GFP: A quantitative indicator of the amount of lipid rafts in yeast cell

Abstract

Lipid rafts (LR) are cholesterol-enriched micro domains of the cell membrane and possess a highly dynamic nature. Lipid rafts have been implicated in signaling pathways and in cancer progression. We emit the hypothesis that the amount of lipid rafts vary from healthy to cancerous cells and that certain conditions such as larger production of cholesterol may be the cause of cancer production. The aim is to analyze the binding property of GFP and cholesterol in Saccharomyces cerevisiae (Sc) cell membrane. Saccharomyces cerevisiae (Sc) cell is very good candidates for studying the lipid contents of their membrane. Finally, the aim of this review is to validate the possibility of studying lipid rafts to make a connection between lipid content of cell membrane with cause of cancer.

Keywords: lipid rafts, green fluorescent protein (GFP), saccharomyces cerevisiae (Sc)

Introduction

Plasma membranes are dynamic compartments with key functions in solute transport, cell shape, and communication between cells and environment. In mammalian cells and yeast the plasma membrane are compartmented into lipid rafts.³ The role of specific lipid domains composed of sterols and sphingolipids for cellular sorting and trafficking is well established.² These membrane microdomains are biochemically. It is hypothesized that the distinct lipid composition of lipid rafts creates a special environment that facilitates protein-protein interactions, protein recruitment in cellular trafficking events, and endocytosis and signaling.⁴ In a mammalian cell, lipid rafts have been shown to play a role in many different processes such as endocytosis via caveolae,⁵ virus budding or pathogen entry,¹ regulation of exocytosis,⁶ actin cytoskeleton organization.⁷ ⁸ Cholesterol dependent segregation of lipid raft proteins from non-raft proteins was visualized in mammalian cells and is consistent with the view of that raft domains in the plasma membrane of cells are usually small and highly dispersed, but their size can be modulated by oligomerization of raft components.⁹ Certain cues for cell growth, survival and other physiological processes are transmitted through lipid rafts.¹⁰ Lipid rafts are the sub-domains of the cell membrane enriched in cholesterol and glycosphingolipids. The structure and function of lipid raft domains depend on their lipid and protein compositions¹¹ as well as temporal stability.¹²

Discussion

The cholesterol concentration in detergent-resistant membranes (rafs) is 3-5 times higher than that in total membrane; sphingomyelin represents 10-15% of total lipid content, while glycosphingolipids such as cerebrosides and gangliosides account for further 10-20%. In contrast, glycerophospholipids comprise less than 30% of raft lipids despite accounting for approximately 60% of total membrane lipids.¹³ Cholesterol and sphingolipids, often found in rafts, are essential for the biological functions of raft embedded proteins. The rafts serve as relay stations in intracellular signaling and play a role in apical protein transport. They also ensure specificity and fidelity during signal transduction and are conceived as part of a mechanism for the intracellular trafficking of lipids and lipid anchored proteins.¹⁴ Alterations on membrane lipid composition have been reported in various disease conditions, including cancer and certain neurological and inflammatory conditions.¹⁵ Recent works suggests that amount of lipid rafts changes in unhealthy cells, particularly cancer cells.¹⁶ They contain unusually large concentration of cholesterol and other sterols (19). Raft disruption is seen due to cholesterol depletion,¹⁷ which proves the decreased raft levels due to decrease in cholesterol. Since cholesterol has been proved to be involved in signaling, it could be vital in formation of specialized membrane domains such as lipid rafts.¹⁸

Lipid rafts (LR)

Lipid rafts are special type lipid domains formed through preferential interactions between sphingolipids and certain type of sterols involved and which are involved in signal transduction and sorting of proteins and lipids.¹⁹ Lipid rafts are plasma membrane domains that are composed of cholesterol, glycosphingolipids, and GPI anchored protein.²⁰ Lipid rafts are low-density, detergent insoluble micro-domains of plasma membrane that are enriched in cholesterol and sphingolipids. They have been shown to contain various signaling related proteins and thus these micro-domains have been involved in many cellular functions, including the regulation of apoptosis and cell proliferation. Physiological functions of lipid rafts include membrane trafficking, cell polarization and signal transduction.

Membrane trafficking and cell polarity of lipid rafts

Membrane trafficking allows exchange of cellular components between cell sites and cellular organelles. In polarized epithelial cells the trafficking machinery is polarised, targeting plasma membrane proteins to separate apical and basolateral domains. Lipid rafts have been proposed to play a decisive role in apical trafficking, which is less understood. Attachment of GPI anchors may also contribute to the polarization of membrane trafficking.²² ²³

Abbreviations: SC, saccharomyces cerevisiae; GFP, green fluorescent protein; LR, lipid rafts

Keywords: lipid rafts, green fluorescent protein (GFP), saccharomyces cerevisiae (Sc)

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Cell signaling by lipid rafts

Lipid rafts have been implicated in a variety of cellular functions including signaling and growth regulation. Lipid raft-mediated trafficking of lipids and proteins facilitates dynamic regulation of cellular signaling cascades. The distribution of lipid rafts over the cell surface depends on the cell type. In polarized epithelial cells and neurons, lipid rafts accumulate in the apical and axonal plasma membrane respectively. The simplest interpretation views lipid rafts as platforms where signaling molecules are co-localized, aiding their structural interactions and influencing downstream signaling. The nature of a signal may be modified by the type of lipid raft, the target molecule is localized in and also the primary location of the raft, which in turn enhances the specificity of the signal. Rafts can also control cellular signaling by altering the function of their affiliated proteins. Some lipid rafts are actively involved in endocytosis, which promotes internalisation of receptors and signaling molecules. At the cellular level, the signaling events that regulate these defensive responses take place in membrane rafts-dynamic microdomains that are enriched in cholesterol and glycosphingolipids that facilitate many protein-protein and lipid-protein interactions at the cell surface.

Lipid rafts in pathogenesis of bacterial infection

Mechanisms of invading host cell through cell membrane to survive, and a wide range of pathogens has evolved replicate including parasites, viruses, and bacteria. Pathogens co-opt the endocytic properties of lipid rafts to enter into host cells. Clustered lipid raft plays an important physiological role in macromolecular transport and are believed to constitute alternate pathway of endocytosis to invade host cells. Lipid raft mediated uptake of pathogens may promote intracellular survival and dissemination within host cell as the pathogens endocytosis via lipid rafts does not generally fuse with traditional lysosomes and the contents are targeted to a range of different intracellular components.

Challenge of studying lipid rafts

Due to differential lipid-lipid interactions lipid domains in cell membrane shows lateral heterogeneity. Membrane bound proteins can also influence membrane lateral organization. Therefore, it becomes difficult to determine the underlying cause of membrane heterogeneity. To address this problem more general term membrane micro domain is often used which indicates the area of cell membrane that contains distinct protein and lipid composition. In mammalian cell apical and basolateral domains of polarized epithelial cells, which have different lipid and protein compositions made it difficult to study lipid rafts unlike yeast cell. Membranes are highly and rapidly adaptable entities, which are very difficult to study.

ABC transporter in cancer cells

ABC transporters are responsible for transport of Exogenous and endogenous substances across the membranes. Influencing pharmacokinetics steps and thus modifying distribution of substances within body and facilitate or limit access to organs and thereby protect against xenobiotics. Over expression of ABC transporters in cancer cell can limit the influx of cytotoxic drug and causes subsequent resistance of cancer cells toward a variety of structurally and functionally diverse cytotoxic drugs.

Cholesterol in cancer cells

Cholesterol and free fatty acids regulate the structure and function of cell membranes and many pathways of lipid metabolism. Maintenance of cholesterol levels is necessary for normal cell function. Cholesterol has also been involved in signaling, and that it is vital in formation of specialized membrane domains. Alteration in cholesterol contents of cell is considered to modify the properties of these domains. Several studies have demonstrated that depletion of cholesterol from the plasma membrane causes disruption of rafts and release of raft constituents into a non-raft membrane, which renders them non-functional. Cholesterol accumulation has also been reported in various solid tumors including prostate and oral cancer. In addition cholesterol metabolism is dys-regulated in many malignancies, including myeloid leukemia, lung and breast cancers.

Why Saccharomyces Cerevisiae

The budding yeast Saccharomyces Cerevisiae has been accepted as an important tool in study of protein interactions because of its powerful and genetic and molecular approaches, a completely sequenced genome and a collection of deletion strain. In particular, the yeast two-hybrid system has the major advantage that interactions are detected in an in vivo setting by the reconstitution of separated domains of a transcription factor, without requiring an in vitro handling of any protein molecules at all.

Florescence spectroscopy

As for labeling with fluorescent lipid analogues, washing after the incubation with such molecules is suggested to avoid high background noise. An important factor to take into account is also that the culture medium is in general much more complex than the solvent used for in vitro studies and some of its components can have strong absorption and/or emission at the wavelengths of interest (an illustrous example is phenol red), and in this case, it is necessary to change the medium prior to fluorescence measurements. Lipid bilayers are the common structural basis of all membranes in a cell, but they distribute differently not only within compartments but also within layers. Lipids carrying a chromophore represent the most “natural” method for labeling cells since they cause only a marginal disturbance of the membrane dynamics. Further, if the properties of the lipid consent its transport through the membrane by means of lipid transporters, such analogues may be suitable as well for a precise visualization of intracellular compartments.

Conclusion

Some studies suggest that cholesterol in lipid rafts is responsible for signaling and also cancer cells are found to have accumulated cholesterol. The main objective of this research is to analyze the alterations in lipid rafts in cell membrane due to incubation of Sc cell in Cholesterol, Green fluorescent protein, and 3:1 ratio mixture of cholesterol and Green fluorescent protein for different time period. Sc cell was incubated with cholesterol and Green fluorescent protein and the amount of absorbed and unabsorbed chemicals were analyzed by UV visible spectroscopy and fluorescent spectroscopy. Emission fluorescent spectroscopy helps the determine the amount of GFP bound in cell membrane and also helps to determine the unabsorbed amount of GFP in outside liquid of cell. Ultraviolet visible spectroscopy-scan reveals that at long incubation time chemicals get absorbed in cell membrane better. Cholesterol gets absorbed in cell membrane at higher incubation time. GFP get absorbed immediately after incubation. When Sc cell are incubated with the mixture of cholesterol and GFP, the absorbed...
chemicals remain constant over the time. The unabsorbed portion of chemicals remains high after long incubation time. The cholesterol amount represents the portion of cholesterol absorbed and native cholesterol of cell membrane.

Emission fluorescence spectroscopy determines the extents of binding of GFP in cell membrane. Extent of binding of GFP in cell membrane increases with increased incubation time. Though, maximum portion of GFP remain unabsorbed inside the cell membrane. All types of chemical get absorbed immediately after incubation and at long incubation the chemicals get detached from cell membrane. The chemicals form equilibrium between the absorbed and unabsorbed amount of chemicals.

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Conflict of interest

The author declares no conflict of interest.

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