Purification and functional analysis of cholesterol transporter ABCG1 and ABCG4

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Cholesterol is essential to the body as a component of cellular membranes and a source of steroid hormones, but excess cholesterol is toxic and a risk factor for arteriosclerosis. Therefore, the removal of cholesterol from peripheral cells is important. ATP Binding Cassette (ABC) protein, a protein family of ATP-dependent transporters, plays important roles in cholesterol homeostasis, and defects in their functions are related to various diseases.

ABCG1 and ABCG4, members of subgroup B of ABC protein, are half-type ABC proteins, and function as homodimers. ABCG1 has been reported to transport free cholesterol and phospholipids to high-density lipoprotein (HDL) and removes excess cholesterol from peripheral cells. Mice lacking ABCG1 accumulate lipids in macrophages and hepatocytes when fed a high-fat and high-cholesterol diet. It has been reported that ABCG4 is expressed in megakryocyte progenitors and that transplantation of ABCG4(-/-) bone marrow into Ldlr(-/-) causes thrombosis and atherosclerosis. Culture cells expressing ABCG4 exhibit cholesterol efflux in the presence of HDL, suggesting that ABCG4 is a cholesterol exporter like ABCG1. However, the physiological roles and transport substrates of ABCG1 and ABCG4 are still unclear.

The purpose of the study presented in this thesis is to reveal the transport substrates of human ABCG1 and ABCG4. For this purpose, the author developed large scale expression and purification systems of these proteins and analyzed their ATPase activity. The results described in each chapter can be summarized as follows.

In chapter 1, the author analyzed the functions of ABCG1 with purified protein. Human ABCG1 was successfully expressed in the plasma membrane of FreeStyle 293-F cells and purified by using Flag-M2 antibody affinity chromatography. Detergent screening analysis revealed that ABCG1 keeps a stable dimer structure in detergent *n*-dodecyl-β-D-maltoside (DDM). After reconstitution in egg lecithin liposome, DDM-purified ABCG1 showed ATPase activity of 150 nmol/min/mg, which was sufficient to function as an active transporter. Without reconstitution, ABCG1-GFP did not show ATPase activity, suggesting that the lipid bilayer environment is important for the function of ABCG1. Next, the author examined whether the ATPase activity of ABCG1 is stimulated by specific lipids. ABCG1 showed higher ATPase activity in liposomes containing cholesterol, SM or PC. The half maximal value of PC for the enhancement of ATPase activity was about 2-hold higher than those of cholesterol and SM. These results suggest that ABCG1 recognizes cholesterol and SM as transport substrates and also recognizes PC as a weak transport substrate, and that the choline head group and a free hydroxy group of SM, which does not exist in PC, are important for interaction with ABCG1. Considering that SM lowered the binding affinity of ABCG1 for cholesterol, the author proposes that the binding sites for SM and cholesterol of ABCG1 are synergistically coupled.

In chapter 2, the author analyzed the functions of ABCG4 with purified protein by modifying the purification procedure for ABCG1. Detergent screening analysis revealed that ABCG4 dimer is unstable in many detergents and dimer structure is retained only in fos-choline-14 (FC-14) and digitonin. After reconstitution in egg lecithin liposome, digitonin-purified ABCG4 showed ATPase activity while FC-14-purified ABCG4 did not.

The properties of ABCG4 ATPase activity, including affinity for ATP, maximum rate constant and inhibitor specificity, were similar to those of ABCG1. The ATPase activity of ABCG4 was stimulated by cholesterol in a dose-dependent manner. Unlike ABCG1, choline phosopholipids showed no stimulatory effects on the ATPase activity of ABCG4. These results suggest that ABCG4 is a cholesterol-selective transporter and does not recognize choline phospholipids as transport substrates.

From these studies, the author proposes that human ABCG1 and ABCG4 are lipid transporters and their substrate specificities are slightly different from each other. Further studies, such as crystallization of these proteins, are necessary to reveal the mechanism of substrate recognition of these proteins. The purification and reconstitution systems developed here should be useful for revealing their transport mechanisms and also discovering the specific inhibitors and activators of these proteins.