Activation of Acid Sphingomyelinase Drives Lysosomal Fusion to Membrane Lipid Raft Clusters in Coronary Endothelial Cells

Jun-Xiang Bao, Si Jin, Justin L Poklis, Min Xia, Christopher Brimson, Pin-Lan Li
Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

ABSTRACT

Fluorescence Resonance Energy Transfer (FRET). An acceptor bleaching protocol was employed to measure the FRET efficiency. After the pre-bleaching image was normally taken, the laser intensity at the excitation wavelength of the acceptor (568 nm) was increased from 50 to 98 to bleach the acceptor fluorescence. After the intensity of the excitation laser of the acceptor was adjusted down to 50 and the post-bleaching image was then taken. The FRET image was obtained by the subtract of the post-bleaching image from the pre-bleaching image (in blue). Before measuring the FITC fluorescence intensity in the pre-, post- and FRET image, the FRET efficiency was calculated through the following formula: E = (FITCpost - FITCpre)/FITCpost X 100%

METHODS

Results

Lipid rafts (LRs) has been reported to be able to cluster LDLR oxidase subunits in endothelial cell membrane to form a LRs-matrix signaling platform in response to death receptor activation which was related to the increased activity of acid sphingomyelinase (ASM), a lysosomal glycoprotein and may catalyze the degradation of membrane-bound sphingomyelin into phosphocholine and ceramide. The fusion of lysosomal vesicles has also been regarded as a participant during this LRs-platform formation process. However, the relationship among ASM, lysosomal fusion and LRs clustering is still far from being understood.

BACKGROUND

Lipid rafts consist of dynamic assemblies of cholesterol, lipids with unaturated acyl chains such as sphingolipids and glycoprophospholipids in the ectoplasmic leaflet of the membrane bilayer. Recent studies in our laboratory have indicated that lipid raft signaling platforms could act as a platform in endocytotic cell signaling events for death receptors (Hypertension 2006;53(7):1748-80 and Hypertension 2006;53(1):16-18).

RESULTS

However, it remains unknown how the ASM is involved in this lysosomal fusion and LRs-platform formation. Given that the fact that hydrolysis of sphingomyelin into ceramide by ASM may lead to the fusion of organelles, the present study was designed to examine whether ASM is first activated in response to stimuli and lead to ceramide production, which drives lysosomes to traffic and fuse to cell membrane and facilitate LRs clustering.

CONCLUSION

We further found that the LRs-platform formation was related to the increased activity of acid sphingomyelinase (ASM) and lysosomal vesicles fusion to the cell membrane (Arterioscler Thromb Vasc Biol 2008; 28: 2056-2062). As shown in Panel A, there was very low FRET in control cell. After stimulation with FasL, PI, Buty, and Ars, significant increase of fluorescence which was the results of lysosomal fusion to cell membrane. The quench and dequench experiments may serve as the direct evidence for lysosomal fusion induced by ASM activation.