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Cholesterol trafficking and raft-like membrane domain composition mediate scavenger receptor class B type 1-dependent lipid sensing in intestinal epithelial cells

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ABSTRACT

Scavenger receptor Class B type 1 (SR-B1) is a lipid transporter and sensor. In intestinal epithelial cells, SR-B1-dependent lipid sensing is associated with SR-B1 recruitment in raft-like/ detergent-resistant membrane domains and interaction of its C-terminal transmembrane domain with plasma membrane cholesterol. To clarify the initiating events occurring during lipid sensing by SR-B1, we analyzed cholesterol trafficking and raft-like domain composition in intestinal epithelial cells expressing wild-type SR-B1 or the mutated form SR-B1-Q445A, defective in membrane cholesterol binding and signal initiation. These features of SR-B1 were found to influence both apical cholesterol efflux and intracellular cholesterol trafficking from plasma membrane to lipid droplets, and the lipid composition of raft-like domains. Lipidomic analysis revealed likely participation of d18:0/16:0 sphingomyelin and 16:0/0:0 lysophosphatidylethanolamine in lipid sensing by SR-B1. Proteomic analysis identified proteins, whose abundance changed in raft-like domains during lipid sensing, and these included molecules linked to lipid raft dynamics and signal transduction. These findings provide new insights into the role of SR-B1 in cellular cholesterol homeostasis and suggest molecular links between SR-B1-dependent lipid sensing and cell cholesterol and lipid droplet dynamics.

1. Introduction

Cellular transport and trafficking of cholesterol, as well as the molecular composition of the plasma membrane, are critically involved in the governance of cell fate and cell metabolism. The scavenger receptor Class B type 1 (SR-B1) is primarily known for its function as a cholesterol transporter, particularly in the liver, where it is responsible for the last step in the reverse cholesterol transport pathway, which entails the selective uptake of cholesterol ester from High Density Lipoprotein (HDL). SR-B1 also mediates the bidirectional transport of cholesterol and phospholipids from peripheral tissues or cells to lipoproteins [1]. In the intestine, the functions of SR-B1 regarding

Abbreviations: AGR2, anterior gradient protein 2 homolog; CALX, calnexin; CTTM, C-terminal transmembrane domain; DRM, detergent-resistant membranes; HDL, high density lipoprotein; HNRH1, heterogeneous nuclear ribonucleoprotein H; HNRPK, heterogeneous nuclear ribonucleoprotein K; ICAM1, intercellular adhesion molecule 1; JAM1, junctional adhesion molecule A; K1C9, keratin, type I cytoskeletal 9; KAP2, cAMP-dependent protein kinase type II-alpha regulatory subunit; LDHB, L-lactate dehydrogenase B chain; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MβCD, methyl- β-cyclodextrin; NDUA2, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 2; PC, phosphatidylcholine; PDE8A, high affinity cAMP-specific and IBMX-insensitive 3′,5′-cyclic phosphodiesterase 8A; PE, phosphatidylethanolamine; PI, phosphoinositide; PL, phospholipids; pPE, plasmalogen phosphatidylethanolamine; PPM, postprandial lipid micelles; PS, phosphatidylserine; SM, sphingomyelin; SR-B1, scavenger receptor Class B type 1; STX16, syntaxin-16; VAMP8, vesicle-associated membrane protein 8; VATD, ion transport, such as V-type proton ATPase subunit D; VP9D1, VPS9 domain-containing protein 1

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cholesterol and lipid metabolism remain unclear. Although SR-B1 is expressed at the apical membrane of enterocytes [2–4], which are the absorptive cells of the intestinal epithelium, its role in intestinal cholesterol uptake has been questioned since NPC1L1 has been demonstrated to be responsible for 70% of cholesterol entry in enterocytes [5–7]. Moreover SR-B1-null mice [8] and mice overexpressing SR-B1 in the proximal intestine [9] do not display alterations in intestinal cholesterol absorption or in trans-intestinal cholesterol efflux [10]. However, other reports showed that SR-B1 is involved in the secretion of triglyceride-rich lipoproteins by enterocytes upon lipid supply [9,11,12].

In addition to serving in cholesterol movement, SR-B1 functions as a signal transducer in certain cell types, particularly in endothelial cells and in intestinal epithelium. In endothelial cells, the binding of HDL to SR-B1 triggers the sequential activation of protein kinases, among which the activating phosphorylation of Src kinase represents the earliest signaling event identified [13]. This signaling cascade leads to the production of nitric oxide and it promotes endothelial cell migration, which both contribute to the maintenance of endothelial monolayer integrity by HDL and SR-B1 [13]. In Caco-2/TC7 enterocyte-like cells, SR-B1 triggers intracellular signaling in response to the supply, at the apical pole of the cells, of postprandial lipid micelles (PPM), which mimic the form and composition of the dietary lipids that are present in the gut lumen following a meal. The sensing of PPM by SR-B1 activates MAP kinases and the trafficking of apolipoprotein B, the structural apolipoprotein required for triglyceride-rich lipoprotein assembly and secretion, from the apical region of the cell to basolateral secretory domains [14,15]. These intracellular events are associated with the recruitment of SR-B1 into raft-like membrane domains [14,15], which are known to be signaling platforms [16].

Studies have been conducted to analyze the molecular mechanisms involved in SR-B1-dependent lipid sensing. It has been established that the C-terminal transmembrane domain (CTTM) and the C-terminal cytosolic domain of SR-B1 are required for its property to sense lipid movement and initiate intracellular signaling [17]. The discovery of the effects of a single point mutation in the SR-B1 CTTM domain, which represents the unique domain of SR-B1 able to interact with plasma membrane cholesterol [17,18], highlighted the importance of plasma membrane cholesterol for SR-B1-dependent lipid sensing and resulting signaling [15]. This mutated form of SR-B1, created by the replacement of the glutamine residue in position 445 of SR-B1 amino acid sequence by an alanine residue (SR-B1-Q445A), provoked a marked decrease in the capacity of SR-B1 to bind plasma membrane cholesterol, whereas the capacities of the receptor to bind HDL and to transport cholesterol were unmodified [15]. In intestinal epithelial cells and endothelial cells expressing SR-B1-Q445A, the addition of ligand (PPM and HDL respectively) failed to activate signaling and subsequent cellular events [15]. Moreover, it was shown that SR-B1-Q445A is not recruited in raft-like membrane domains of intestinal epithelial cells after a PPM supply, contrary to what is observed for wild-type SR-B1 [14,15]. The initiating cellular events involved in SR-B1-dependent lipid sensing remain unknown. However, plasma membrane cholesterol movement, through cyclodextrin treatment, is sufficient to reproduce, in intestinal epithelial cells and endothelial cells, all the signaling cascades and subcellular events in wild-type SR-B1-expressing cells while SR-B1-Q445A transfected cells are unresponsive [15]. Altogether these results emphasize the critical importance of plasma membrane cholesterol in SR-B1-dependent intracellular signaling, and they suggest that the initiating events in SR-B1-related lipid sensing take place at the plasma membrane and may involve cholesterol trafficking. Although it has been shown that SR-B1 reorganizes the free cholesterol pool in the plasma membrane [19–21], the modifications in the plasma membrane microenvironment that occur upon SR-B1 lipid sensing remain largely unknown.

The purpose of the present study was to analyze the early cellular events involved in intestinal epithelial SR-B1-dependent lipid sensing by analyzing cholesterol trafficking and the remodeling of raft-like membrane domains upon supply of lipid micelles.

2. Materials and methods

2.1. Cell culture and lipid micelle supply

Caco-2/TC7 cell line is a clonal population of the human colon carcinoma-derived Caco-2 cells, which reproduces to a high degree most of the morphological and functional characteristics of enterocytes [22,23]. These cells were stably transfected with constructs expressing mouse WT SR-B1 or SR-B1-Q445A as previously described [15]. The presence of SR-B1 at plasma membrane (Suppl. Fig. 1) was shown by FACS analysis (BD LSRII FACS system and FlowJo software) performed on unpermeabilized cells as described in [24], using an anti-SR-B1 antibody (NB400–113 Novus Biologicals, Lille, France) and an anti-rabbit antibody conjugated to phycoerythrin (111–116 − 144 Jackson ImmunoResearch Laboratories Inc. West Grove, PA, USA). As previously described [15], SR-B1-Q445A has a dominant negative effect on signaling triggered by endogenous SR-B1 in both endothelial and Caco-2/TC7 cells. Cells were tested for mycoplasma contamination and found clean. In all experiments, cells were cultured on filter supports for 3 weeks to obtain fully differentiated enterocyte-like cells. Lipid micelles (2 mM sodium taurocholate, 0.6 mM oleic acid, 0.2 mM lysophosphatidylcholine, 0.05 mM cholesterol, and 0.2 mM monoacylglycerol) were prepared in serum-free medium as previously described [14,15,25] and added to the upper compartment for the indicated times. When appropriate, lipid micelles were supplemented with 5 μM BODIPY-cholesterol (Avanti polar lipids, Alabaster, AL, USA) or with 5 μCi/ml [1,2-^{3}H(N)]-cholesterol (57,6 Ci/mmol, PerkinElmer, Villebon-sur-Yvette, France). Transfection of Caco-2/TC7 cells with control RNAi and SR-B1 RNAi were performed as previously described [26].

2.2. Cholesterol labelling, trafficking and distribution

The intracellular distribution of cholesterol was analyzed in pulse-chase experiments. Caco-2/TC7 cells were incubated with BODIPY-cholesterol-containing lipid micelles for 1 h hour, washed twice with warm phosphate-buffered saline (PBS), and then incubated with non-fluorescent lipid micelles for one more hour. At the end of the experiment cells were rinsed with cold PBS and prepared for confocal microscopy analysis.

The trafficking of cholesterol from the plasma membrane towards intracellular domains was analyzed as described previously [27]. Briefly, BODIPY-cholesterol was combined with 370 mM methyl- β-cyclodextrin (MβCD, Sigma, Saint-Quentin Fallavier, France) in a 100:1 M ratio (MβCD/BODIPY-chol). Caco-2/TC7 cells were incubated at their apical pole with MβCD/BODIPY-chol complex in serum-free medium for 1 min at 37 °C. The final concentration of MβCD and BODIPY-cholesterol were 0.185 mM and ~1 μM, respectively. Cells were rinsed three times in warm PBS and then incubated with serum-free medium for the indicated times and analyzed by confocal microscopy.

Free cholesterol was visualized by filipin staining. Caco-2/TC7 cells were incubated or not with 3 μg/ml U-18666A (Sigma, Saint-Quentin Fallavier, France) added in both compartments of the filter support for 18 h. Cells were then rinsed three times with PBS, fixed with 4% paraformaldehyde (PFA), and incubated with 0.05 mg/ml filipin (Sigma, Saint-Quentin Fallavier, France) in PBS/10%serum for 15 min. After extensive washing with PBS, cells were analyzed by confocal microscopy using a UV filter set.

2.3. Cholesterol efflux analysis

Caco-2/TC7 cells were cultured on filter supports and incubated