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Review

Cholesterol ester droplets and steroidogenesis

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ABSTRACT

Intracellular lipid droplets (LDs) are dynamic organelles that contain a number of associated proteins including perilipin (Plin) and vimentin. Cholesteryl ester (CE)-rich LDs normally accumulate in steroidogenic cells and their mobilization is the preferred initial source of cholesterol for steroidogenesis. Plin1a, 1b and 5 were found to preferentially associate with triacylglycerol-rich LDs and Plin1c and Plin4 to associate with CE-rich LDs, but the biological significance of this remains unanswered. Vimentin null mice were found to have decreased ACTH-stimulated corticosterone levels, and decreased progesterone levels in females, but normal hCG-stimulated testosterone levels in males. Smaller LDs were seen in null cells. Lipoprotein cholesterol delivery to adrenals and ovary was normal, as was the expression of steroidogenic genes; however, the movement of cholesterol to mitochondria was reduced in vimentin null mice. These results suggest that vimentin is important in the maintenance of CE-rich LDs and in the movement of cholesterol for steroidogenesis.

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Contents

1. Introduction

When increased amounts of cholesterol accumulate within the ER, the integral ER membrane protein fatty acyl coA:cholesterol acyltransferase converts unesterified cholesterol to cholesteryl ester (CE) for storage as lipid droplets (LDs). The prevailing view of LD formation has been based primarily on analogy with triacylglycerol (TAG) LDs and can be interpreted to state that CE are synthesized within the ER and, as CEs accumulate and coalesce within the ER membrane bilayer, the LD buds off from the ER forming a nascent LD possessing a phospholipid monolayer on its surface within the cytosol (Walther and Farese, 2009), though continued physical communication between the ER and the LD is possible. During this process of nascent LD formation, several proteins, primarily of the perilipin family (Kimmel et al., 2010), associate with the LD either during budding from the ER or derived from a soluble pool within the cytosol. Evidence suggests that some perilipin family members such as Plin3 and Plin4 associate with and coat very small, nascent LDs; subsequently, they are replaced by Plin2 as

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LDs enlarge and finally by perilipin (Plin1) as LD size enlarges further (Wolins et al., 2005). Adrenal LDs containing CE are similarly known to be coated by Plin1 (Servetnick et al., 1995) and Plin2 (Fong et al., 2002). In addition to these dynamic changes in surface proteins associated with LDs, there is temporal and spatial movement of TAG-rich LDs within the cell as they enlarge both by accumulation of newly synthesized TAG and via fusion of cytosolic LDs (Nagayama et al., 2007) independent of TAG biosynthesis; this fusion appears to require microtubules and the motor protein dynein (Boström et al., 2005), as well as the soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) complex (Boström et al., 2007) and other related SNARE proteins. Whereas TAG-rich LDs tend to coalesce into large LDs, particularly in adipose cells where large LD formation is dependent on fat specific protein of 27 kDa (FSP27) (Nishino et al., 2008; Puri et al., 2007), CEs have a propensity to form multiple small LDs in adrenals and gonads (Reaven et al., 1995), although LD enlargement also occurs. Proteomic analyses of LDs from yeast, mouse mammary gland, Chinese hamster ovary cells, human hepatoma cells, human squamous epithelial carcinoma cells, mouse 3T3-L1 adipocytes, fly cells and leukocytes have revealed the existence of many LD-associated proteins including specific marker proteins, structural proteins, enzymes involved in various aspects of cholesterol and fatty acid metabolism, and proteins that function as regulators of membrane traffic (Athenstaedt et al., 1999; Wu et al., 2000; Liu et al., 2004; Fujimoto et al., 2004; Umlauf et al., 2004; Brasaemle et al., 2004; Miura et al., 2002; Paciga et al., 2003; Wolins et al., 2003; Bartz et al., 2007; Beller et al., 2006; Cermelli et al., 2006; Sato et al., 2006; Wan et al., 2007). In recent studies, functional genome-wide screens of *Drosophila* cells using RNA interference revealed that approximately 1.5% of the fly genome is involved in TAG LD formation and regulation (Guo et al., 2008; Beller et al., 2008). The genes identified as involved in LD homeostasis represent a wide variety of different and diverse cellular processes, including genes involved with TAG and phospholipid synthesis, the proteosome and spliceosome, vesicular transport machinery, translational machinery, and several cytoskeletal genes and motor proteins. While it seems reasonable to assume that there are substantial similarities, and perhaps even identity between many of the processes involved in the formation and behavior of predominantly TAG-containing and CE-containing LDs, the fact remains that there is very limited direct evidence proving this. Indeed, there are clearly differences between the synthesis of CE and TAG, as well as differences in the pathways in which TAG and CE enter cells and the cell types where TAG-rich and CE-rich LDs form under normal physiological conditions, with CE-rich LDs forming predominantly in the adrenal and ovary where the stored cholesterol is utilized as a primary source for steroidogenesis (Kraemer, 2007).

2. Differential protein expression of LDs

Investigators have only very recently begun to examine whether the proteomes of TAG-rich and CE-rich LDs differ. The first published study to address this issue specifically examined whether members of the perilipin family were differentially expressed in TAG-rich and CE-rich LDs (Hsieh et al., 2012). The Plin family consists of five distinct genes (designated 1–5), with Plin1 having four different splice variants (designated a–d) that are essentially C-terminal truncations of the full length Plin1a (Brasaemle, 2007).

2.1. Plin regulation by TAG or CE

To address this issue, the investigators (Hsieh et al., 2012) incubated Y1 adrenocortical cells with either fatty acids to enhance TAG-rich LDs or with cholesterol to enhance CE-rich LDs. An increased expression of Plin1a and Plin5 was observed with accumulation of TAG-rich LDs, and increased expression of Plin1c and Plin4 was observed with accumulation of CE-rich LDs. Expression of Plin2 and Plin3 did not differ whether cells accumulated TAG-rich or CE-rich LDs, and Plin1b and Plin1d were only weakly detected. This differential expression of Plin family members was observed with lipid loading of other cell types in culture and also when the Plin members were over-expressed in cells using a constitutive promoter.

2.2. Distinct LDs

Using specific fatty acid and cholesterol fluorescent probes to label LDs, the investigators observed relatively limited mixing of lipid species in LDs when loaded simultaneously with fatty acids and cholesterol and observed a distinctive separation of TAG-rich and CE-rich LDs in discrete LDs (Hsieh et al., 2012). Though in most cells studied the different LDs were intermingled, this separation was most prominent in cultured McArdle liver cells where TAG-rich and CE-rich LDs formed in completely separate subcellular locations within the cells. Fluorescent-activated cell sorting was used to separate the differentially fluorescently labeled LDs, and the expression of the different Plin species was examined. Plin1a, Plin1b and Plin5 were preferentially expressed on isolated TAG-rich LDs, whereas Plin1c and Plin4 were preferentially expressed on isolated CE-rich LDs. Plin1b, Plin2 and Plin3 did not display any distinct lipid preference. Ectopic expression of Plin members was found to alter TAG/CE cellular distribution; however, it is unclear whether inhibiting expression of individual Plin members would alter lipid composition in LDs since previous reports have shown that deletion of one Plin member results in the compensatory increase in other Plin members. Nonetheless, these studies document that TAG-rich and CE-rich LDs contain different Plin members and further suggest that each of the Plin members may have unique functions that reflect their differential LD expression; however, the biological significance as it relates to steroidogenesis remains as yet unexplored.

3. Vimentin

As opposed to Plin, vimentin, a protein identified to be associated with LDs, has been proposed for a number of years to play a role in steroidogenesis (Almahbobi and Hall, 1990). Vimentin is an intermediate filament that constitutes part of the network of the cytoskeleton (Fuchs and Weber, 1994). It is expressed in mesenchymal cells, including adrenal cells where it is attached to and forms a capsule around LDs (Almahbobi and Hall, 1990; Almahbobi et al., 1992; Hall, 1997), as opposed to the cage or scaffold it has been described to form around LDs in adipose cells (Franke et al., 1987), although it does not appear to surround all LDs. Vimentin has been reported to interact with a number of different proteins, including some with motor-like properties (Chou et al., 2007) and sterol binding properties (Wang et al., 2002; Wyles et al., 2007), as well as with agonist-stimulated ß$_3$-adrenergic receptors, where this interaction appears to be important for activation of ERK and stimulation of lipolysis (Kumar et al., 2007). Moreover, we recently reported that hormone sensitive lipase (HSL) can interact with vimentin and that the interaction affects lipolysis, as well as the translocation of HSL to the LD (Shen et al., 2010). Thus, recent studies examined the importance of vimentin in CE-rich LDs and the utilization of CE for steroidogenesis (Shen et al., 2012).

3.1. Steroid production in vivo

These studies were carried out in vimentin null mice, which are known to develop and reproduce normally (Colucci-Guyon et al.,