), and Janus kinase 2 (JAK2) (8–11). Both PKA and PKC directly phosphorylate ABCA1 and thereby control its expression level and activity (10, 12–14). It has not been unequivocally established, however, whether the binding of apolipoproteins to ABCA1 is directly involved in the activation of intracellular signaling pathways by apolipoproteins.

We previously demonstrated that exposure of cells to apoA-I induces the activation of Rho family small G-proteins, including Cdc42 and Rac1, as well as actin polymerization, which is known to be controlled by Cdc42 (15). In addition, protein kinases localized downstream of Cdc42 in the signaling cascade, such as PAK-1 and p54^{JNK}, were activated in the presence of apoA-I. Inhibition of Cdc42 or p54^{JNK} partially inhibited apoA-I-induced cholesterol efflux, indicating that these signal transduction pathways are obligatory for the optimal ABCA1-dependent

Manuscript received 15 November 2005 and in revised form 5 January 2006.

Published, JLR Papers in Press, January 28, 2006.
DOI 10.1194/jlr.M500502-JLR200

Abbreviations: apoA-I, apolipoprotein A-I; GEF, GDP exchange factor; GFP, green fluorescent protein; GST-PBD, glutathione *S*-transferase-p21 binding domain; JAK2, Janus kinase 2; LXR, liver X receptor; PC, phosphatidylcholine; PKA, protein kinase A; PKC, protein kinase C; RXR, retinoid X receptor; WS, Werner syndrome.

[1] To whom correspondence should be addressed.
e-mail: nofer@uni-muenster.de

transport of lipids from cells to apolipoproteins. However, it is not known whether ABCA1 is directly required for Cdc42 activation or is localized distally to the Cdc42 signaling cascade, where it functions solely as a cholesterol and/or phospholipid transporter. In this study, we provide evidence that ABCA1 directly links apoA-I binding to activation of the Cdc42 signaling cascade and thereby to cholesterol efflux in fibroblasts.

EXPERIMENTAL PROCEDURES

Materials

Phosphospecific antibodies against $p54^{JNK}$ were purchased by Cell Signaling Technologies (Beverly, MA). Polyclonal antibodies against nonphosphorylated $p54^{JNK}$ and ABCA1 were from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies against green fluorescent protein (GFP) were from Roche (Mannheim, Germany). Anti-Cdc42 antibodies were from BD Biosciences (Erembodegem, Belgium). Glutathione *S*-transferase-p21 binding domain (GST-PBD) beads were purchased by Upstate Biotechnology (Chicago, IL). ApoA-I, apoC-III, and apoE were obtained from Merck Biosciences (Schwalbach, Germany). 4,4-difluror-4-bora-3a,4a-diaza-*s*-indacene (BODIPY)-phalloidin and Alexa Fluor 594 succinimidyl ester were from Molecular Probes (Eugene, OR). [^{3}H]cholesterol was obtained from Amersham (Braunschweig, Germany). DMEM, FBS, and BSA were from PAA Laboratories (Pasching, Germany). The liver X receptor (LXR) agonist, T0901317, and the retinoid X receptor (RXR) agonist, R0264456, were generous gifts of Dr. Michael Pech (F. Hoffman-La Roche, Basel, Switzerland). All others chemicals were from Sigma (Deisenhofen, Germany).

Tangier disease patient

Dermal fibroblasts were obtained from a 65 year old male patient with Tangier disease, who presented with dyslipidemia (total cholesterol < 100 mg/ml, HDL-cholesterol < 2 mg/ml) and coronary heart disease. Characterization of the ABCA1 defect at the molecular level revealed a homozygous 1 bp deletion in exon 14, leading to a stop codon at amino acid position 635 and the deletion of most of the protein sequence, including both ATP cassettes.

Cell culture

Human skin fibroblasts cultured from biopsies of adult human hip skin were grown and maintained in DMEM containing 10% (v/v) FBS, 2 mmol/l L-glutamine, and 1% (v/v) antibiotic/antimycotic solution. Once separated, the dermis was cut into small pieces (0.5 mm on each side) and placed in a flask in DMEM. When these primary cultures were confluent, they were expanded by passage. For experiments, cells between passage levels three and six were used. HEK293 cells were purchased from the American Tissue Cell Culture Collection and grown to confluence in DMEM supplemented with FBS (10%, v/v). For experiments, cells were plated on 6- or 24-well plates coated with collagen. Both human fibroblasts and HEK293 cells were enriched with cholesterol by incubation in serum-free DMEM with 2 mg/ml BSA and 30 μg/ml nonlipoprotein cholesterol for 24 h.

Plasmid constructs, and generation of cell lines stably expressing ABCA1-GFP

The coding sequence of human ABCA1 (NM_005502.2, bp 311–7,096) without stop codon was amplified from human macrophage cDNA in three ~2 kb fragments, which were reassembled in pBluescriptII KS+, using endogenous restriction sites *Bcl*I and *Bsu*36I, at positions 1,961 and 4,187, respectively, within the coding sequence of the ABCA1-cDNA. The whole coding sequence of ABCA1 was cut with *Apa*I and *Not*I restriction sites, which had been introduced during amplification of the cDNA fragments at the 5′ and 3′ ends and ligated into pcDNA3.1(−) vector. hGFP was amplified from phrGFP (Stratagene, La Jolla, CA) with flanking *Not*I and *Pme*I restriction sites and introduced in-frame with the ABCA1 open reading frame into the pcDNA3.1(−)-ABCA1 plasmid. ABCA1-W-GFP variant with the disrupted first Walker A motif of ABCA1 was constructed by PCR-based mutagenesis, creating a missense mutation of K939M. ABCA1-ΔC-GFP variant with a deleted C terminus was generated by PCR using a reverse primer annealing at nucleotide position 5,559 and introducing a stop codon adjacent to amino acid 1,873, which led to a deletion of the terminal 388 amino acids. Plasmid DNA was checked by DNA sequencing, using the ABI PRISM® BigDye™ Terminator 3.0 cycle sequencing kit and the ABI-Prism 3700 DNA analyzer (Applied Biosystems, Weiterstadt, Germany). HEK293 cells were transfected by electroporation, using commercially available reagent (Amaxa, Köln, Germany), and selected with 0.5 mg/ml G418. Antibiotic-resistant cells were screened for the expression of the fusion protein by fluorescence microscopy, and positive clones were purified by limiting dilution.

ApoA-I cell binding assay

ApoA-I was labeled with Alexa Fluor 594 according to the manufacturer's protocol. Briefly, apoA-I (2 mg) was mixed with 1 mg of the dye, followed by 1 h of incubation at room temperature with continuous stirring. The unbound dye was separated from the conjugate using a Sephadex G-25 column (Amersham) equilibrated with PBS buffer. Human fibroblasts or HEK293 cells stably expressing ABCA1 (5×10^5 cells/ml) were incubated with labeled apoA-I (0.01 mg/ml) for 15 min, washed twice with PBS, and collected for fluorescence measurement using a Hitachi F-2000 fluorescence spectrometer (excitation wavelength, 590 nm; emission wavelength, 620 nm).

Assay for actin polymerization

Actin polymerization was quantified as described by Ha and Exton (16). Briefly, cells were treated with agonists and fixed with 4% (v/v) formaldehyde containing 2.5% (v/v) octyl glucopyranoside for 15 min on ice. Cells were then stained with 0.6 ml/well BODIPY-phalloidin (15 nmol/l) for 30 min, and bound BODIPY-phalloidin was extracted with 0.1 ml of methanol for 1 h on ice. Fluorescence intensity measurements were performed using a Hitachi F-2000 fluorescence spectrometer with excitation and emission wavelengths of 505 and 515 nm, respectively.

SDS-PAGE and Western blotting

SDS-PAGE and Western blotting were performed exactly as described previously (17). For each blot with anti-phosphospecific antibodies, loading controls were used, with an antibody against a nonphosphorylated isoform of $p54^{JNK}$.

Pulldown assay for Cdc42 activation

Stimulated fibroblasts were lysed in a buffer containing 20 mmol/l HEPES (pH 7.4), 150 mmol/l NaCl, 2% (v/v) Nonidet P-40, 20% (v/v) glycerol, 8.0 mmol/l EGTA, 8.0 mmol/l EDTA, 10 mmol/l $MgCl_2$, 1 mmol/l orthovanadate, and the Complete® protease inhibitor mixture. Cell lysates were homogenized by three freeze-thaw cycles, cleared by centrifugation (14,000 rpm,

4°C), and incubated for 1 h at 4°C with 10 μg/sample GST-PBD beads for Cdc42 precipitation. The beads were collected by centrifugation (14,000 rpm, 4°C) and washed, and captured proteins were removed by boiling for 5 min in Laemmli sample buffer. Samples were then subjected to Western blotting as described above.

Coimmunoprecipitation studies

Human dermal fibroblasts treated with LXR/RXR agonists or HEK293 cells stably expressing ABCA1-GFP were stimulated with apoA-I (0.01 mg/ml) for 10 min, washed, and scraped into 0.4 ml of assay buffer (20 mmol/l Tris-HCl, 250 mmol/l NaCl, 3 mmol/l EDTA, and 3 mmol/l EGTA, pH 7.6) containing 0.5% (v/v) Nonidet P-10 and protease inhibitors. After lysis on ice (10 min) and three freeze-thaw cycles, insoluble material was cleared by centrifugation. The soluble fraction was incubated for 1 h on ice with polyclonal anti-ABCA1 antibody (5.0 μg) or anti-GFP antibody (2.0 μg). Thereafter, 0.01 ml of protein agarose G was added, and samples were incubated overnight. Agarose beads were washed four times with assay buffer and once with high-salt assay buffer containing 350 mmol/l NaCl. Captured proteins were resuspended in Laemmli buffer, boiled, and separated using 12% SDS-PAGE. Proteins were analyzed by Western blotting using polyclonal antibodies against ABCA1 and Cdc42, as described above.

Efflux of cellular cholesterol

Cholesterol efflux was measured according to established methods (15). Briefly, [^{3}H]cholesterol (1 μCi/well) was added to cells grown on 24-well plates for 24 h. Cells were washed and incubated for 4 h at 37°C with DMEM containing BSA (0.2%, v/v) and apoA-I at desired concentrations. The efflux medium was collected and centrifuged to remove cell debris. Cells were solubilized in 0.1 mol/l NaOH. Radioactivity in efflux medium and cell lysates was determined by scintillation counting. The results are reported as percentages of efflux relative to the radioactivity fraction present in efflux medium in the absence of apoA-I.

General procedures

Data are presented as means ± SD from three separate experiments or as representative immunoblots for at least three repetitions, unless indicated otherwise.

RESULTS

ABCA1 ligands activate Cdc42 signaling in human fibroblasts

Recent investigations have demonstrated that in addition to apoA-I, several other exchangeable apolipoproteins, such as apoC-I, apoC-II, and apoC-III, as well as apoE are capable of effluxing cholesterol from cells via the ABCA1-dependent pathway (18, 19). Moreover, in cross-linking and competition experiments, these apolipoproteins were shown to physically bind to the extracellular domains of ABCA1 (7, 19). To assess the role of ABCA1 in the generation of intracellular signals, we first examined the effect of ABCA1-interacting apolipoproteins on the activation of Cdc42 and its downstream effector kinases PAK-1 and p54^{JNK}, as well as on the process of actin polymerization, which is directly regulated by Cdc42. As expected, in addition to apoA-I, two other exchangeable apolipoproteins, apoC-III and apoE, also stimulated cholesterol efflux to a similar extent. Under similar conditions, exposure of fibroblasts to all apolipoproteins tested increased the amount of extractable BODIPY-phalloidin, indicating that the intracellular content of polymerized actin increased after stimulation (**Fig. 1B**). To assess directly whether exchangeable apolipoproteins affect the activity of small G-proteins, we examined the amount of activated Cdc42 in fibroblasts treated with apoA-I, apoC-III, and apoE. The GST-PBD beads precipitated only marginal amounts of Cdc42 from unstimulated fibroblasts. By contrast, exposure of cells to apolipoproteins markedly stimulated the amount of active Cdc42. In addition, autophosphorylation of PAK-1 and phosphorylation of p54^{JNK} were observed in fibroblasts exposed to apoA-I, apoC-III, and apoE (Fig. 1C).

The ability of apolipoproteins to act as a lipid acceptor is most likely related to the shared secondary structure of these proteins, characterized by the presence of amphipathic helices. Small synthetic peptides, which do not have significant sequence homology with apolipoproteins but contain at least two amphipathic helices, are capable of interacting directly with ABCA1 and thereby inducing cholesterol egress from cells (7, 20, 21). To establish whether ligand amphipathicity is required for the effective induction of intracellular signaling in fibroblasts, we made use of two synthetic peptides: D-37pA, which was synthesized solely from D amino acids and contains two octadecameric A class amphipathic helices linked by a proline, and L3D-37pA, which contains both D and L amino acids (20). The introduction of D stereoisomers into a peptide that otherwise contains L stereoisomers is known to interfere with its ability to form an α-helical structure. Accordingly, only D-37pA, and not L3D-37pA, has been reported previously to induce cholesterol efflux and to compete with apoA-I for ABCA1 binding (20). In our hands, D-37pA fully mimicked apoA-I in its capacity to induce cholesterol efflux and actin polymerization in fibroblasts (Fig. 1A, B). Furthermore, Cdc42 activation, autophosphorylation of PAK-1, and phosphorylation of p54^{JNK} were observed in fibroblasts exposed to D-37pA (Fig. 1C). By contrast, L3D-37pA remained totally inactive with respect to all responses tested.

The heterogeneous distribution of cholesterol in the cell membrane contributes to the formation of transverse domains, which are potentially involved in transmembrane signaling. For instance, cholesterol-rich membrane rafts host several signal transduction intermediates, such as receptors, protein kinases, and adaptor molecules. To investigate the possibility that apolipoprotein- or amphipathic peptide-induced signaling arises solely as a result of changes in the domain structure of the plasma membrane brought about by the reduction of its cholesterol content, we examined the intracellular signal generation in cells exposed to cyclodextrin or phosphatidylcholine (PC)-containing liposomes, which deplete membrane cholesterol by passive diffusion that does not involve ABCA1 (22). Both cyclodextrin and PC liposomes were applied in

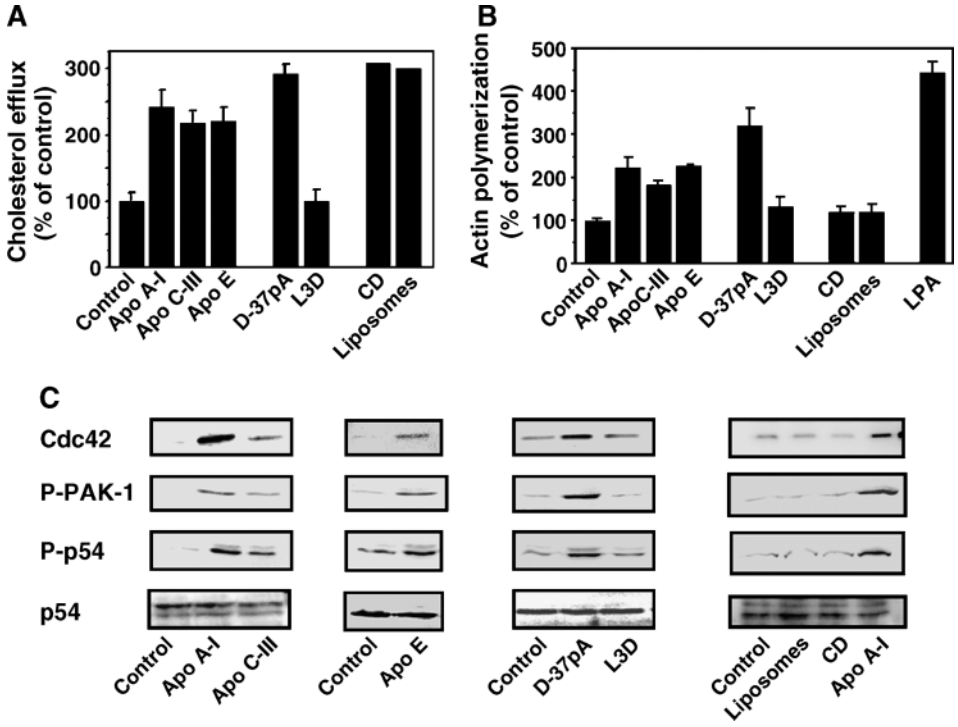

Fig. 1. ABCA1 ligands induce Cdc42 signaling in human dermal fibroblasts. Human dermal fibroblasts grown to confluence were treated with 10 μg/ml apolipoprotein A-I (apoA-I), apoC-III, or apoE, 5 μg/ml apoA-I mimetic peptides D-37pA and L3D-37pA (L3D), 1.5 mg/ml cyclodextrin (CD), or 0.5 mg/ml phosphatidylcholine (PC)-containing liposomes. A: Cells were loaded for 24 h with [^{3}H]cholesterol, washed, and incubated for 4 h with agonists as indicated. The radioactivity released into the medium or associated with cells was determined by liquid scintillation spectroscopy. Results from three to six separate experiments are shown (means ± SD). B: Cells were starved, treated for 10 min with agonists as indicated, fixed, permeabilized, and stained with BODIPY-phalloidin, as described in Experimental Procedures. The stain was extracted and quantified fluorimetrically. Cells treated with lysophosphatidic acid (LPA), a potent stimulator of actin polymerization, were used as a positive control. Results from three to five independent experiments are shown (means ± SD). C: Starved and washed cells were exposed to agonist for 10 min. Active Cdc42 was precipitated from the lysates using glutathione *S*-transferase-p21 binding domain (GST-PBD) beads, as described in Experimental Procedures. Captured proteins were separated by SDS-PAGE and analyzed by Western blotting using anti-Cdc42 antibodies. Phosphorylated isoforms (P-) of PAK-1 and p54^{JNK} were analyzed using phosphospecific antibodies. Loading equality was controlled using antibody against the unphosphorylated isoform of p54^{JNK} (p54). Blots shown are representative of three to five experiments.

sufficient concentrations to induce cholesterol efflux at a magnitude similar to that of apoA-I (Fig. 1A). Under these experimental conditions, no effect of cyclodextrin or PC liposomes on the polymerization of actin was observed (Fig. 1B). In addition, both compounds failed to induce Cdc42 activation and PAK-1 and p54^{JNK} phosphorylation in human fibroblasts.

ABCA1 inactivation or absence abolishes Cdc42 signaling in human fibroblasts

To further evaluate the relationship between ABCA1 and apoA-I-induced signal transduction, we sought to examine Cdc42 signaling under conditions in which ABCA1 activity is eliminated. To this aim, we used glyburide, a sulfonylthiourea derivative, which binds to and effectively blocks several ABC transporters, including cystic fibrosis transmembrane conductance regulator and multidrug resistance proteins. Previous studies demonstrated inhibitory effects of glyburide on ABCA1-dependent cholesterol and phospholipid efflux, as well as on apoA-I binding to ABCA1 in HEK293 cells (4, 23). In agreement with this report, glyburide treatment markedly reduced apoA-I-induced cholesterol efflux in human fibroblasts (**Fig. 2A**). The inhibitory effects of glyburide were dose-dependent, with a maximum seen at a concentration of 1.0 mmol/l. As shown in Fig. 2B, at the same concentration range, glyburide inhibited apoA-I-induced actin polymerization, as inferred from the reduced amount of BODIPY-phalloidin extracted from pretreated cells. Moreover, a reduced amount of activated Cdc42 could be precipitated from fibroblasts exposed to 1.0 mmol/l glyburide before stimulation with apoA-I (Fig. 2C). In addition, glyburide (1.0 mmol/l) markedly suppressed the apoA-I-induced autophosphorylation of PAK-1 and phosphorylation of p54^{JNK} in human fibroblasts (Fig. 2C).

To confirm the importance of ABCA1 for apoA-I-induced intracellular signaling, we conducted experiments in fibroblasts obtained from a Tangier disease patient, in which a

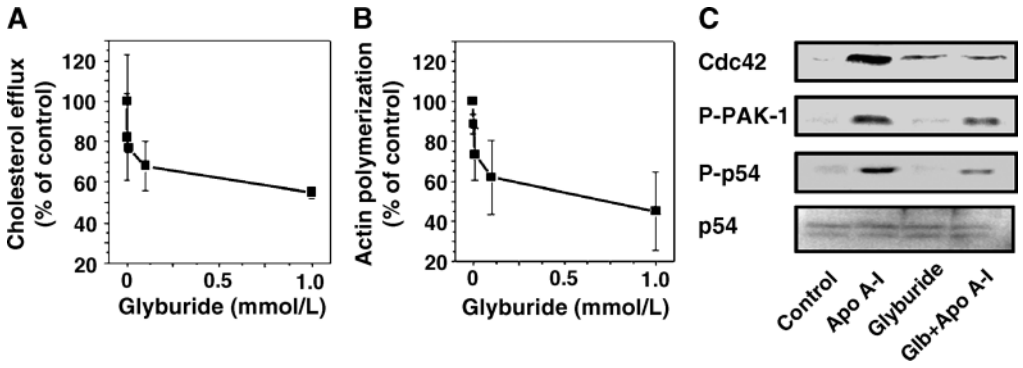

Fig. 2. Glyburide inhibits apoA-I-induced Cdc42 signaling in human dermal fibroblasts. Human dermal fibroblasts grown to confluence were exposed for 60 min to increasing concentrations (A, B) or to 0.5 mmol/l glyburide and then stimulated with apoA-I (10 μg/ml). A: Cholesterol efflux was determined in cells loaded for 24 h with [^{3}H]cholesterol and exposed to apoA-I for 4 h, as described in Experimental Procedures. Results from three independent experiments are shown (means ± SD). B: Actin polymerization was estimated after labeling fixed and permeabilized cells with BODIPY-phalloidin and extracting dye, as described in Experimental Procedures. Results from three to five independent experiments are shown (means ± SD). C: Confluent fibroblasts were stimulated for 10 min with apoA-I (10 μg/ml). Cdc42 was precipitated from the lysates using GST-PBD beads, and captured proteins were separated by SDS-PAGE and analyzed by Western blotting using anti-Cdc42 antibodies. Phosphorylated isoforms (P-) of PAK-1 and p54^{JNK} were analyzed using phosphospecific antibodies. Loading equality was controlled using antibody against the unphosphorylated isoform of p54^{JNK} (p54). Blots shown are representative of three to five experiments. Glb, glyburide.

stop codon at amino acid position 635 prevents ABCA1 expression (**Fig. 3A**, inset). As a consequence, cell binding of apoA-I conjugated to Alexa 596 was completely abolished in Tangier fibroblasts, whereas the fluorescence-labeled apolipoprotein bound to normal fibroblasts in a concentration-dependent manner (Fig. 3A). As shown in Fig. 3B, for all concentrations tested, apoA-I failed to induce cholesterol efflux from Tangier fibroblasts, unlike normal fibroblasts, which efficiently effluxed cholesterol in the presence of apoA-I. Next, the time course of actin polymerization in control and Tangier fibroblasts exposed to apoA-I was examined. Figure 3B demonstrates that the amounts of polymerized actin were increased significantly in response to apolipoprotein stimulation in normal but not in Tangier cells. Finally, the effect of apoA-I on the activation of Cdc42 and its downstream kinases was investigated (Fig. 3D). In marked contrast to normal fibroblasts, Tangier fibroblasts failed to respond to apoA-I stimulation with Cdc42 activation, autophosphorylation of PAK-1, or phosphorylation of p54^{JNK}.

ABCA1 expression potentiates Cdc42 signaling in human fibroblasts and HEK293 cells

The prediction from the postulated role of ABCA1 in facilitating signal transduction in response to apoA-I is that apoA-I-induced Cdc42 signaling should be enhanced in cells expressing this transporter. To test this proposition, we applied T0901317 and R0264456, which are synthetic agonists of LXR and RXR, respectively, two ubiquitously expressed nuclear transcription factors known to control ABCA1 gene expression (24). As shown in **Fig. 4A** (inset), combined treatment for 24 h with T0901317 (1.0 μmol/l) and R0264456 (0.1 μmol/l) considerably increased ABCA1 expression in human fibroblasts. Moreover, apoA-I-induced cholesterol efflux was increased in T0901317- and R0264456-pretreated cells. Figure 4B demonstrates that induction of ABCA1 expression with T0901317 and R0264456 increased the amount of actin polymerized in fibroblasts in response to apoA-I. Moreover, larger amounts of active Cdc42 could be precipitated from fibroblasts in which ABCA1 expression was induced by pretreatment with T0901317 and R0264456. In addition, combined exposure of fibroblasts to both compounds enhanced the autophosphorylation of PAK-1 and the phosphorylation of p54^{JNK} in response to apoA-I.

As the expression of several genes apart from ABCA1 is controlled by LXR and RXR (24), we sought to more specifically examine the effect of ABCA1 overexpression on apoA-I-induced signal transduction. To this aim, we stably expressed human ABCA1-GFP in HEK293 cells, which do not produce ABCA1 in a wild state. In addition, we made a Walker motif mutant (ABCA1-W-GFP) of ABCA1 that disrupts the first ATP binding site of the transporter and an ABCA1 variant lacking the C-terminal domain (ABCA1-ΔC-GFP). Both wild-type ABCA1 and ABCA1 variants were abundantly expressed in HEK293 cells and localized at the plasma membrane, as determined by immunoblot analysis with anti-GFP antibodies and fluorescence microscopy, respectively (data not shown). However, concentration-dependent binding of Alexa 596-conjugated apoA-I was seen in cells overexpressing ABCA1-GFP and ABCA1-ΔC-GFP but not ABCA1-W-GFP (**Fig. 5A**). Cholesterol efflux was observed only in cells overexpressing ABCA1-GFP (Fig. 5B). By contrast, ABCA1-W-GFP and ABCA1-ΔC-GFP failed to promote cholesterol efflux to apoA-I. Figure 5C, D demonstrates that apoA-I increased the cellular amount of

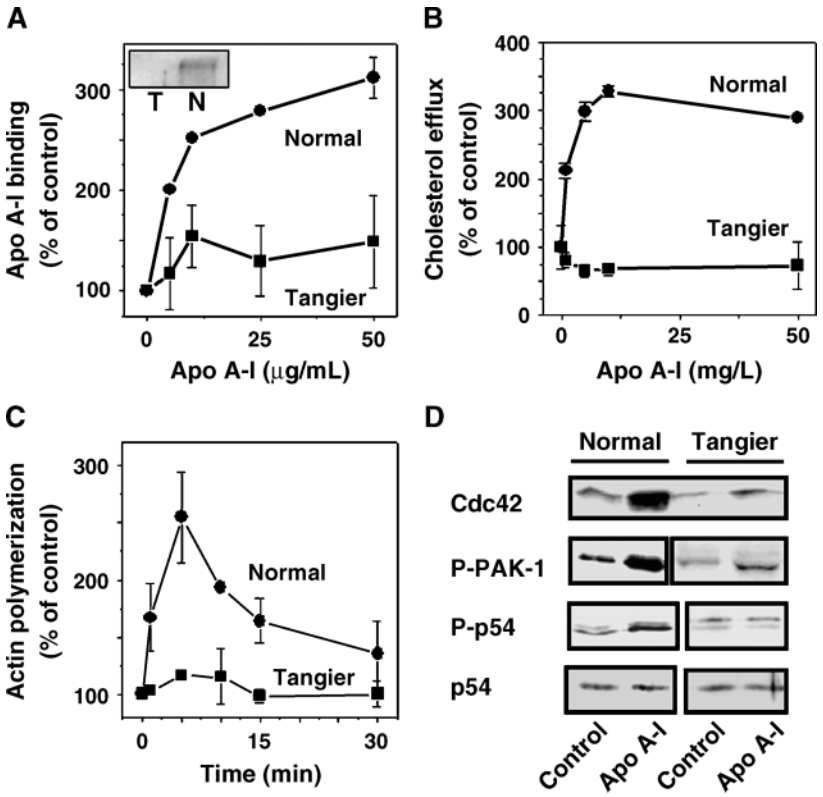

Fig. 3. ApoA-I-induced Cdc42 signaling is impaired in human dermal fibroblasts from a Tangier disease patient. A: Normal and Tangier fibroblasts (5×10^5 cells/ml) were incubated for 15 min with increasing concentrations of Alexa Fluor 594-labeled apoA-I. Cells were then washed three times with PBS, and fluorescence intensity was determined using a fluorescence spectrometer. Results from five separate experiments are shown (means ± SD). Inset: Western blot demonstrating the expression of ABCA1 in Tangier (T) and normal (N) fibroblasts. B, C: Normal and Tangier fibroblasts grown to confluence were loaded with [^{3}H]cholesterol and exposed for 4 h to increasing concentrations of apoA-I (B) or for the indicated times to apoA-I (10 μg/ml) (C). Cholesterol efflux and actin polymerization were determined as described in Experimental Procedures. Results from three to five independent experiments are shown (means ± SD). D: Normal and Tangier fibroblasts grown to confluence were stimulated for 10 min with apoA-I (10 μg/ml). Cdc42 activation was determined using a pulldown assay, as described in Experimental Procedures. Phosphorylated isoforms (P-) of PAK-1 and p54^{JNK} were analyzed using phosphospecific antibodies. Loading equality was controlled using antibody against the unphosphorylated isoform of p54^{JNK} (p54). Blots shown are representative of three to five experiments.

polymerized actin and activated Cdc42 in HEK293 cells overexpressing ABCA1-GFP but not in cells transfected with the mock vector. In addition, apoA-I induced the autophosphorylation of PAK-1 and the phosphorylation of p54^{JNK} in these cells. The exposure of ABCA1-W-GFP- or ABCA1-ΔC-GFP-expressing HEK293 cells to apoA-I failed to induce actin polymerization, Cdc42 activation, PAK-1 autophosphorylation, and p54^{JNK} phosphorylation (Fig. 5C, D).

ApoA-I induces Cdc42-ABCA1 complex formation in human fibroblasts and HEK293 cells

The absence of Cdc42 activation in ABCA1-ΔC-GFP-expressing HEK293 cells in response to apoA-I suggests that this small G-protein may interact directly with ABCA1. To test this proposition, we examined whether Cdc42 coimmunoprecipitates with ABCA1 in cells exposed to apoA-I. To this purpose, lysates from T0901317- and R0264456-pretreated human fibroblasts or HEK293 cells expressing ABCA1-GFP, ABCA1-W-GFP, or ABCA1-ΔC-GFP were immunoprecipitated with anti-ABCA1 or anti-GFP antibodies and analyzed by Western blot. As shown in Fig. 5E, treatment of cells for 10 min with apoA-I (10 μg/ml) substantially increased the amount of Cdc42 immunoprecipitated from lysates obtained from skin fibroblasts or from ABCA1-GFP lysates but not from ABCA1-W-GFP or ABCA1-ΔC-GFP lysates. These results indicate that ABCA1 interacts with Cdc42 and that the exposure of ABCA1-expressing cells to apoA-I results in the formation of an Cdc42-ABCA1 complex.

DISCUSSION

It is well documented that a wide variety of peptides and protein hormones, neurotransmitters, chemokines, growth factors, and other ligands elicit specific cellular responses by binding to plasma membrane receptors that are coupled to intracellular signaling intermediates, such as trimeric or small G-proteins or protein kinases. Several studies have demonstrated that ABCA1 acts as a binding

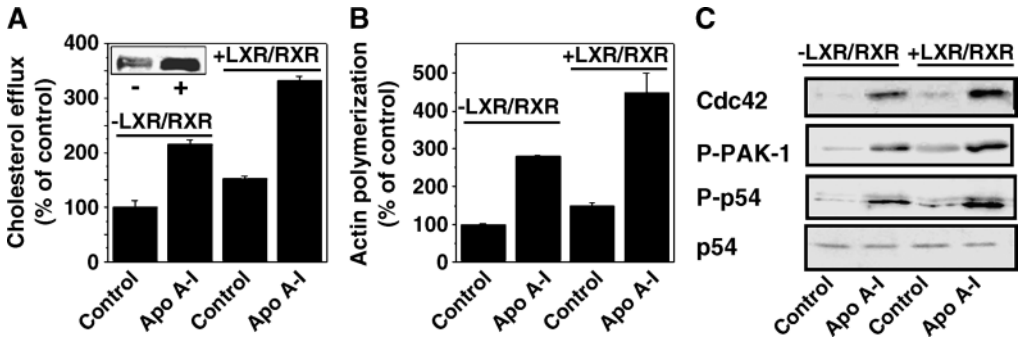

Fig. 4. Enhanced ABCA1 expression amplifies apoA-I-induced Cdc42 signaling in human dermal fibroblasts. Human dermal fibroblasts grown to confluence were incubated for 24 h with the liver X receptor (LXR) agonist T0901317 (1.0 μmol/l) and the retinoid X receptor (RXR) agonist R0264456 (0.1 μmol/l) and then exposed to apoA-I (10 μg/ml). A: Cholesterol efflux was determined in cells loaded for 24 h with [^{3}H]cholesterol and exposed to apoA-I for 4 h, as described in Experimental Procedures. Results from three independent experiments are shown (means ± SD). Inset: Western blot demonstrating the expression of ABCA1 in fibroblasts pretreated (+) or not pretreated (−) with LXR and RXR agonists. B: Actin polymerization was estimated after labeling fixed and permeabilized cells with BODIPY-phalloidin and extracting dye, as described in Experimental Procedures. Results from four to five independent experiments are shown (means ± SD). C: Fibroblasts incubated in the presence or absence of LXR and RXR agonists were stimulated for 10 min with apoA-I (10 μg/ml). Cdc42 activation was determined using a pulldown assay, as described in Experimental Procedures. Phosphorylated isoforms (P-) of PAK-1 and p54^{JNK} were analyzed using phosphospecific antibodies. Loading equality was controlled using antibody against the unphosphorylated isoform of p54^{JNK} (p54). Blots shown are representative of three independent experiments.

partner to apoA-I and other exchangeable apolipoproteins (4–7), but its contribution to the outside-in signaling has not been completely resolved. This study provides several lines of evidence that apoA-I functions as a signaling ligand that modulates its ability to remove lipids from cells via ABCA1-dependent signal transduction. First, both lipid-free apolipoproteins and amphipathic peptides known to specifically bind to ABCA1 were able to induce a cascade of signaling events, including activation of Cdc42 and subsequent phosphorylations of PAK-1 and p54^{JNK} and polymerization of actin. Second, apoA-I-induced cellular signaling was attenuated in the presence of glyburide, a nonselective ABC transport inhibitor, and abolished completely in Tangier cells with a nonfunctional ABCA1 transporter. Third, the augmentation of ABCA1 expression by a combined exposure of dermal fibroblasts to LXR and RXR agonists led to an amplification of Cdc42-dependent signaling in response to apoA-I stimulation. In addition, HEK293 cells, which do not produce ABCA1 on their own, responded to apoA-I with Cdc42 activation, PAK-1 and p54^{JNK} phosphorylation, and actin polymerization only after transfection with a functional ABCA1 transporter.

Results of previous studies provide additional evidence that initial apoA-I binding to ABCA1 is coupled to the activation of signal transduction pathways, allowing apoA-I lipidation and cholesterol removal from cells. For instance, ABCA1 expression was demonstrated to be required for the apoA-I-dependent generation of cAMP and activation of PKA in CHO cells (8). Likewise, apoA-I-triggered JAK2 autophosphorylation was reported to be abolished in BHK cells lacking the expression of ABCA1 (11). More recently, synthetic peptides containing one or two amphipathic helices were shown to emulate apoA-I in its ability to stimulate JAK2 phosphorylation, and greatly reduced peptide-mediated cholesterol efflux was found after the inhibition of JAK2, indicating that this process requires an active kinase (25). Together, the results of the current and previous studies strongly support the contention that ABCA1 mediates outside-in signaling in response to ligand binding; hence, ABCA1 should be considered a full apolipoprotein receptor.

The mechanisms by which ABCA1 promotes lipid removal from cells are not completely understood. The demonstration of a direct interaction between apoA-I and ABCA1 gave rise to a model in which the binding of apoA-I to ABCA1 allows for the spatial proximity required by the apolipoprotein to microsolubilize lipids in the plasma bilayer (26). Consistent with this model, we found that an impaired apoA-I binding attributable to structural changes in ABCA1, such as with mutations in the ATP binding domains, is accompanied by reduced cholesterol efflux from cells. However, a partial deletion of the C-terminal ABCA1 fragment led to a complete abolition of both apoA-I-induced Cdc42-dependent cell signaling and cholesterol efflux, despite there being only moderately decreased apoA-I binding at the cell surface. These findings suggest that ABCA1-ΔC is able to bind apoA-I in a nearly normal manner but that this process can be uncoupled from the ABCA1-mediated outside-in signaling and the transfer of cholesterol to apoA-I. Likewise, Fitzgerald et al. (7) reported that the W590S ABCA1 mutant is fully competent to form complexes with apoA-I but fails to efflux cholesterol normally. Another ABCA1 variant, C1477R, was shown by Haidar et al. (8) to be fully ineffective at mediating apoA-I-dependent cAMP formation and effluxing cholesterol, despite there being only partially decreased apoA-I binding. These observations, together with the findings of this study, imply that apoA-I binding to ABCA1 is necessary

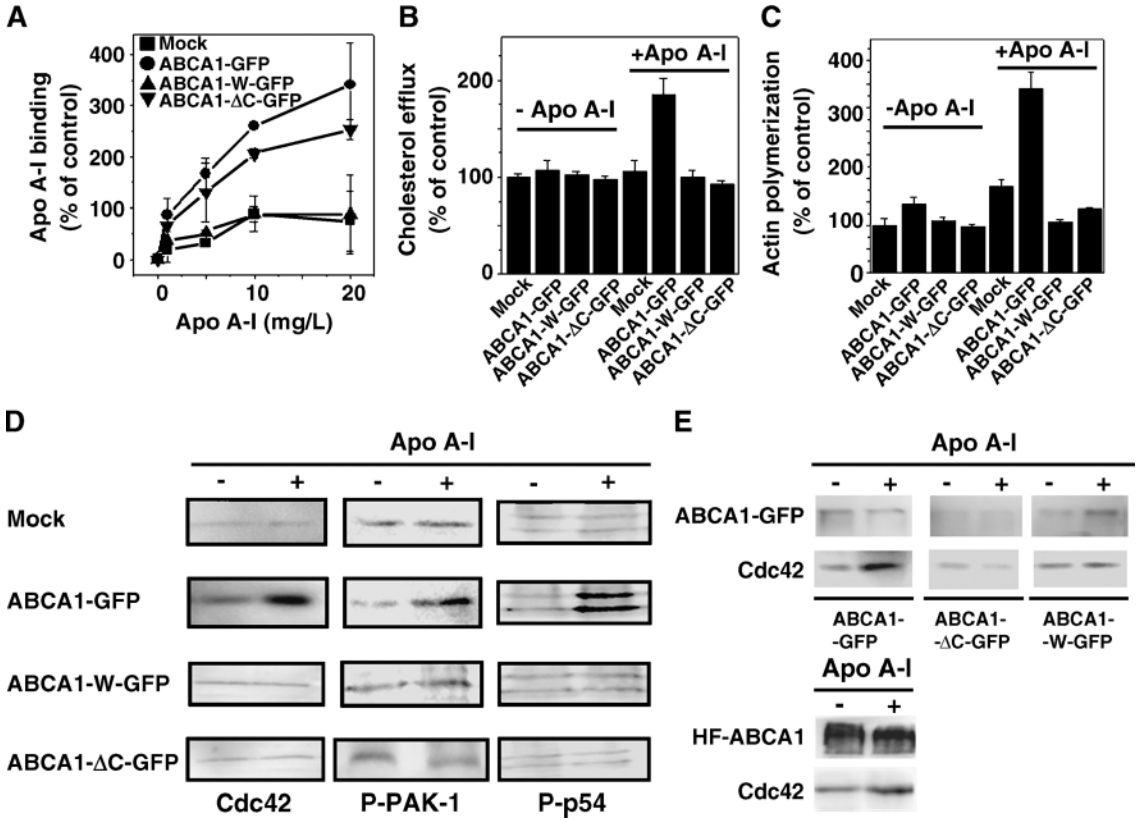

Fig. 5. ApoA-I induces Cdc42 signaling and the formation of Cdc42-ABCA1 complexes in cells expressing ABCA1-green fluorescent protein (GFP). A: HEK293 cells stably transfected with ABCA1-GFP, ABCA1-ΔC-GFP, ABCA1-W-GFP, or mock vector (5×10^5 cells/ml) were incubated for 15 min with increasing concentrations of Alexa Fluor 594-labeled apoA-I. Cells were then washed three times with PBS, and fluorescence intensity was determined using a fluorescence spectrometer. Results from five separate experiments are shown (means ± SD). B, C: HEK293 cells expressing ABCA1-GFP fusion proteins were loaded with [^{3}H]cholesterol and exposed for 4 h to apoA-I (10 μg/ml) (B) or for 10 min to apoA-I (10 μg/ml) (C). Cholesterol efflux and actin polymerization were determined as described in Experimental Procedures. Results from three to five independent experiments are shown (means ± SD). D: HEK293 cells expressing ABCA1-GFP fusion proteins were stimulated for 10 min with apoA-I (10 μg/ml). Cdc42 activation was determined using a pulldown assay, as described in Experimental Procedures. Phosphorylated isoforms (P-) of PAK-1 and p54^{JNK} were analyzed using phosphospecific antibodies. Blots shown are representative of three to five experiments. E: After stimulation for 10 min with apoA-I (10 μg/ml), ABCA1 in human fibroblasts (HF-ABCA1) was precipitated from T0901317-pretreated (1.0 μmol/l) and R0264456-pretreated (0.1 μmol/l) cells using anti-ABCA1 polyclonal antibody. ABCA1-GFP fusion proteins were immunoprecipitated from HEK293 cells using anti-GFP monoclonal antibody. Immunoprecipitates were separated by SDS-PAGE and analyzed by Western blot using polyclonal antibodies against ABCA1 (upper panels) or Cdc42 (lower panels). Blots shown are representative of one experiment out of three.

but not sufficient for effective lipid removal from cells, and they are consistent with the notion that ABCA1-mediated outside-in signaling may be an important step preceding apoA-I-induced cholesterol efflux.

We previously demonstrated that apoA-I induces Cdc42-dependent actin polymerization in human fibroblasts (15). Here, we have confirmed this finding and identified ABCA1 as an upstream signaling component functionally linking the extracellular signal to Cdc42 activation. As a consequence, Cdc42-dependent actin polymerization was found to be abolished in Tangier fibroblasts that lack functional ABCA1. The impaired formation of actin fibers, resulting in an abnormal cell shape and a reduced number of filopodia, was observed previously in Tangier cells, and this phenomenon could be reversed by exogenous expression of a constitutively active Cdc42 variant (27). A similar phenotype characterized by impaired Cdc42 activation and abnormal reorganization of the actin cytoskeleton was also reported in fibroblasts obtained from aged subjects or patients with Werner syndrome (WS), which is characterized by the early onset of senescent phenotypes, including premature atherosclerosis (28, 29). In this regard, it is of interest that HDL- and apolipoprotein-inducible cholesterol efflux is substantially reduced in nearly senescent normal fibroblasts and in patients with premature ageing syndromes, such as WS or Hutchison-Gifford progeria (29, 30). Moreover, the impaired cholesterol efflux in WS fibroblasts can be corrected by complementation with Cdc42 (29). Therefore, it seems that the dysfunction of the apoA-I/ABCA1/Cdc42 signaling pathway may at least partially account for the less efficient cholesterol efflux and, thereby, the lower HDL levels encountered in aged individuals.

The exact molecular mechanisms by which ABCA1 couples apoA-I binding to activation of the Cdc42 signaling cascade remain to be elucidated. Tsukamoto et al. (31) demonstrated that Cdc42 closely colocalizes with

ABCA1 in plasma membrane and perinuclear compartments that correspond to the Golgi apparatus. In addition, Cdc42 could be immunoprecipitated with ABCA1, indicating that these proteins interact with each other. In this study, we extend this observation to show that the exposure of cells to apoA-I markedly amplifies the ABCA1-Cdc42 interaction. The observations that Cdc42-ABCA1 complex formation is absent in cells expressing a C-terminally truncated ABCA1 variant and that Cdc42-dependent signaling is abolished in these cells suggest that the C-terminal domain is indispensable for the interaction between ABCA1 and Cdc42. Several structural motifs mediating protein-protein interactions, such as the PDZ domain and the VFVNFA motif, were previously found within the ABCA1 C terminus (32, 33). In this regard, it is of interest that several GDP exchange factors (GEFs), which increase the rate of GTP binding to Cdc42 and thereby specifically control its activity, contain the PDZ domain and are, therefore, potential ABCA1-interacting partners. Recently, several proteins interacting with the PDZ domain derived from the C-terminal part of ABCA1 were isolated by Okuhira et al. (34). Whereas no Cdc42-specific GEFs were identified by these authors, their data suggest that ABCA1 avidly interacts with GEFs specific for RhoA, a small G-protein activated in parallel to Cdc42 upon apoA-I stimulation (15). In addition, both Okuhira et al. (34) and other authors (35, 36) have reported that the C-terminal PDZ domain is involved in the interaction between ABCA1 and α- and/or $\beta_{1/2}$-syntrophin. The latter proteins were demonstrated to interact with and to activate several small G-proteins inducible by apoA-I, including Rac1 and RhoA (15, 37).

Although direct interaction between ABCA1 and Cdc42 appears to be required for effective outside-in signaling by apoA-I, it remains unclear whether this process is also dependent on the ABCA1-mediated translocation of lipids across the cell membrane. In this study, we found that compounds inducing cholesterol efflux by means of unspecific desorption, such as PC-containing liposomes or cyclodextrins, failed to activate Cdc42-dependent signaling. This observation argues against the notion that the apolipoprotein- and amphipathic peptide-induced Cdc42 signaling arises as a consequence of membrane cholesterol depletion. However, it has to be kept in mind that both cholesterol and phospholipids are unevenly distributed within the cell membrane and that membrane domains with distinct physicochemical properties may be targeted for lipid efflux by various acceptors. Actually, previous studies have provided evidence for the existence of separate membrane domains specifically supplying cholesterol and phospholipids for the ABCA1-mediated efflux (38). Therefore, it cannot be entirely excluded that apolipoprotein treatment induces a localized change in membrane structure by depleting cholesterol from specific domains and that this process is required for effective induction of the Cdc42 signaling cascade. Clearly, further studies are necessary to fully understand the molecular mechanisms accounting for the ABCA1-mediated transduction of cellular signaling.

The establishment of ABCA1 as a full apoA-I receptor that not only executes inside-out effector functions, such as cholesterol and/or phospholipid efflux, but also mediates outside-in signal transduction may have far-reaching consequences for our understanding of the atheroprotective mechanisms of ABCA1. It is conceivable that by generating intracellular signals via ABCA1, antiatherogenic apolipoproteins, such as apoA-I and apoE, could not only promote cholesterol efflux from cells but also modulate other cellular responses. Some observations indeed suggest that antiatherogenic mechanisms of ABCA1 may not be confined solely to the initiation of cholesterol efflux. For instance, ABCA1-deficient macrophages exhibit increased recruitment into the arterial wall of atherosclerosis-prone animals and enhanced responsiveness to chemotactic factors, such as macrophage chemoattractant protein-1 (39). Finally, the results of this study also suggest that a complete understanding of the antiatherogenic mechanisms of HDL will likely require future investigations aimed at characterizing ABCA1-mediated cell signaling pathways.

This work was supported in part by Grant-in-Aid EC116/3-6 from the Deutsche Forschunggemeinschaft to A.v.E. and G.A.

REFERENCES

1. Hersberger, M., and A. von Eckardstein. 2003. Low high-density lipoprotein cholesterol: physiological background, clinical importance and drug treatment. *Drugs.* **63:** 1907–1945.
2. Assmann, G., and J-R. Nofer. 2003. Atheroprotective effects of high-density lipoproteins. *Annu. Rev. Med.* **54:** 321–341.
3. Nofer, J-R., and A. T. Remaley. 2005. Tangier disease: still more questions than answers. *Cell. Mol. Life Sci.* **62:** 2150–2160.
4. Wang, N., D. L. Silver, P. Costet, and A. R. Tall. 2000. Specific binding of apoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *J. Biol. Chem.* **275:** 33053–33058.
5. Oram, J. F., R. M. Lawn, M. R. Garvin, and D. P. Wade. 2000. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *J. Biol. Chem.* **275:** 34508–34511.
6. Fitzgerald, M. L., A. L. Morris, J. S. Rhee, L. P. Andersson, A. J. Mendez, and M. W. Freeman. 2002. Naturally occurring mutations in the largest extracellular loops of ABCA1 can disrupt its direct interaction with apolipoprotein A-I. *J. Biol. Chem.* **277:** 33178–33187.
7. Fitzgerald, M. L., A. L. Morris, A. Chroni, A. J. Mendez, V. I. Zannis, and M. W. Freeman. 2004. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *J. Lipid Res.* **45:** 287–294.
8. Haidar, B., M. Denis, M. Marcil, L. Krimbou, and J. Genest, Jr. 2004. Apolipoprotein A-I activates cellular cAMP signaling through the ABCA1 transporter. *J. Biol. Chem.* **279:** 9963–9969.
9. Mendez, A. J., J. F. Oram, and E. L. Bierman. 1991. Protein kinase C as a mediator of high density lipoprotein receptor-dependent efflux of intracellular cholesterol. *J. Biol. Chem.* **266:** 10104–10111.
10. Yamauchi, Y., M. Hayashi, S. Abe-Dohmae, and S. Yokoyama. 2003. Apolipoprotein A-I activates protein kinase C alpha signaling to phosphorylate and stabilize ATP binding cassette transporter A1 for the high density lipoprotein assembly. *J. Biol. Chem.* **278:** 47890–47897.
11. Tang, C., A. M. Vaughan, and J. F. Oram. 2004. Janus kinase 2 modulates the apolipoprotein interactions with ABCA1 required for removing cellular cholesterol. *J. Biol. Chem.* **279:** 7622–7628.
12. See, R. H., R. A. Caday-Malcolm, R. R. Singaraja, S. Zhou, A. Silverston, M. T. Huber, J. Moran, E. R. James, R. Janoo, J. M. Savill,

et al. 2002. Protein kinase A site-specific phosphorylation regulates ATP-binding cassette A1 (ABCA1)-mediated phospholipid efflux. *J. Biol. Chem.* **277:** 41835–41842.
13. Haidar, B., M. Denis, L. Krimbou, M. Marcil, and J. Genest, Jr. 2002. cAMP induces ABCA1 phosphorylation activity and promotes cholesterol efflux from fibroblasts. *J. Lipid Res.* **43:** 2087–2094.
14. Martinez, L. O., B. Agerholm-Larsen, N. Wang, W. Chen, and A. R. Tall. 2003. Phosphorylation of a PEST sequence in ABCA1 promotes calpain degradation and is reversed by apoA-I. *J. Biol. Chem.* **278:** 37368–37374.
15. Nofer, J. R., R. Feuerborn, B. Levkau, A. Sokoll, U. Seedorf, and G. Assmann. 2003. Involvement of Cdc42 signaling in apoA-I-induced cholesterol efflux. *J. Biol. Chem.* **278:** 53055–53062.
16. Ha, K. S., and J. H. Exton. 1993. Activation of actin polymerization by phosphatidic acid derived from phosphatidylcholine in IIC9 fibroblasts. *J. Cell Biol.* **123:** 1789–1796.
17. Nofer, J. R., B. Levkau, I. Wolinska, R. Junker, M. Fobker, A. von Eckardstein, U. Seedorf, and G. Assmann. 2001. Suppression of endothelial cell apoptosis by high density lipoproteins (HDL) and HDL-associated lysosphingolipids. *J. Biol. Chem.* **276:** 34480–34485.
18. Remaley, A. T., J. A. Stonik, S. J. Demosky, E. B. Neufeld, A. V. Bocharov, T. G. Vishnyakova, T. L. Eggerman, A. P. Patterson, N. J. Duverger, S. Santamarina-Fojo, et al. 2001. Apolipoprotein specificity for lipid efflux by the human ABCAI transporter. *Biochem. Biophys. Res. Commun.* **280:** 818–823.
19. Krimbou, L., M. Denis, B. Haidar, M. Carrier, M. Marcil, and J. Genest, Jr. 2004. Molecular interactions between apoE and ABCA1: impact on apoE lipidation. *J. Lipid Res.* **45:** 839–848.
20. Remaley, A. T., F. Thomas, J. A. Stonik, S. J. Demosky, S. E. Bark, E. B. Neufeld, A. V. Bocharov, T. G. Vishnyakova, A. P. Patterson, T. L. Eggerman, et al. 2003. Synthetic amphipathic helical peptides promote lipid efflux from cells by an ABCA1-dependent and an ABCA1-independent pathway. *J. Lipid Res.* **44:** 828–836.
21. Arakawa, R., M. Hayashi, A. T. Remaley, B. H. Brewer, Y. Yamauchi, and S. Yokoyama. 2004. Phosphorylation and stabilization of ATP binding cassette transporter A1 by synthetic amphiphilic helical peptides. *J. Biol. Chem.* **279:** 6217–6220.
22. Yancey, P. G., A. E. Bortnick, G. Kellner-Weibel, M. de la Llera-Moya, M. C. Phillips, and G. H. Rothblat. 2003. Importance of different pathways of cellular cholesterol efflux. *Arterioscler. Thromb. Vasc. Biol.* **23:** 712–719.
23. Nieland, T. J., A. Chroni, M. L. Fitzgerald, Z. Maliga, V. I. Zannis, T. Kirchhausen, and M. Krieger. 2004. Cross-inhibition of SR-BI- and ABCA1-mediated cholesterol transport by the small molecules BLT-4 and glyburide. *J. Lipid Res.* **45:** 1256–1265.
24. Millatt, L. J., V. Bocher, J. C. Fruchart, and B. Staels. 2003. Liver X receptors and the control of cholesterol homeostasis: potential therapeutic targets for the treatment of atherosclerosis. *Biochim. Biophys. Acta.* **1631:** 107–118.
25. Tang, C., A. M. Vaughan, G. M. Anantharamaiah, and J. F. Oram. 2006. Janus kinase 2 modulates the lipid-removing but not protein-stabilizing interactions of amphipathic helices with ABCA1. *J. Lipid Res.* **47:** 107–114.
26. Vedhachalam, C., L. Liu, M. Nickel, P. Dhanasekaran, G. M. Anantharamaiah, S. Lund-Katz, G. H. Rothblat, and M. C. Phillips. 2004. Influence of apoA-I structure on the ABCA1-mediated efflux of cellular lipids. *J. Biol. Chem.* **279:** 49931–49939.
27. Hirano, K., F. Matsuura, K. Tsukamoto, Z. Zhang, A. Matsuyama, K. Takaishi, R. Komuro, T. Suehiro, S. Yamashita, Y. Takai, et al. 2000. Decreased expression of a member of the Rho GTPase family, Cdc42Hs, in cells from Tangier disease—the small G protein may play a role in cholesterol efflux. *FEBS Lett.* **484:** 275–279.
28. Tsukamoto, K., K. Hirano, S. Yamashita, N. Sakai, C. Ikegami, Z. Zhang, F. Matsuura, H. Hiraoka, A. Matsuyama, M. Ishigami, et al. 2002. Retarded intracellular lipid transport associated with reduced expression of Cdc42, a member of Rho-GTPases, in human aged skin fibroblasts: a possible function of Cdc42 in mediating intracellular lipid transport. *Arterioscler. Thromb. Vasc. Biol.* **22:** 1899–1904.
29. Zhang, Z., K. Hirano, K. Tsukamoto, C. Ikegami, M. Koseki, K. Saijo, T. Ohno, N. Sakai, H. Hiraoka, I. Shimomura, et al. 2005. Defective cholesterol efflux in Werner syndrome fibroblasts and its phenotypic correction by Cdc42, a RhoGTPase. *Exp. Gerontol.* **40:** 286–294.
30. Nofer, J-R., M. Walter, and G. Assmann. 2005. Current understanding of the role of high density lipoproteins (HDL) in atherosclerosis and senescence. *Expert Rev. Cardiovasc. Ther.* **3:** 1071–1086.
31. Tsukamoto, K., K. Hirano, K. Tsujii, C. Ikegami, Z. Zhongyan, Y. Nishida, T. Ohama, F. Matsuura, S. Yamashita, and Y. Matsuzawa. 2001. ATP-binding cassette transporter-1 induces rearrangement of actin cytoskeletons possibly through Cdc42/N-WASP. *Biochem. Biophys. Res. Commun.* **287:** 757–765.
32. Buechler, C., S. M. Bared, C. Aslanidis, M. Ritter, W. Drobnik, and G. Schmitz. 2002. Molecular and functional interaction of the ATP-binding cassette transporter A1 with Fas-associated death domain protein. *J. Biol. Chem.* **277:** 41307–41310.
33. Fitzgerald, M. L., K. Okuhira, G. F. Short 3rd, J. J. Manning, S. A. Bell, and M. W. Freeman. 2004. ATP-binding cassette transporter A1 contains a novel C-terminal VFVNFA motif that is required for its cholesterol efflux and apoA-I binding activities. *J. Biol. Chem.* **279:** 48477–48485.
34. Okuhira, K., M. L. Fitzgerald, D. A. Sarracino, J. J. Manning, S. A. Bell, J. L. Goss, and M. W. Freeman. 2005. Purification of ATP-binding cassette transporter A1 and associated binding proteins reveals the importance of beta1-syntrophin in cholesterol efflux. *J. Biol. Chem.* **280:** 39653–39664.
35. Buechler, C., A. Boettcher, S. M. Bared, M. C. Probst, and G. Schmitz. 2002. The carboxyterminus of the ATP-binding cassette transporter A1 interacts with a beta2-syntrophin/utrophin complex. *Biochem. Biophys. Res. Commun.* **293:** 759–765.
36. Munehira, Y., T. Ohnishi, S. Kawamoto, A. Furuya, K. Shitara, M. Imamura, T. Yokota, S. Takeda, T. Amachi, M. Matsuo, et al. 2004. Alpha1-syntrophin modulates turnover of ABCA1. *J. Biol. Chem.* **279:** 15091–15095.
37. Oak, S. A., Y. W. Zhou, and H. W. Jarrett. 2003. Skeletal muscle signaling pathway through the dystrophin glycoprotein complex and Rac1. *J. Biol. Chem.* **278:** 39287–39295.
38. Drobnik, W., H. Borsukova, A. Bottcher, A. Pfeiffer, G. Liebisch, G. J. Schutz, H. Schindler, and G. Schmitz. 2002. Apo AI/ABCA1-dependent and HDL3-mediated lipid efflux from compositionally distinct cholesterol-based microdomains. *Traffic.* **3:** 268–278.
39. van Eck, M., I. S. Bot, W. E. Kaminski, E. Orso, G. Rothe, J. Twisk, A. Bottcher, E. S. van Amersfoort, T. A. Christiansen-Weber, W. P. Fung-Leung, et al. 2002. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. *Proc. Natl. Acad. Sci. USA.* **99:** 6298–6303.