Functional Roles of Cholesterol and Microdomains in the Plasma Membrane of HaCaT Keratinocytes

Dina Vind-Kezunovic
Department of Dermatology, University of Copenhagen, Bispebjerg Hospital, Bispebjerg bakke 23, DK-2400 Copenhagen NV, Denmark. E-mail: dinavind@hotmail.com

Dina Vind-Kezunovic, Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark defended her thesis on March 31, 2009 at Copenhagen University Hospital. Assessment Committee: Associate Professor Erik Michael Danielsen (Chairman), University of Copenhagen, Professor Lars E. French, Zürich University Hospital, Switzerland and Professor Berit Johansen, Norwegian intitute of Science and Technology, Trondheim. Supervisor: Robert Gniadecki, University of Copenhagen, Denmark.

Lipid membranes consisting of a mixture of phospholipids, sphingolipids and cholesterol may organize spontaneously under certain conditions into liquid-ordered (L_o) or liquid-disordered (L_d) microdomains. The L_o domains are enriched in cholesterol, which condenses the phospholipids and retards their diffusion. It has been proposed that L_o-like nanometer-size domains exist in plasma membranes of living cells (so-called lipid rafts). Since many membrane proteins have an affinity for lipid rafts, these domains are thought to play a critical role in the regulation of plasma membrane function in normal cells and in pathological conditions.

The aim of this PhD project was to investigate some of the aspects of the functional role of cholesterol and lipid raft-like microdomains in the keratinocyte cell line HaCaT. Initially, we aimed to visualize raft-like structures by confocal fluorescence microscopy employing known markers such as ganglioside GM_1, caveolins and flotillins, detected by fluorescein-isothiocyanate conjugated cholera toxin B subunit (CTB-FITC) and the specific antibodies. In HaCaT keratinocytes, CTB-FITC stained micrometre GM_1 assemblies (the CTB^{bright} domains), which were also enriched in caveolin 1 and 2 and, flotillin 1 and 2. The receptor for epidermal growth factor (EGFR) co-localized with the CTB^{bright} domains. With the technique of fluorescence recovery after photobleaching (FRAP), we confirmed that the lateral movement of EGFR was restricted within these domains. Since EGFR is the most important growth factor receptor in epidermal cells, we examined whether L_o-like domain disruption by cholesterol-depleting agents influenced its function. Indeed, methyl-β-cyclodextrin (MβCD) and filipin III caused a redistribution of EGFR from the CTB^{bright} domains to the surrounding membrane and a ligand-independent stimulation of the EGFR signalling.

On the basis of the above data, we hypothesized that the CTB^{bright} domains in the plasma membrane of HaCaT cells represented aggregates of lipid rafts. Unexpectedly, however, L_d markers Alexa Fluor (AF)-conjugated transferrin, which labels the transferrin receptor, and the lipophilic dye 1,1´-dioctadecyl-3,3,3´,3´-tetramethylindocarbocyanine perchlorate (DiI-$C_{18:0}$) were also enriched within the CTB^{bright} domains. Since the CTB^{bright} domains were mostly visible on the basal portion of the membrane where cells adhere to the glass, we speculated that they might represent one of the adhesive structures. Indeed, an adhesion protein α4β1 integrin spatially overlapped with the CTB-FITC and DiI-$C_{18:0}$, suggesting that CTB^{bright} L_o-like domains represented focal junctions. FRAP experiments further showed that the lateral mobilities of CTB-FITC, AF-transferrin and DiI-$C_{18:0}$ were diminished in CTB^{bright} domains, which led us to the development of a model of focal junctions as molecular sieves restricting the lateral mobility of the molecules which happen to migrate through them.

In the apical HaCaT keratinocyte plasma membrane stained with CTB-FITC and DiI-$C_{18:0}$, we observed spherical 1–2 µm budding vesicles which were predominantly enriched in DiI-$C_{18:0}$, whereas GM_1 was weakly stained. Cholesterol depletion with MβCD enhanced the vesiculation of DiI-$C_{18:0}$ and other selected DiI L_d markers, including short-tailed 1,1´-didodecyl-3,3,3´,3´-tetramethylindocarbocyanine perchlorate and di-unsaturated 1,1´-dilinoleyl-3,3,3´,3´-tetramethylindocarbocyanine perchlorate, suggesting that cholesterol plays a role in the control of exocytic vesiculation. It is conceivable that the line tension arising at the edge of the L_o/L_d boundaries could provide a force strong enough to cause membrane bending and vesicle formation. An analysis of the energetic requirements for vesiculation confirmed this hypothesis and revealed that a coalescence of smaller L_d-like domains at a micrometre scale makes it energetically more favourable for the vesicles to be composed of L_d-like lipids than of L_o-like lipids.

In conclusion, the studies described in this thesis shed new light on the organization of the HaCaT keratinocyte plasma membrane and point to the potential importance of the cholesterol and membrane microdomains in the regulation of diverse processes such as EGFR signalling, cell adhesion and vesicle formation. Membrane cholesterol can provide an interesting target for pharmacological manipulation of keratinocyte metabolism.