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Controlled cholesterol efflux from the aortic smooth muscle cells triggers microheterogeneity of plasma membrane lipids and induces modification of the mitochondrial topology

C. Tukaj[1], E. Syta-Książek[2], A. Zauszkiewicz[1], K. Drózd[3], A. Figarski[3], J.H. Spodnik[4], M. Woźniak[3]

[1]*Department of Electron Microscopy, Medical University of Gdańsk, Poland*
[2]*Department of Physics and Biophysics, Medical University of Gdańsk, Poland*
[3]*Department of Medical Chemistry, Medical University of Gdańsk, Poland*
[4]*Department of Anatomy and Neuroscience, Medical University of Gdańsk, Poland*

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It is generally accepted that phospholipids of plasma membrane display lateral segregation into small microdomains commonly known as lipid rafts. Such lateral lipid organization is under the control of cholesterol. Cholesterol depletion evolved by methyl-β-cyclodextrin (MCD) has been found to induce further marked perturbation in lateral lipid organization, evidenced in the high field part of electron paramagnetic resonance spectra of plasma membranes labelled with a spectroscopic probe, namely 5-doxyl-stearic acid (5DOXS). Such perturbation of surface lipid topo-logy has been found to induce distinct changes in the mitochondrial morpho-logy, i.e. switch from filamentous form into small granular form. (Folia Morphol 2009; 68, 4: 244–246)

Key words: lipid raft, electron paramagnetic resonance, methyl-β-cyclodextrin, mitochondria

INTRODUCTION

Eukaryotic cell membranes are known to display lateral microheterogeneity caused by differences of acyl chain lengths in binary phospholipid mixtures as well as cholesterol. In mammalian cells, as much as 90% of all cholesterol is localized within the plasma membrane [4].

Cholesterol is now recognized as one of the most important constituents of membrane structural lipids modulating fluidity of biological membranes and responsible for the segregation of cell membrane sphingomyelin molecules into discrete lipid microdomains [2, 5, 7]. It is generally accepted that plasma membrane lipids and protein consist of a highly ordered assay of microdomenal topology [6]. Among different plasma membranes of eukaryotic cell origin, sarcolemma displays its individual periodic pattern of regional microdomenal organization owing to its functional force-transmitting demands [1]. It has been demonstrated that caveolae-like membrane organization topology can be disrupted in smooth muscle cells (SMCs) by cholesterol depletion using β-cyclodextrin [10]. Based on these observations, we tested controlled cholesterol efflux from SMCs using methyl-β-cyclodextrin (MCD), and inspected the topology of the plasma membrane by electron paramagnetic resonance (EPR) spectroscopy and mitochondrial tridimensional structure by confocal microscopy.

Address for correspondence: C Tukaj, Department of Electron Microscopy, Medical University of Gdańsk, Dębinki 1, 80–211 Gdańsk, Poland, tel: +48 58 349 15 00, e-mail: ctukaj@amedec.amg.gda.pl

MATERIAL AND METHODS

Cell culture

SMCs were obtained from the media of neonatal Wistar rat aortas according to the procedure of Tukaj et al. [9]

SMCs were examined from 5 to 9 days after inoculation when logarithmic growth in the primary culture was seen.

Electron paramagnetic resonance studies

Into Eppendorf tube 1 uL of 6.5 mM 5-doxyl-stearic acid (5DOXS) in 96% ethanol was pipetted and left for ethanol evaporation. SMCs from the primary culture were harvested by trypsinization, washed twice with PBS, and the cell count was adjusted to 10^7/mL. The cells were treated for 30 min with 2% methyl-β-cyclodextrin (MCD) in PBS or with PBS alone for control, pelleted, and washed two times with PBS. The treated and the control cells were transferred to tubes with dry 5DOXS and gently pipetted back and forth. The EPR spectrum of the cell suspension was scanned. All chemicals were obtained from Sigma-Aldrich, USA.

Immunofluorescence staining

The analysis of mitochondria of in vitro cultivated SMCs was performed employing a direct immunofluorescence method with Mito Tracker CMXRos (Molecular Probes, USA). Samples were examined by the confocal system Radiance 2100 (Bio-Rad, UK), equipped with a krypton/argon laser and mounted on a light microscope Eclipse 600 (Nikon, Japan). The confocal microscopy (CLSM) images were obtained using 60 × oil immersion objective lenses of N.A. = 1.4.

Plasma membrane cholesterol depletion

We depleted the cholesterol content of the SMCs by 30 minutes of incubation of the cells with 2% methyl-β-cyclodextrin.

RESULTS

The high field part of ESR spectra of plasma membrane labelled with 5DOXS revealed a marked movement of the minimum toward lower values of the magnetic field (Fig. 1). Such changes indicate marked perturbation of phospholipid organization upon cholesterol removal.

Unexpectedly, we noticed big impact of enhanced cholesterol efflux induced by (methyl-β-cyclodextrin) treatment on the structural integrity and shape of mitochondria inside the cells. Mitochondria have been found to change the typical elongated filamentous shape into fragmented and distinctly granular shapes (Fig. 2B). They were also shorter than the control (Fig. 2A). We observed an increased number of mitochondria in cytoplasm after methyl-β-cyclodextrin treatment.

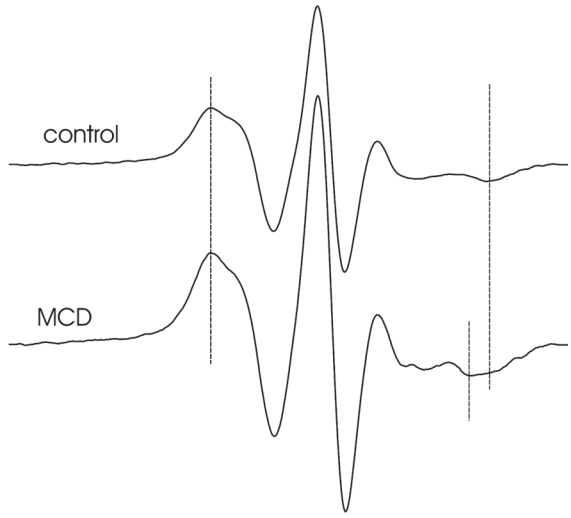

Figure 1. Electron paramagnetic resonance spectrum of liposoluble 5-doxyl-stearic acid in plasma membrane of native and 2% methyl-β-cyclodextrin (MCD) treated rat smooth muscle cells.

DISCUSSION

Lateral assemblies of sphingomyelin and cholesterol so-called lipid rafts are thought to display their own impact on important cellular processes like signal transduction, cell adhesion, and membrane sorting [3]. In contractile cells [1], T cells stimulated by peptide presenting antigen [11], as well as in neutrophils [8], specialized microdomains seems to be linked to actin cytoskeleton. Changes in the shape of smooth muscle cells during contraction relaxation cycles require subtle coordination of cytoskeletal and sarcolemmal rearrangement to protect the cell from mechanical damage.

Our results point to an important role of cell membrane cholesterol in preserving the integrity of sarcolemma as well as energy conserving organelle — mitochondria.

In addition to the crucial role of cholesterol-rich microdomains in spatial organization of SMC plasma membranes, the increase in the number of these organelles seems to be an adaptive response to stress conditions initiated by rapid efflux of cholesterol from cell membrane.

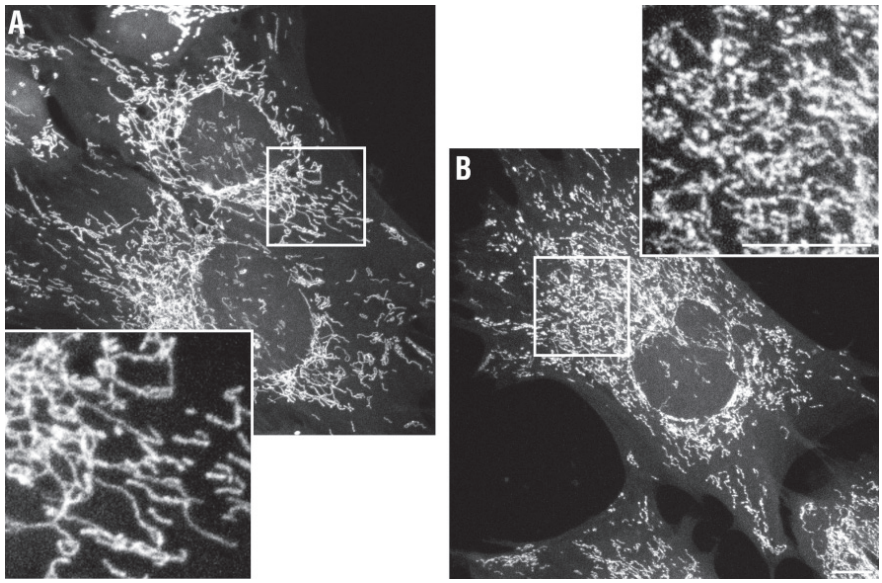

Figure 2. Distribution of active mitochondria detected by Mito Tracker CMXRos in (**A**) control smooth muscle cells and (**B**) cells treated with 2% methyl-β-cyclodextrin for 30 minutes. Scale bar: 10 μm.

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