Identification of an Amino Acid Residue Critical for Plasma Membrane Localization of ATP-Binding Cassette Transporter G1—Brief Report

Hong-mei Gu,* Faqi Wang,* Adekunle Alabi, Shijun Deng, Shucun Qin, Da-wei Zhang

Objective—ATP-binding cassette transporter G1 (ABCG1) mediates cholesterol efflux to lipidated lipoproteins. Conflicting data about cellular localization of ABCG1 and its effect on cholesterol efflux have been reported. Confocal microscopy and biotinylation were used to assess cell surface localization of ABCG1. We found that mouse ABCG1 (mABCG1) used in one previous study has a substitution of Leu to Pro at position 550 (mG1-L550P). When the corresponding Leu at position 562 in human ABCG1 (hABCG1) was mutated to Pro (hG1-L562P), the mutant hABCG1, like mG1-L550P, mainly resided intracellularly, whereas wild-type mABCG1 and hABCG1 were localized on the plasma membrane. However, replacement of this Leu with Pro had no significant effect on mABCG1- and hABCG1-mediated cholesterol efflux.

Conclusions—Leu at position 550/562 in mABCG1/hABCG1 is critical for their plasma membrane localization but not for ABCG1-mediated cholesterol efflux. Our findings indicate that the substitution of Leu to Pro at position 550 in mABCG1 may contribute to the non–cell surface localization of mABCG1 observed in the previous study. (Arterioscler Thromb Vasc Biol. 2016;36:253-255. DOI: 10.1161/ATVBAHA.115.306592.)

Key Words: ATP-binding cassette transporter G1 ■ cholesterol efflux ■ lipoprotein ■ mutation ■ protein trafficking

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

When compared with the canonical hABCG1 sequence, mABCG1 lacks a highly positively charged intracellular segment of 12 amino acid residues (VKQT九龙KGLKL) (Figure 1A), even though the overall sequence homology between the 2 proteins is high with 97% amino acid identity. It has been reported that lacking the 12 amino acid segment in hABCG1 has no effect on hABCG1 trafficking and cholesterol efflux activity.1,13 To assess the possibility that this positively charged segment might facilitate association of the NH2 terminal cytoplasmic region of mABCG1 with negatively charged cell membranes and consequently affect its cellular localization, we inserted the segment of 12 amino acid residues into the corresponding site in mABCG1 (mG1-Ins) that was cloned from mouse macrophage RAW264 cells. We also deleted the 12 amino acid segment from hABCG1 (hG1-del). HEK293 (human embryonic kidney 293) cells transiently expressing wild-type or mutant hABCG1 or mABCG1 were subjected to confocal microscopy. We found that the distribution patterns of wild-type
hABCG1 and mABCG1, hG1-del, and mG1-Ins were similar (Figure 1B). The transporter was detected on the cell periphery and co-localized with the plasma membrane marker, Na+/K+-ATPase (right). Thus, the 12 amino acid segment does not influence cellular localization of both hABCG1 and mABCG1, consistent with previous reports.1,13

To elucidate the difference between our observation and Tarling’s finding,12 we obtained the mouse cDNA used in their study (mG1-T) and found that mG1-T was mainly localized intracellularly (Figure 1B). DNA sequencing revealed that mG1-T has Pro at position 550 (mG1-T/mG1-L550P), whereas our hABCG1 and mABCG1 contain Leu at the corresponding position that is consistent with the sequences of hABCG1 and mABCG1 found in the database of Swiss-Prot and National Center for Biotechnology Information. This Leu is highly conserved in ABCG1 from different species (Figure 2A). We mutated the corresponding Leu at position 562 in hABCG1 to Pro (hG1-L562P) and found that hG1-L562P, like mG1-L550P, was mainly localized intracellularly (Figure 2B). To confirm this finding, we performed a biotinylation experiment. As shown in Figure 2C, the expression levels of wild-type and mutant mABCG1 and hABCG1 were comparable in whole cell lysates. However, we observed wild-type but not mutant mABCG1 and hABCG1 in the biotinylated cell surface proteins. Thus, Leu at position 550/562 in mABCG1/hABCG1 is

Nonstandard Abbreviations and Acronyms

<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>ABCG1</td>
<td>ATP-binding cassette transporter G1</td>
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<tr>
<td>hABCG1</td>
<td>human ABCG1</td>
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<tr>
<td>Ldlr</td>
<td>low-density lipoprotein receptor</td>
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<td>mABCG1</td>
<td>mouse ABCG1</td>
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Figure 1. Cellular localization of ATP-binding cassette transporter G1 (ABCG1). A, Schematic structure of ABCG1. B, Confocal microscopy. HEK293 (human embryonic kidney 293) cells were transiently transfected with empty (control) or wild-type (WT) or mutant mouse/human ABCG1 as indicated in the figures. ABCG1 was detected with a polyclonal anti-ABCG1 antibody, H-65 (green). Monoclonal anti-Na+/K+-ATPase antibody was visualized in red. Nuclei were visualized with DAPI (blue). Co-localization of ABCG1 with Na+/K+-ATPase is in yellow (right, overlap column).

Figure 2. Cholesterol efflux. A, Sequence alignment of ATP-binding cassette transporter G1 (ABCG1) performed by ClustalW2. Only parts of sequence alignment that includes intracellular loop 2 (CL2) were shown. B, Confocal microscopy. Immunofluorescence was performed on HEK293 (human embryonic kidney 293) cells transiently expressing hG1-L562P, as described in legend to Figure 1B. H-65 was used to detect ABCG1 (green). Na+/K+-ATPase was visualized in red and nuclei in blue. C, Biotinylation. HEK293 cells transiently expressing wild-type (WT) or mutant mouse/human ABCG1 were labeled with biotin. Biotinylated proteins were pull down by Neutravidin agarose and detected by immunoblotting. D, Cholesterol efflux. Cells labeled with [3H]cholesterol were incubated with reconstituted high-density lipoprotein, followed by the cholesterol efflux assay. Values are mean±SD of 3 independent experiments. Each experiment was performed in triplicate. Total numbers in each group were 9. *P<0.05 vs control. Bottom figure is representative one showing protein levels. Expression of WT and mutant mouse/human ABCG1 (G1) was detected with a monoclonal antibody. Similar results were obtained from at least one more independent experiment.
critical for plasma membrane localization of ABCG1. Next, we
examined whether replacement of this Leu with Pro affected
ABCG1-mediated cholesterol efflux. We found that cells
expressing hG1-L562P or mG1-L550P showed similar cho-
lesterol efflux activity as the wild-type hABCG1/mABCG1-
expressing cells (Figure 2D), indicating a negligible role of
this Leu in ABCG1-mediated cholesterol efflux.

Discussion
We found that replacement of Leu at position 550/562 in
mABCG1/hABCG1 to Pro caused undetectable cell surface
ABCG1, whereas both wild-type hABCG1 and mABCG1
resided on the plasma membrane. Thus, the substitution of Leu
at position 550 in mABCG1 to Pro may contribute to the non–cell
surface localization of mABCG1 observed in Tarling’s study.12

Both mG1-L550P and hG1-L562P mediated cellular cho-
lesterol efflux as efficiently as the wild-type proteins even
though they were mainly localized intracellularly, consistent
with previous findings.12 Tarling et al proposed that ABCG1
acts as an intracellular cholesterol transporter.12 However, it
has been shown that liver X receptor activation increases cell
surface localization of endogenous macrophage ABCG1 that
mainly resides intracellularly at the basal condition, enhanc-
ing cholesterol efflux to high-density lipoprotein.8 Further,
Pagler et al14 demonstrated that endogenous ABCG1, together
with ABCA1, facilitated the translocation of cholesterol from
the inner leaflet to the outer leaflet of the plasma membrane,
where it was removed by high-density lipoprotein. Lacking
both ABCA1 and ABCG1 in macropathes caused accumu-
lation of cholesterol in the plasma membrane. Vaughan et al
also reported that ABCG1 overexpressed in cultured cells
was localized on the cell surface and increased the accessibility
of cholesterol in plasma membrane to cholesterol oxidase.9
Together, these findings clearly reveal the important role of
cell surface ABCG1 in cholesterol efflux. Thus, ABCG1 may
facilitate cholesterol translocation across both the plasma
membrane and the membranes of intracellular vesicles depen-
don on where the transporter is localized. However, how the
intracellular vesicles deliver cholesterol to extracellular ac-
tors is unclear. It has been proposed that these vesicles are
fused with the plasma membrane by unknown mechanisms
and then deliver cellular cholesterol to exogenous acceptors.12
It will be of interest to see whether liver X receptor activation
affects intracellular vesicle trafficking.

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Disclosures
None.

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Significance
Our findings provide an explanation for the conflicting observations on ATP-binding cassette transporter G1 cellular localization in the litera-
ture. The substitution of Leu at position 550 to Pro in mouse ATP-binding cassette transporter G1 appears to cause undetectable cell-surface
ATP-binding cassette transporter G1 in one previous study.
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