Roles of ATP-Binding Cassette Transporter A7 in Cholesterol Homeostasis and Host Defense System

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ATP-binding cassette transporter (ABC) A7 is an ABC family protein that is a so-called full-size ABC transporter, highly homologous to ABCA1, which mediates the biogenesis of high-density lipoprotein (HDL) with cellular lipid and helical apolipoproteins. ABCA7 mediates the formation of HDL when exogenously transfected and expressed; however, endogenous ABCA7 was shown to have no significant impact on the generation of HDL and was found to be associated with phagocytosis regulated by sterol regulatory element binding protein 2. Since phagocytosis is one of the fundamental functions of animal cells as an important responsive reaction to infection, injury and apoptosis, ABCA7 seems to be one of the key molecules linking sterol homeostasis and the host defense system. In this context, HDL apolipoproteins were shown to enhance phagocytosis by stabilizing ABCA7 against calpain-mediated degradation and increasing its activity, shedding light on a new aspect of the regulation of the host-defense system.


Key words; ABCA7, ApoA-I, HDL, Phagocytosis, ABCA1, Cholesterol
phospholipid ratio and small diameter on average\(^\text{23}\).

However, endogenous ABCA7 was shown to have no significant impact on the generation of HDL\(^\text{15, 24, 25}\). No genetic defect was reported on the human ABCA7 gene. A high-density single nucleotide polymorphism (SNP) map of ABC transporters in Japan has been constructed and 67 SNPs identified at the ABCA7 locus\(^\text{26}\), but no association has been indicated between the variations and pathological phenotypes. A homozygous ABCA7 knockout mouse was found to be embryonically lethal\(^\text{25}\) by one laboratory, but the other group reported the generation of ABCA7 null mice\(^\text{24}\). Production of homozygous mice by intercrossing heterozygotes was at the expected rate, and its development, including feeding behavior and weight gain up to 10 weeks, was normal\(^\text{24}\). No marked phenotype was observed in these animals, except that the white adipose tissue mass was about 50% less and the serum HDL cholesterol level was somewhat but significantly lower than that of the wild-type control only in females at the age of 10 weeks. No such change was detected in male knockout mice\(^\text{24}\). The details of the roles of ABCA7 in cholesterol homeostasis are summarized in the previous review\(^\text{23}\).

**Role of ABCA7 in host defense systems**

Several ABC transporters, including ABCA1, ABCA7, and ABCC7, were reported to relate to the phagocytic function of cells. Although ABCA1 is an ortholog of C. elegans ced-7, a gene involved in the phagocytosis of apoptotic cells\(^\text{27}\), both positive\(^\text{28, 29}\) and negative regulation\(^\text{30}\) of cellular phagocytic function by ABCA1 was observed. ABCA7\(^\text{15, 31}\) and ABCC7\(^\text{32}\) were reported to be phagocytic potentiators.

ABCC7, the another function is chloride ion channel, was reported as the cause of Cystic Fibrosis\(^\text{33, 34}\). Phagocytosis of amine-coated latex beads triggered the proteolytic activation of sterol regulatory element binding protein-1a (SREBP-1a) and SREBP-2 without lipid deprivation\(^\text{35}\), and phagocytosis induced ABCA7 up-regulation via SREBP2 activation under similar experimental conditions\(^\text{15}\).

Structural\(^\text{36}\) and functional characteristics of ABCA1, ABCA7, and ABCC7 are summarized in Table 1.

### Table 1. Controversy over ABC transporters involved in phagocytosis

<table>
<thead>
<tr>
<th>Gene (alias)</th>
<th>ABCA1</th>
<th>ABCA7</th>
<th>ABCC7 (CFTR)</th>
</tr>
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<tbody>
<tr>
<td><strong>Structure</strong> (36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of transmembrane helices</td>
<td>12</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Number of nucleotide binding domains</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Mendelian disorder (pathological physiology)</td>
<td>Tangier disease, (Dysfunction of Cholesterol efflux) (6, 7, 8)</td>
<td>unknown</td>
</tr>
<tr>
<td>Regulation of phagocytosis</td>
<td>Positive (28, 29)</td>
<td>Positive (15, 31)</td>
<td>Positive (32)</td>
</tr>
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</table>

**Subcellular localization of ABCA7**

Overexpressed ABCA1 protein is localized mainly in the plasma membrane\(^\text{37-39}\) consistent with the localization of endogenous ABCA1 in the plasma membrane\(^\text{40}\). ABCA7 proteins resulting from transfected cDNA (wild type, GFP-tagged, and Flag-tagged) were found to be localized predominantly in the plasma membrane, but were also detected in the intracellular membranes\(^\text{19, 20, 22, 41}\). Probing cell surface protein by biotinylation of HEK 293 cells transfected with ABCA7 cDNA confirmed the cell surface expression of the transfected and expressed ABCA7 protein\(^\text{20}\). In contrast, rat ABCA7 with no tag and with a GFP tag at the N-terminus were both detected mainly in the plasma membrane when expressed in CHO cells, while ABCA7 with GFP fusion to the C-terminus was localized by probing with the ABCA7 epitope and GFP fluorescence in the perinuclear membrane\(^\text{41}\). Immunofluorescence microscopy analysis of non-permeabilized cells with the antibody against the first putative extracellular domain of human ABCA7 (amino acids 45-549) confirmed the cell surface expression of
the overexpressed ABCA7 protein\textsuperscript{22}. The latter experiment also revealed that this domain is exposed to the outside of the cell, indicating the same topological arrangement as ABCA1\textsuperscript{37, 38, 42}. On the other hand, immunofluorescence confocal microscopy with rabbit anti-mouse ABCA7 antibody detected no signal of ABCA7 in the plasma membrane, but rather in the intracellular space in peritoneal macrophages\textsuperscript{25}, or redistribution into phagocytosis cups during phagocytosis\textsuperscript{31}. Our previous study with immunofluorescent probing indicated intracellular localization of endogenous ABCA7\textsuperscript{19}, however, estimation of cell surface ABCA7 in J774 and mouse fibroblasts by a biotinylation technique directly demonstrated that endogenous ABCA7 mainly existed in the plasma membrane and apoA-I increased cell surface ABCA7 (Fig. 1A)\textsuperscript{43}. This discrepancy may be consistent with the assumption that ABCA7 turns over rapidly on the cell surface.

**Glycosylation of ABCA7**

Exogenously transfected ABCA1-GFP in HEK293 cells was highly glycosylated\textsuperscript{37, 38}. Peptide N-glucosidase F treatment revealed that ABCA2\textsuperscript{44}, ABCA3\textsuperscript{45}, and ABCA4\textsuperscript{46} proteins were also glycosylated. We therefore investigated the glycosylation of endogenous ABCA7 as well as ABCA1. The results indicated that ABCA7 contains N-glycoside-linked oligosaccharides as ABCA1 (Fig. 1B)\textsuperscript{43}. This modification of ABCA7 was not influenced by extracellular apoA-I\textsuperscript{43}.

**Turnover and degradation of ABCA7**

ABCA1 is stabilized against its calpain-mediated degradation by helical apolipoproteins when the HDL biogenesis reaction is ongoing\textsuperscript{47-50}. With the biotinylation method, ABCA1 on the surface was shown to be internalized and intracellularly degraded. Exposure of ABCA1 to extracellular apoA-I before its endocytosis retarded ABCA1 internalization protected ABCA1 against this calpain-mediated proteolysis and consequently recycled more ABCA1 to the surface to increase surface ABCA1, perhaps by forming a complex prior to endocytotic internalization for its intracellular proteolysis\textsuperscript{51}. Direct inhibition of ABCA1 endocytosis also led to a decrease of its degradation and increased surface ABCA1. When HEK293 cells stably expressing ABCA7-GFP were treated by N-acetyl-Leu-Leu-norleucinal (ALLN) or apoA-I, ABCA7 protein increased\textsuperscript{50}. Our recent study showed that endogenous ABCA7 was also stabilized against its calpain-mediated degradation by helical apolipoproteins (Fig. 1C)\textsuperscript{43}. Thus, both ABCA7 and ABCA1 seem to be processed essentially in a similar manner.

**Helical apolipoprotein-mediated stabilization of ABCA7 and phagocytosis enhancement**

Effects of helical apolipoproteins on the stabilization of ABCA7 and enhancement of phagocytosis were examined\textsuperscript{43}. Decay of ABCA7 protein examined in the presence of the protein synthesis inhibitor cycloheximide was retarded by apoA-I and apoA-II, similarly to the findings with ABCA1 (Fig. 1C, upper panel)\textsuperscript{43}. Calpeptin increased ABCA7 protein as well as ABCA1 protein by retarding their decay rate (Fig. 1C, lower panel)\textsuperscript{43}.

ApoA-I and apoA-II enhanced phagocytosis (Fig. 1D)\textsuperscript{43}. Expression of ABCA1 or ABCA7 was down-regulated by the respective siRNA in J774 and a quantitative phagocytosis assay was performed. Phagocytosis enhancement by apoA-I and apoA-II was ablated in the cells by knockdown of ABCA7, but remained after knockdown of ABCA1 (Fig. 1E)\textsuperscript{43}. Phagocytic activity of macrophages in the mouse peritoneal cavity in vivo was examined by measuring the uptake of carbon microparticles after injecting them into the peritoneal cavity. Uptake of the particles by peritoneal cells was decreased in ABCA7-knockout mice compared to wild-type mice (Fig. 1F). Helical apolipoproteins are carried by HDL and may dissociate from lipoprotein particles in equilibrium\textsuperscript{12}. It is not completely clear yet whether helical apolipoproteins directly bind to ABCA1 or ABCA7 to achieve these effects\textsuperscript{20, 53}, and it is still possible that ABC transporters may be stabilized by alteration of the membrane microenvironment by modification of its lipid composition.

**Conclusion**

This review is summarized in Table 2 and Fig. 2. ABCA7 is highly homologous to ABCA1 and mainly exists in the plasma membrane, as does ABCA1. Its post-transcriptional regulation, glycosylation and degradation are also quite similar to ABCA1\textsuperscript{43}; however, the function of the two transporters is different. ABCA1 generates HDL with apolipoproteins, but endogenous ABCA7 does not contribute to HDL biogenesis. ABCA7 is rather involved in the enhancement of phagocytosis, and apolipoproteins further increase this function\textsuperscript{43}.

Contribution of HDL to the host defense system has been previously indicated as the inhibition of LPS.
Fig. 1. Phagocytic enhancement by stabilizing ABCA7

(A) ABCA7 is localized on the cell surface. After overnight incubation of J774 with apoA-I, biotinylated (surface) and non-biotinylated (intracellular) proteins were prepared for Western blotting after the treatment of cells with 1 mM biotin. W indicates analysis of the whole cell lysate without biotinylation.

(B) ABCA7 is glycosylated. After overnight incubation of J774 with apoA-I, membrane proteins treated with each enzyme (Endoglycosidase H (endoH) or peptide: N-glycosidase F (PNGase F)) were analyzed by Western blotting.

(C) ABCA7 is degraded by calpain, and degradation is inhibited by helical apolipoproteins. (Upper panel) After overnight incubation of J774 with apoA-I or apoA-II, protein synthesis was inhibited by cycloheximide, and proteins were analyzed by Western blotting at 0, 3, 6 h of incubation. (Lower panel) After overnight starvation of J774 cells, the cells were preincubated with and without calpeptin for 1 h, and then incubated for 0, 3, or 6 hours under inhibition of protein synthesis by cycloheximide.

(D) and (E) Helical apolipoproteins enhance phagocytosis through ABCA7. After overnight incubation with apoA-I or apoA-II of J774 (D) or J774 knocked-down ABCA7 and ABCA1 (E), a quantitative phagocytosis assay was performed.

(F) Phagocytic function is attenuated in ABCA7-knockout mice. Diluted carbon ink was injected into the mouse peritoneal cavity. After overnight starvation of mice, peritoneal macrophages were recovered and over 400 cells were counted to calculate the phagocytosis index as a relative number of cells that engulfed carbon ink particles. Statistical analysis was performed to give significance levels as ***, p < 0.001 to the control or between groups is indicated. See reference 39 for detailed methods.
HDL traps LPS\textsuperscript{54, 55} and HDL apolipoproteins and amphiphilic \alpha helical peptides block the uptake of LPS through SR-BI\textsuperscript{50} as its competitive ligands. Involvement of ABCA7 in phagocytosis and its modulation by helical apolipoproteins of HDL revealed a more direct molecular mechanism of HDL contribution to the host-defense system. The finding would provide new insight into the relationship between cholesterol homeostasis and phagocytosis in host-defense systems.

Table 2. Summary of findings

<table>
<thead>
<tr>
<th>ABCA1</th>
<th>ABCA7</th>
</tr>
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<tbody>
<tr>
<td>Structure</td>
<td>12 transmembrane spans (12, 37)</td>
</tr>
<tr>
<td>Localization</td>
<td>Plasma membrane (40)</td>
</tr>
<tr>
<td>Transcription</td>
<td>LXR (16)</td>
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<tr>
<td>Glycosylation</td>
<td>N-glycosylated (37, 38)</td>
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<tr>
<td>Degradation</td>
<td>Calpain (20, 49, 50, 51)</td>
</tr>
<tr>
<td>apoA-I-mediated stabilization</td>
<td>Yes (47, 49, 50, 51)</td>
</tr>
<tr>
<td>&amp; Exogenous: Yes (19, 21, 38, 42)</td>
<td>Exogenous: Yes (19, 20, 21, 22)</td>
</tr>
<tr>
<td>apoA-I-mediated phagocytosis</td>
<td>No (43)</td>
</tr>
</tbody>
</table>

Fig. 2. Relationships among cholesterol homeostasis, HDL generation, and phagocytosis.
References


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