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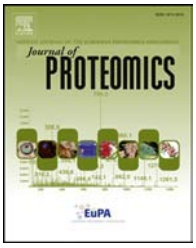

Localization and proteomic characterization of cholesterol-rich membrane microdomains in the inner ear

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ABSTRACT

Biological membranes organize and compartmentalize cell signaling into discrete microdomains, a process that often involves stable, cholesterol-rich platforms that facilitate protein–protein interactions. Polarized cells with distinct apical and basolateral cell processes rely on such compartmentalization to maintain proper function. In the cochlea, a variety of highly polarized sensory and non-sensory cells are responsible for the early stages of sound processing in the ear, yet little is known about the mechanisms that traffic and organize signaling complexes within these cells. We sought to determine the prevalence, localization, and protein composition of cholesterol-rich lipid microdomains in the cochlea. Lipid raft components, including the scaffolding protein caveolin and the ganglioside GM1, were found in sensory, neural, and glial cells. Mass spectrometry of detergent-resistant membrane (DRM) fractions revealed over 600 putative raft proteins associated with subcellular localization, trafficking, and metabolism. Among the DRM constituents were several proteins involved in human forms of deafness including those involved in ion homeostasis, such as the potassium channel KCNQ1, the co-transporter SLC12A2, and gap junction proteins GJA1 and GJB6. The presence of caveolin in the cochlea and the abundance of proteins in cholesterol-rich DRM suggest that lipid microdomains play a significant role in cochlear physiology.

Biological significance

Although mechanisms underlying cholesterol synthesis, homeostasis, and compartmentalization in the ear are poorly understood, there are several lines of evidence indicating that cholesterol is a key modulator of cochlear function. Depletion of cholesterol in mature sensory cells alters calcium signaling, changes excitability during development, and affects the biomechanical processes in outer hair cells that are responsible for hearing acuity. More recently, we have established that the cholesterol-modulator beta-cyclodextrin is capable of inducing significant and permanent hearing loss when delivered subcutaneously at high doses. We hypothesize that proteins involved in cochlear homeostasis and otopathology are partitioned into cholesterol-rich domains. The results of a large-scale proteomic analysis point to metabolic processes, scaffolding/trafficking, and ion homeostasis as particularly associated with

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cholesterol microdomains. These data offer insight into the proteins and protein families that may underlie cholesterol-mediated effects in sensory cell excitability and cyclodextrin ototoxicity.

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1. Introduction

The membrane lipid bilayer serves as a gatekeeper in cell physiology, segregating biological reactions to specific extracellular and intracellular spaces and serving as a hub of cell signaling. Fittingly, over 30% of the genes in most genomes are estimated to code for integral membrane proteins [1], and the proportion of proteins associated with the membrane is even greater when including those anchored by glycosylphosphatidylinositol (GPI) [2], fatty acid modifications [3,4], or other proteins. According to the fluid mosaic model, these membrane proteins diffuse freely within a fluid lipid bilayer unless compartmentalized by protein–protein interactions [5]. However, an accumulating amount of evidence suggests that lipid–protein and lipid–lipid interactions in the plasma membrane constrain the motion of membrane proteins and aid in their localization [6–8]. These observations and others gave rise to the concept of lipid rafts, discrete 10–200 nm lipid-ordered microdomains enriched with sphingomyelin, glycosphingolipids, GM1 ganglioside, and cholesterol [6,7,9–12]. The partitioning of specific proteins within these microdomains appears to be a primary means for compartmentalizing various processes in many, if not most, cell types. A large diversity in the structure and dynamics of lipid rafts [9,10,13] underlies their ability to organize a wide array of processes including protein and lipid trafficking, endocytosis, cellular recognition, and signaling [12].

The unique composition of lipid rafts renders them insoluble in micelles of non-ionic detergents. These so-called detergent-resistant membrane (DRM) fragments can be separated from soluble membrane by sucrose density gradient fractionation [9,14]. Although DRM fragments and in situ lipid rafts are not to be equated, the analysis of DRM composition traditionally is the first step in identification of putative raft components. Within the DRM, there are at least two domain types, those that incorporate the scaffolding protein caveolin and those that do not. Moreover, caveolin-based domains can be further classified into two subtypes based on whether the scaffold assembles into planar rafts or morphologically distinct membrane invaginations termed caveolae [15].

In hair cells in the inner ear, observations of caveola-like structures were reported decades ago [16,17], but little is known about the role of cholesterol-enriched microdomains in the inner ear. Hair cells contain diverse machinery, with mechanotransduction of sound energy occurring at the apical end, synaptic transmission occurring at the basolateral end, and a complex network of ion channels functioning together to shape excitability. The polarity of these cells, their role in signal transduction, and the interplay of these ion channels necessitates the localization and co-localization of membrane proteins to specific regions of the cell. Multiple observations including the presence of caveolae, inhomogeneous distribution of membrane cholesterol [18,19], and detection of extensive segregation of lipids in the membrane of the hair bundle [20] suggest that the lipid bilayer of these cells serves to modulate membrane protein distribution and functionality. While only caveolin and BK-type potassium channels have been biochemically identified in DRMs of cochlea [18], there is growing evidence that cholesterol-enriched microdomains may be involved in a wide range of processes in the inner ear, including sensory transduction [20] and cochlear mechanics [21–24]. Moreover, disruption of these microdomains with cholesterol-chelating cyclodextrins causes aberrant electrophysiological behavior [18,22,23,25,26], while systemic delivery of cyclodextrins causes profound hearing loss and outer hair cell death with no apparent effect on other systems [27].

To explore what processes may be affected by changes in membrane cholesterol distribution in the inner ear, and the mechanisms behind the inner ear's unique sensitivity, the localization and composition of cholesterol-enriched membrane microdomains were analyzed. The presence of raft-like domains is supported by localization of the raft-associated ganglioside GM1 and the raft-scaffolding protein caveolin to sensory and non-sensory cells in the ear. Additionally, over 600 proteins were found in triplicate preparations of cochlear DRM, and gene ontology analysis suggested DRM involvement in cell signaling, protein localization, and metabolism. Three gene products associated with syndromic hearing loss and eight gene products associated with non-syndromic hearing loss were also found in all three samples.

2. Methods

2.1. Tissue preparations

All animal procedures were conducted with the express approval of the University Animal Care and Use Committee at the University of Michigan. White Leghorn chickens (*Gallus gallus*), 18–28 days old, were anesthetized with a ketamine/xylazine solution and euthanized by decapitation. Whole auditory organs and microdissected subcompartments were collected essentially as described previously [28]. Pectoral skeletal muscle, proventricular smooth muscle, heart, lung, and brain (cerebellum) were dissected rapidly on ice.

2.2. Gene expression assays

Total RNA was extracted from basilar papilla (10–12 per sample) and auditory nerve (8–12 per sample) using an RNEasy Micro Kit (Qiagen), and from 50 to 200 mg samples of all other organ tissues using Trizol reagent (Invitrogen) after mechanical homogenization. RNA integrity was assessed using an Agilent 2100 Bioanalyzer. Only samples with a 28S:18S ratios greater than 1.0 were included in the analyses. First-strand cDNA was synthesized using Superscript III

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