Letter to the Editor

Cell Surface Localization of ABCG1 Does Not Require LXR Activation

To the Editor:

In a recent article in this journal entitled "LXR-Induced Redistribution of ABCG1 to Plasma Membrane in Macrophages Enhances Cholesterol Mass Efflux to HDL", Nan Wang and colleagues showed that in nonstimulated macrophages ABCG1 protein is predominantly intracellular, and that on treatment with a synthetic liver X-receptor (LXR) ligand it increased in amount and appeared to relocate from this intracellular pool to the plasma membrane.[1] Based on our findings outlined below, we suggest an alternative interpretation of these results.

Experiments performed in our laboratory showed that transiently expressed N-terminally EGFP-tagged ABCG1 fusion protein localized in the Golgi apparatus and in intracellular vesicles of epithelial HeLa cells a few hours after transfection. Then, between 6 and 24 hours after transfection, increasing amounts of EGFP-ABCG1 appeared in the plasma membrane (Figure, A). In contrast to the data of Wang et al,[1] our Tet-Off HeLa cells stably expressing an ABCG1 protein tagged at the N terminus with a triple repeat of the influenza virus hemagglutinin epitope tag YPYDVPDYA [$(HA)_3$-ABCG1] showed increased efflux of cholesterol to HDL_3 compared with a negative control (Figure, B). Further, human macrophages derived from the monocytic THP-1 leukemia cell line that transiently expressed the N-terminally EGFP-tagged ABCG1 protein not only showed intracellular perinuclear localization of the EGFP-ABCG1 protein, but also prominent staining of the plasma membrane 12 hours after transfection even in the absence of LXR ligands (Figure, C).

Based on our findings, we therefore suggest that ABCG1 is targeted to the plasma membrane via the Golgi apparatus directly after synthesis and the insertion in the endoplasmic reticulum. Clearance of the EGFP-ABCG1 protein from the plasma membrane of macrophages may then occur because of degradation or internalization by, for example, endocytosis. The latter is corroborated by the finding that ABCG1 colocalizes with recycling endosomes.[1] Because the expression of ABCG1 is strongly induced by LXR and retinoid X-receptor (RXR) activation,[2] it is possible that on LXR activation large amounts of newly synthesized ABCG1 are rapidly transported to the plasma membrane. Thus, the observation described by Wang et al may reflect targeting and transport of newly synthesized ABCG1 via the Golgi apparatus to the plasma membrane, rather than a redistribution to the plasma membrane of preexisting ABCG1 from intracellular pools.

We used an N-terminally tagged protein, whereas the ABCG1-GFP or ABCG1-Flag fusion proteins used by Wang and colleagues were tagged at their C end. The members of the ABCG subfamily lack N-terminal signal peptides and display characteristics of tail-anchored transmembrane proteins which are inserted into membranes from their C terminus.[3,4] The C-terminal tag is very close to the last transmembrane helix of the ABCG1 protein and may disturb its correct processing or folding. This might explain failure of human embryonic kidney (HEK) 293 cells transiently expressing C-terminal ABCG1-GFP or ABCG1-Flag proteins in the study by Wang et al to show increased cholesterol efflux. In this respect it is important to recall that transient overexpression of nontagged ABCG1 in HEK 293 cells increased cholesterol efflux to HDL particles (Wang et al, 2004 and 2006).[1,5]

To sum up, ABCG1 localizes in the plasma membrane even in the absence of LXR and RXR activators. This indicates that further studies are necessary to elucidate the exact modes of intracellular trafficking and action of ABCG1.

Disclosures

None.

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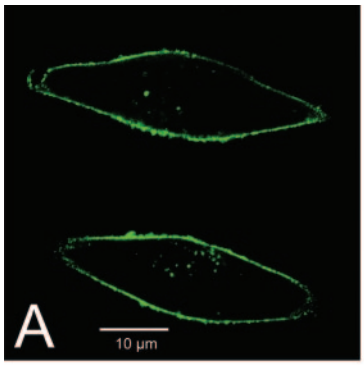
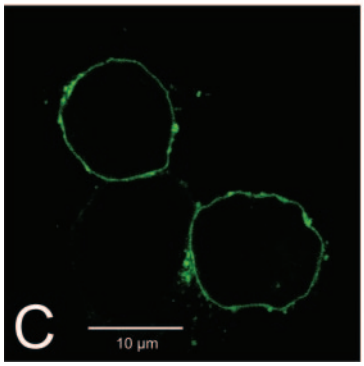
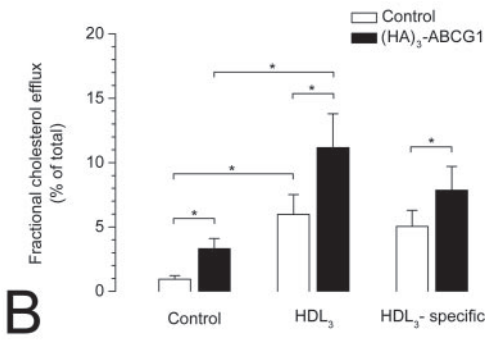
(***Arterioscler Thromb Vasc Biol*. 2006;26:e143-e144.**)

Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000245790.47112.b2**







Cellular localization and cholesterol efflux capability of expressed EGFP-ABCG1. A, Transiently expressed EGFP-ABCG1 fusion protein is present at the cell surface of epithelial HeLa cells in the absence of LXR and RXR ligands. B, Cholesterol efflux to HDL_3 is increased in Tet-Off HeLa cells stably expressing $(HA)_3$-ABCG1 fusion protein compared with control Tet-Off HeLa cells not expressing $(HA)_3$-ABCG1 (n=3). C, In THP-1 cells transiently expressed EGFP-ABCG1 is also localized in the plasma membrane in the absence of LXR ligands. Representative figures of EGFP-ABCG1 expressing cells are shown. $^*P<0.05$.