ATP-binding cassette transporter A7 (ABCA7) Binds Apolipoprotein A-I and Mediates Cellular Phospholipid Efflux*

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ATP-binding cassette transporter 1 (ABCA1), the defective transporter in Tangier disease, binds and promotes cellular cholesterol and phospholipid efflux to apolipoprotein I (apoA-I). Based on a high degree of sequence homology between ABCA1 and ABCA7, a transporter of unknown function, we investigated the possibility that ABCA7 might be involved in apolipoprotein binding and lipid efflux. Similarly to cells expressing ABCA1, HEK293 cells overexpressing ABCA7 showed specific binding and cross-linking of lipid-poor apoA-I. ABCA7 expression increased cellular phosphatidylcholine and sphingomyelin efflux to apoA-I in a manner similar to ABCA1 but had no effect on cholesterol efflux. Western analysis showed a high protein level of ABCA7 in mouse spleen, lung, adrenal, and brain but low expression in liver. In contrast to ABCA1, ABCA7 showed moderate basal mRNA and protein levels in macrophages and lymphocytes but no induction by liver X receptor activation. These results show that ABCA7 has the ability to bind apolipoproteins and promote efflux of cellular phospholipids without cholesterol, and in this study suggest a possible role of ABCA7 in cellular phospholipid metabolism in peripheral tissues.

ATP-binding cassette (ABC) transporters are a superfamily of highly conserved membrane proteins that transport a wide variety of substrates including ions, amino acids, peptides, sugars, lipids, and sterols across cell membranes (1). Mutations of ABC transporters have been identified as the cause of at least 12 different genetic disorders (1). Among these genetic diseases, several are caused by mutations of the ABC transporters involved in lipid transport, including Tangier disease (2–4), Stargardt disease (5), and sitosterolemia (6). Tangier disease is characterized by very low HDL levels and increased accumulation of tissue macrophage foam cells (7) and is caused by mutations in the ABCA1 gene (2–4). Heterozygous ABCA1 deficiency causes familial hypo-α-lipoproteinemia and is associated with increased risk of cardiovascular diseases (8). ABCA1 functions to promote cellular phospholipid and cholesterol efflux to apolipoproteins, particularly apolipoprotein A-I (apoA-I), initiating the formation of HDL. ABCA1-mediated lipid efflux to apolipoproteins is the initial step in the transport of cholesterol by HDL from peripheral tissues back to the liver for disposal, mainly as biliary cholesterol (9). This function of ABCA1 likely involves a direct binding of apoA-I to the transporter at the cell surface (10) which appears to be essential for ABCA1-mediated lipid efflux to apolipoproteins (11). ABCA1 encodes a large transmembrane protein with six predicted extracellular domains (12), and the extracellular domains have been implicated in the interaction between apoA-I and ABCA1 (13).

In this study, we identified ABCA7, a member of ABCA family, as a novel transporter mediating cellular phospholipid efflux to apolipoproteins. In contrast to ABCA1, ABCA7 does not promote cellular cholesterol efflux, reflecting a distinct substrate specificity for ABCA1 and ABCA7. The findings from this study suggest a possible role of ABCA7 in the regulation of cellular phospholipid homeostasis.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—Human apoA-I (BioDesign, Brockville, ON, Canada) was dialyzed against phosphate buffered saline. M2 anti-Flag antibody was obtained from Sigma; polyclonal anti-ABC7 antisera were raised against the last 15 amino acids of mouse ABCA7 in rabbits. Affinity-purified anti-ABC7 antibody was prepared by using a peptide-affinity column, and the control antibody was purified from the pre-bleed serum using the protein A/G-agarose column.

Plasmid Constructs and Cell Transfection—The plasmid constructs expressing mouse ABCA7 or ABCA7-Flag were prepared by cloning mouse ABCA7 full-length cDNA into pCMV-sport6 vector, and the cDNA sequence was confirmed by DNA sequencing. The plasmid construct expressing ABCA4 was kindly provided by Dr. Hui Sun. For transient transfection of HEK293 cells, cells in 12- or 24-well collagen-coated plates were transfected with various plasmid constructs at indicated DNA concentrations with LipofectAMINE 2000 (Invitrogen) at 37 °C overnight (~20 h). To estimate transfection efficiency, a construct expressing green fluorescent protein was routinely used in the experiment to visually monitor for transfection efficiency. The transfection efficiency of HEK293 cells was in the range of 50–80% of cells. Although transfection efficiency did vary from experiment to experiment, we found that the variation within the same experiment was small.

Cellular Lipid Efflux Assays, apoA-I Cell Association, and Chemical Cross-linking—The assays were carried out as in Ref. 10. Generally, HEK293 cells were labeled by culturing for 24 h in 10% fetal bovine serum/Dulbecco's modified Eagle's medium. The plasmid constructs containing the apoA-I coding sequence were transfected into HEK293 cells using LipofectAMINE transfection reagent. After washing three times with fresh media, cells were lysed with 0.1% SDS and 0.1 N NaOH lysis buffer, and radioactivity counter.
Immunoprecipitation and Immunoblot Analysis—For immunoblot analysis of ABCA1, ABCA7, ABCA7-Flag, or ABCA4, transfected HEK293 cells, Chinese hamster ovary cells, or mouse tissue homogenates were lysed in radioimmune precipitation assay buffer (10 mM Tris-HCl, pH 7.3, 1 mM MgCl₂, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, and 5 mM EDTA in the presence of protease inhibitors (0.5 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin A); Roche Applied Science). Postnuclear supernatants containing the indicated amounts of protein were subjected to Western analysis using an anti-ABCA1 or anti-ABCA7 antiserum and chemiluminescence detection. The relative intensity of the bands was determined by densitometry. For cell surface ABCA7 analysis, cells were first biotinylated with 0.5 mg/ml EZ-Link™ sulfo-NHS-LC-biotin (Pierce) at 4 °C for 30 min. Then, cells were lysed with radioimmune precipitation assay buffer at 4 °C. After centrifugation, the supernatant of cell lysates was incubated with streptavidin-agarose beads overnight at 4 °C. After centrifugation and washing, the collected agarose beads were subjected to SDS-PAGE sample buffer with 100 mM 2-mercaptoethanol. The biotinylated ABCA1 or ABCA7 was detected by Western blotting by using antibodies against the transporters.

Immunofluorescence Confocal Microscopy—Cells were fixed with 3.7% formaldehyde for 10 min and then incubated with 0.1% Triton X-100 in phosphate-buffered saline for 2 min. After washing with phosphate-buffered saline, cells were incubated with primary antibody in 4 mg/ml normal goat globulin and 0.1% saponin in phosphate-buffered saline at room temperature for 30 min. Alexa 488-labeled goat anti-rabbit IgG was used as the secondary antibody. After washing and fixing with 3% formaldehyde, cells were examined by confocal microscopy.

RESULTS
ABCA7 Binds and Cross-links apoA-I—The efflux of cellular lipids to apoA-I seems to require binding of apoA-I to ABCA1 (10, 13). Because several mutations in the first and fourth extracellular loops of ABCA1 disrupt the interaction between apoA-I and ABCA1 and cause Tangier disease, these loops may be involved in the binding of apoA-I (11). We noticed that ABCA1 and ABCA7 have a high degree of sequence homology, including the first and fourth extracellular loops (~60% identity), suggesting that ABCA7 might be able to bind apolipoproteins and mediate cellular lipid efflux. To test this hypothesis, we carried out an apoA-I binding assay using [125I]apoA-I in control or in ABCA1- or ABCA7-transfected HEK293 cells. Cells expressing ABCA7 displayed increased specific apoA-I binding, as compared with the empty vector-transfected control cells; the levels of binding were similar to those observed in cells expressing ABCA1 (Fig. 1A). Because several mutations in the first and fourth extracellular loops of ABCA1 disrupt the interaction between apoA-I and ABCA1 and cause Tangier disease, these loops may be involved in the binding of apoA-I (11). We noticed that ABCA1 and ABCA7 have a high degree of sequence homology, including the first and fourth extracellular loops (~60% identity), suggesting that ABCA7 might be able to bind apolipoproteins and mediate cellular lipid efflux. To test this hypothesis, we carried out an apoA-I binding assay using [125I]apoA-I in control or in ABCA1- or ABCA7-transfected HEK293 cells. Cells expressing ABCA7 displayed increased specific apoA-I binding, as compared with the empty vector-transfected control cells; the levels of binding were similar to those observed in cells expressing ABCA1 (Fig. 1A). Cell surface biotinylation of ABCA7 in transfected HEK293 cells confirmed that ABCA7 was expressed at the cell surface (Fig. 1B). Previously, we showed apoA-I could be chemically cross-linked to ABCA1 (10). To test the idea that ABCA7 also directly binds apoA-I, we performed the chemical cross-linking using [125I]apoA-I and transfected HEK293 cells. Indeed, [125I]apoA-I was readily cross-linked to ABCA7, similarly to ABCA1 (Fig. 1C).
was blocked by excess unlabeled apoA-I (Fig. 1C). Reduction of the cross-linker resulted in the release of free apoA-I from the complex (Fig. 1C). These data suggest the direct binding of apoA-I to ABCA7 at the cell surface. Confocal fluorescence microscopy was carried out by using a specific ABCA7 antibody in permeabilized HEK293 cells. This confirmed a plasma membrane staining pattern as well as an intracellular punctate distribution (Fig. 1D).

**ABCA7 Mediates Cellular Phospholipid Efflux**—To explore the functional significance of the interaction of apoA-I with ABCA7, we measured apoA-I-mediated cellular phospholipid efflux. Cells expressing ABCA7 showed a marked increase in apoA-I-mediated phospholipid efflux at similar levels to cells expressing ABCA1 (Fig. 2A). As controls, we also transfected cells with ABCA4, which is also homologous to ABCA1 but has restricted distribution in the retina, where it appears to be involved in the transport of retinylidene phosphatidylethanolamine (14) and with ABCC3, which we have identified as a potential LXR target gene in murine macrophages.2 In contrast to ABCA7, ABCA4 and ABCC3 failed to facilitate efflux of cellular choline-containing lipids to apoA-I (Fig. 2A), despite robust expression of ABCA4 and ABCC3 protein in the transfected HEK293 cells (not shown). ABCA7 promotes phospholipid efflux to apoA-I in a time- and dose-dependent fashion (Fig. 2, B and C). The EC₅₀ for apoA-I-mediated phospholipid efflux was 4.5 μg/ml (Fig. 2B), a value comparable with that of apoA-I-mediated cellular lipid efflux to ABCA1 (10). Time-course experiments revealed a continuous increase in phospholipid efflux over 8 h (Fig. 2C), which was similar to the kinetics of ABCA1-mediated phospholipid efflux (15). Like ABCA1, ABCA7 expression also promoted both phosphatidylcholine and sphingomyelin efflux to apoA-I (Fig. 2D). ABCA1 can bind and promote cellular lipid efflux to lipid-poor apolipoproteins like apoA-I and apoE but not HDL₂ (16). ABCA7 expression also promoted phospholipid efflux to apoA-I and apoE but not HDL₂ (Fig. 2E), indicating a similar ligand specificity for ABCA1 and ABCA7. Thus, ABCA7 is very similar to ABCA1 in its ability to bind apoA-I and apoE and to promote efflux of PC and SM to these lipid-poor apolipoproteins.

**ABCA7 Does Not Promote Cellular Cholesterol Efflux to apoA-I**—ABCA1 promotes both phospholipid and cholesterol efflux to apolipoproteins (10). Remarkably, ABCA7 failed to promote cellular cholesterol efflux to apoA-I under conditions similar to that of ABCA1-promoted cellular phospholipid efflux to apoA-I (Fig. 3A). Neither prolonged incubation (24 h) with apoA-I nor increased apoA-I concentration (50 μg/ml) resulted in increased cholesterol efflux to apoA-I in ABCA7-transfected HEK293 cells (not shown). Similar to ABCA7, ABCA4 and ABCC3 expression had no effect on cholesterol efflux to apoA-I (Fig. 3A). In an attempt to force ABCA7 to mediate cholesterol efflux, cells were loaded with cholesterol-cyclodextrin, using an 8/1 molar ratio of cyclodextrin/cholesterol that resulted in loading of cells with extra cholesterol mass (not shown). However, transfection of ABCA7 still failed to stimulate cholesterol efflux.

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2 Y. Luo and A. Tall, unpublished data.
Because SR-BI promotes efflux of cellular cholesterol to HDL particles (17), we also considered that ABCA7 might act in conjunction with SR-BI to promote cellular cholesterol efflux. However, co-expression of ABCA7 and SR-BI did not lead to any synergistic or additive increase in cholesterol efflux to HDL (not shown). Overexpression of ABCA7 in Chinese hamster ovary cells, a cell line expressing high levels of ABCA1 in the basal state and showing a robust apoA-I-mediated cholesterol efflux (18), did not result in any higher level of cholesterol efflux to apoA-I than control cells transfected with empty vector alone (Fig. 3C), thus suggesting no interaction between ABCA1 and ABCA7 in cellular cholesterol efflux.

These data indicate that despite its ability to promote cellular phospholipid efflux at similar levels to ABCA1, ABCA7 was not able to promote cholesterol efflux. This result was surprising, as previous studies had suggested a two-step mechanism in which an initial phospholipid-apoA-I complex formed by ABCA1 would promote cholesterol efflux in an autocrine or paracrine fashion in HEK293 cells (19). Part of the evidence for this was a media transfer experiment, in which phospholipid-apoA-I complexes formed by ABCA1 in cyclodextrin-pre-treated ABCA1-transfected HEK293 cells would promote cholesterol efflux when transferred to a second set of cells. However, we found that phospholipid-apoA-I complexes made by ABCA7 had no ability to promote cholesterol efflux in a media transfer experiment (Fig. 3D). This finding led us to repeat the earlier media transfer experiments carried out with ABCA1 (19). We found that when we added an additional washing procedure with media containing bovine serum albumin on the donor cells prior to adding apoA-I, there was no subsequent stimulation of cholesterol efflux when media were transferred to recipient cells (Fig. 3D). Thus, the earlier positive result was likely because of carry-over of residual cyclodextrin released from the cells during incubation with apoA-I. These experiments indicate that complexes formed by ABCA1 and ABCA7 do not promote passive cholesterol efflux in HEK293 cells and suggest that the lipid efflux specificity resides in the transporter itself.

**ABCA7 Is Not a Target Gene of LXR**—ABCA1 gene expression is induced in cholesterol-loaded cells as a result of LXR and retinoid X receptor activation of the ABCA1 promoter (20). Kaminski et al. (21) showed that ABCA7 mRNA and protein levels were increased by acetyl-LDL and down-regulated by HDL₃ in human monocyte-derived macrophages, suggesting that ABCA7 was a sterol-regulated gene. This result would seem to be in conflict with our observations that ABCA7 was not directly involved in cellular cholesterol transport. To test this idea further, we treated mouse peritoneal macrophages

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**Fig. 3. ABCA7 does not promote cellular cholesterol efflux to apoA-I.** A, cholesterol efflux to apoA-I (15 μg/ml) for 6 h in HEK293 cells expressing the transporters. Specific apoA-I-mediated cholesterol efflux was determined by subtracting the background cholesterol efflux in the absence of apoA-I. B, HEK293 cells were cholesterol-loaded and labeled with [³H]cholesterol/cyclodextrin as described under “Experimental Procedures.” ApoA-I-mediated cholesterol efflux was conducted by incubating apoA-I (15 μg/ml) with the cells for 30 min. C, Chinese hamster ovary cells were transiently transfected with the indicated dose of ABCA7-expressing plasmid DNA plus empty vector DNA to maintain a constant DNA mass. ApoA-I-mediated cholesterol efflux was carried out similarly as in A. D, HEK293 cells were transiently transfected with mock vector (control) or constructs expressing ABCA1 or ABCA7. Cells were treated with 20 mM cyclodextrin for 30 min, washed four times with a last wash of 30 min, and apoA-I (15 μg/ml) was then added to incubate with the cells for 5 h. Then, the media was transferred to a second set of [³H]cholesterol-labeled recipient cells and incubated for 6 h for cholesterol efflux.
with TO-901317, a potent LXR agonist that induces ABCA1 expression. Indeed, TO-901317 alone and in combination with 9-cis-retinoic acid increased ABCA1 mRNA expression (42- and 85-fold, respectively) in mouse peritoneal macrophages (Fig. 4A) but had no significant effect on ABCA7 mRNA levels (Fig. 4B). This observation was further confirmed by Western analysis that showed no change of ABCA7 protein levels in response to TO-901317 or TO plus 9-cis-retinoic acid treatment (Fig. 4C). Moreover, similar results were obtained in CD4+ T-lymphocytes that were found to have a relatively high expression of ABCA7 mRNA and protein (not shown). Thus, consistent with its failure to induce cholesterol efflux from cells, ABCA7 is not an LXR target gene.

**Tissue Distribution of ABCA7**—The tissue distribution of ABCA7 was assessed by Western analysis using a specific antibody that only gave rise to a single band on Western blots (Fig. 1D). ABCA7 showed a widespread expression and was detected in all of the tissues tested. The highest ABCA7 protein levels were detected in brain, lung, adrenal gland, and spleen (Fig. 5). However, ABCA7 protein was expressed at a low level in liver. Recently, Sasaki et al. (22) showed that ABCA7 was highly expressed in rat platelets and red blood cells. We confirmed their findings by showing a high level of ABCA7 protein in mouse platelets and red blood cells (Fig. 5). The tissue distribution pattern of ABCA7 protein is generally similar to the ABCA7 mRNA tissue distribution reported previously (23) and confirmed by us using real-time PCR (not shown). High protein expression of ABCA7 was noted in both platelets and brain and in each case was associated with a band of higher Mr than the main band. This finding could reflect alternative splicing of ABCA7 mRNA or alternative post-translational modifications. ABCA7 protein was readily detected in primary cultures of mouse B cells, CD4+ T cells, mouse peritoneal macrophages, and RAW macrophages (Fig. 5).

**DISCUSSION**

Patients with Tangier disease have almost no detectable HDL in plasma, suggesting that ABCA1 has a unique function in initiating cellular lipid efflux and HDL formation (7). Unexpectedly, we have identified a second transporter, ABCA7 that has an ability similar to ABCA1 to promote efflux of cellular phospholipids to apolipoproteins but does not promote cellular cholesterol efflux. ABCA7 has a widespread distribution in peripheral tissues but has low expression in liver. Thus, ABCA7 may have a role in promoting efflux of cellular phospholipids to lipid-poor apolipoproteins in peripheral tissues and possibly in the brain. This could serve as a way to rid cells of excess choline-containing phospholipids without a concomitant release of cellular cholesterol.

Although the need of cells to rid themselves of excess cholesterol has been the paradigm driving HDL research, the possibility that cells have a distinct mechanism to dispose of phospholipids extracellularly has not received much attention. Cellular plasma membranes may expand rapidly as a result of the movement of pools of phospholipid from inside the cell to the surface, e.g., in preparation for phagocytic engulfment of large particles in macrophages (24) or after fusion of secretory vesicles with the plasma membrane (25). Although the general view is that excess phospholipids are removed by endocytosis, phospholipid efflux to extracellular apolipoproteins could also help to trim excess phospholipids from the membrane.

It is intriguing that ABCA1 and ABCA7 are the closest mammalian orthologs of Ced-7, the *Caenorhabditis elegans* molecule that may be required for disposal of apoptotic cells. There is preliminary evidence to support a role of ABCA1 in the disposal of apoptotic cells during embryonic development (26). We speculate that ABCA1 and ABCA7 may have overlapping roles in cellular phospholipid metabolism, perhaps reflecting a common
basic property of translocation of membrane choline-phospholipids. This basic property could be involved in both the disposal of apoptotic cell lipid efflux when added membrane lipids to apoA-I by ABCA7 was insufficient to promote the formation of an HDL particle. Surprisingly, the addition of phospholipid to apoA-I by ABCA7 was insufficient to promote passive efflux of cholesterol from transfected HEK293 cells, even when co-expressed with SR-BI, which is known to enhance this process (17). This result indicates that the initial phospholipid added to apoA-I by ABCA7 is not in a form such as a phospholipid bilayer that is able to stimulate passive uptake of cholesterol (29).

This discovery led us to re-assess our earlier conclusion that ABCA1 promotes cholesterol efflux in a two-step process (19). The new experiments that included an additional washing step for CD4+ T cells, B cells, RAW cells, and mouse peritoneal macrophages (MPM), ~25 μg of protein was used.

X receptor. This is apparently restricted to ABC transporters directly involved in cholesterol transport, i.e. ABCG5/8 for biliary cholesterol and ABCA1 for cellular cholesterol. The concept of specificity of lipid transport and gene regulation suggested in the present study is thus consistent with an emerging picture for ABC transporters involved in lipid secretion and reverse cholesterol transport.

REFERENCES

ABCA7 Promotes Phospholipid Efflux to Apolipoproteins

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