Review

MOLECULAR METABOLISM

Microscopy tools for the investigation of intracellular lipid storage and dynamics

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ABSTRACT

Background: Excess storage of lipids in ectopic tissues, such as skeletal muscle, liver, and heart, seems to associate closely with metabolic abnormalities and cardiac disease. Intracellular lipid storage occurs in lipid droplets, which have gained attention as active organelles in cellular metabolism. Recent developments in high-resolution microscopy and microscopic spectroscopy have opened up new avenues to examine the physiology and biochemistry of intracellular lipids.

Scope of review: The aim of this review is to give an overview of recent technical advances in microscopy, and its application for the visualization, identification, and quantification of intracellular lipids, with special focus to lipid droplets. In addition, we attempt to summarize the probes currently available for the visualization of lipids.

Major conclusions: The continuous development of lipid probes in combination with the rapid development of microscopic techniques can provide new insights in the role and dynamics of intracellular lipids. Moreover, *in situ* identification of intracellular lipids is now possible and promises to add a new dimensionality to analysis of lipid biochemistry, and its relation to (patho)physiology.

Keywords Metabolic disease; Lipid droplets; Fluorescent lipid probes; Super-resolution; Live-cell imaging; Vibrational microscopy

1. INTRODUCTION

Lipids are vital to the cell and have key roles in multiple cellular processes. Lipids are structural components of cellular membranes, involved in cellular signaling, and serve as a critical energy source. In current society, where obesity is a global epidemic, partly because of the overconsumption of lipid-dense foods, the role of lipids in human health and disease has taken central stage in biomedical research. In obesity, an overflow of lipids in circulation can lead to excess lipid storage in ectopic tissues, such as skeletal muscle, liver, and heart. This ectopic fat storage is thought to contribute to the development of various metabolic disorders e.g. insulin resistance, non-alcoholic fatty liver disease, and heart failure [1].

More recently, however, a different view has emerged: augmented storage of lipids in peripheral tissues — despite its close association with metabolic and cardiac disorders — does not necessarily detrimentally affect cellular and tissue function. Not only is the amount of intracellular lipid potentially important for the development of metabolic disease but so is the way that lipids are handled and stored [2]. Intracellular lipid storage occurs in lipid droplets (LDs), which are classically viewed as energy depots but now considered to be active organelles in cellular metabolism. The shape and localization of the LDs appear to correlate with the development of metabolic disorders [3,4]. In addition to shape and size, proteins coating the LD affect LD dynamics as well as the composition of the lipids in the LD [2].

Lipidomic approaches to analyze lipids extracted from tissues have yielded novel information on global lipid composition from many tissues and under a variety of (patho)physiological conditions [5]. However, more detailed information at the individual LD level is warranted to examine the putative differential effects of localization, size, and composition of the LD on cellular function. Recent developments in (high-resolution) microscopy and microscopic spectroscopy open new avenues to examine intracellular lipids. The aim of this review is to give an overview of recent developments in microscopy with a focus on

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Abbreviations: BODIPY, Boron-dipyrromethene; CARS, coherent anti-stokes Raman scattering; CLSM, confocal laser scanning microscopy; CLEM, correlative light electron microscopy; DIC, differential interference microscopy; FA, fatty acid; FLIP, fluorescence loss in photobleaching; FRET, fluorescence resonance energy transfer; FRAP, fluorescent recovery after photobleaching; FIB-SEM, focused ion beam scanning electron microscopy; GFP, green fluorescent protein; HCV, hepatitis C virus; LD, lipid droplet; NBD, nitro-benzoxadiazolyl; PALM, photoactivation localization microscopy; SIMS, Secondary Ion Mass Spectrometry; SBEM, serial block face scanning electron microscopy; STED, stimulated emission depletion; SRS, Stimulated Raman Scattering; STORM, stochastic optical reconstruction microscopy; TOF-SIMS, time-of-flight SIMS; TEM, transmission electron microscopy; TAG, triacylglycerol; TPLSM, two-photon laser scanning microscopy

Received December 3, 2015 • Revision received December 19, 2015 • Accepted December 27, 2015 • Available online 31 December 2015

http://dx.doi.org/10.1016/j.molmet.2015.12.005

visualization, identification, and quantification of intracellular lipids. We will focus on the practical application of and the information gleaned from different microscopy tools. This review starts with description of probes currently available to visualize lipids and the dynamics of intracellular lipids. Thereafter, we focus on the available microscopy tools to study LD shape, localization, remodeling, and coating proteins. Finally, we discuss new possibilities in the field of microscopy to elucidate intracellular lipid composition in detail.

2. VISUALIZING LIPIDS

The vast majority of lipids do not possess intrinsic fluorescence. Hence a wide range of probes has been developed that allows visualization of intracellular lipids by microscopy. Many of these probes are suitable for live-cell imaging and therefore allow for detailed study of both the subcellular location of lipids and cellular lipid dynamics. The ongoing development of such probes and the rapid advances in super-resolution microscopy permitting imaging at resolution below the diffraction limit create a powerful combination to further study the dynamics of cellular lipids. Visualization of lipids can be performed via (i) direct labeling of lipids with a fluorophore, (ii) incorporation of (fluorescent) lipid-binding probes, and (iii) the introduction of fluorescent lipid analogs and dyes. The choice for the best probe is dependent not only on the experimental setting (static measurements on histological slices or fixed cells or live imaging on cells, tissues, or organs) but also on the type of microscopy used (given that certain microscopy techniques require probes with specific properties).

2.1. Fluorophore-conjugated lipids

When studying the intracellular localization and mobility of lipid species, covalent linking of a fluorophore to the lipid of interest can be performed. The fluorophore can be linked either to the polar hydrophilic head group, e.g. via polar rhodamine or cyanide dyes, or to the acyl chains or hydrophobic groups. Since large fluorophores like rhodamine and cyanide are not suited to link to acyl chains, frequently used fluorophores include boron-dipyrromethene (BODIPY) and nitrobenzoxadiazolyl (NBD) [6]. Inherent to fluorophore-conjugation is the concern that biophysical properties of the lipids might be altered and/or (yet unknown) functional groups of the lipids are modified or masked [7]. For example, labeling of the acyl chains of phospholipids with NBD can result in looping of the acyl tail to the surface of the lipid bilayer due the moderate polarity of NBD [8]. As a consequence, the intracellular motility and localization of these conjugated lipids can be affected [9–11]. This could be especially problematic for live-cell imaging but may also have implications in fixed samples, as lipids can retain mobility after fixation [12].

Probe development should aim for minimal functional interaction to allow for optimal investigation of native lipid dynamics. Therefore, lipids have been labeled with an alkyne or azide group, which are most often bound to the terminus of the acyl chains. The advantage of these chemical groups is their small size and bio-orthogonality, meaning that they do not occur in nature and are therefore biologically inert. These lipid probes can subsequently be visualized by 'clicking' a fluorophore to the alkyne or azide group via established chemical reactions. This can be done after the lipid probe has reached its cellular destination [13] (Figure 1). Clickable lipid probes have so far been developed and used for various fatty acids, cholesterol, and sphinganine [13]. Besides the clickable group, the lipid probe can have a small photoactivatable group grafted, e.g. in the alkyl chain, which can be used for the investigation of lipid–protein interactions. Activation of this photoactivatable moiety with UV light leads to covalent linking of the lipid to protein binding partners. Covalent cross-linking of the protein–lipid complex before clicking the fluorophore ensures that the fluorophore does not disturb the interaction of lipid and protein [13]. Lipid probes containing both a photoactivatable and clickable group are referred to as bifunctional lipids.

2.2. Lipid-binding probes

Lipid-binding probes comprise a group of fluorescently-tagged lipid-binding proteins, toxins, and (conjugated) antibodies. Lipid-binding proteins can especially be used for visualizing the location of lipids and lipid dynamics within living cells. Lipid-binding proteins are often labeled with fluorophores like green fluorescent protein (GFP) or its color variants. Fluorescently-tagged lipid-binding proteins can be directly expressed in the cells of interest, via plasmid transfection or genetic manipulation, or introduced into the cells. The latter requires permeabilization of the plasma membrane to allow entrance of the lipid-binding proteins. A classic example of fluorescently-tagged lipid-binding proteins is the use of the pleckstrin domains of phospholipase Cδ and Akt to visualize the phosphoinositides PI(2,4)P_2 and PI(3,4,5)P_3, respectively [14]

Several lipid-binding toxins can be used to visualize lipids in cellular lipid bilayers. Again, these molecules, or the lipid-binding domains thereof, can be tagged with fluorescent moieties. Lysenin and cholera toxin subunit B have been used to visualize sphingomyelin [15] and ganglioside GM1 [16], respectively. Pore-forming cytolysins specifically bind to sterols and are used to visualize cholesterol in lipid membranes [17]. It is important to note, however, that the use of the toxin-based lipid probes is mostly restricted to imaging fixed cells, given the cytolytic activity of these probes and that their toxic nature may hamper examination of physiological processes.

Another lipid-binding probe is filipin, which is an intrinsically fluorescent antibiotic that specifically binds to free cholesterol, but not to esterified cholesterol [18]. Because filipin can penetrate through the cell membrane, it can also be easily used for staining of intracellular cholesterol [7]. However, filipin cannot be used in live-cell imaging

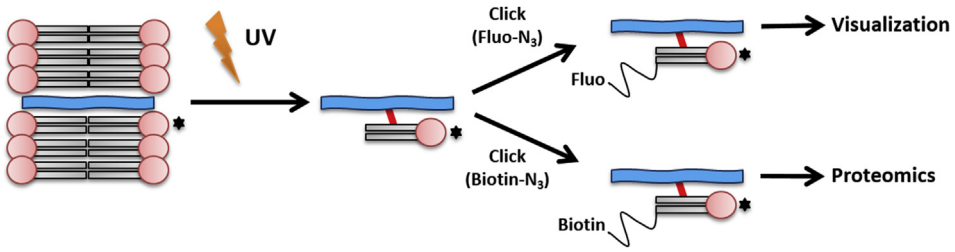

Figure 1: Example of a bifunctional lipid. Upon activation of a photoactivatable (clickable) fatty acid, the fatty acid can be turned into a variety of bifunctional phospholipids. These functional phospholipids can be linked to proteins e.g., by irradiation with ultraviolet light. Conjugation of the clickable group with reporter molecules (potentially of a wide range) subsequently facilitates imaging of the lipid bound proteins.

