Experimental Cell Research ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Review Article

Lipid droplets and steroidogenic cells

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ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form
23 November 2015
Accepted 25 November 2015

Keywords:
Lipid droplet proteins
PLIN
Adrenal gland
Gonads
Steroid synthesizing enzymes
Steroidogenesis
Cholesteryl esters

ABSTRACT

Lipid droplets (LDs) in steroidogenic tissues have a cholesteryl ester (CE) core surrounded by a phospholipid monolayer that is coated with associated proteins. Compared with other tissues, they tend to be smaller in size and more numerous in numbers. These LDs are enriched with PLIN1c, PLIN2 and PLIN3. Both CIDE A and B are found in mouse ovary. Free cholesterol (FC) released upon hormone stimulation from LDs is the preferred source of cholesterol substrate for steroidogenesis, and HSL is the major neutral cholesterol esterase mediating the conversion of CEs to FC. Through the interaction of HSL with vimentin and StAR, FC is translocated to mitochondria for steroid hormone production. Proteomic analyses of LDs isolated from loaded primary ovarian granulosa cells, mouse MLTC-1 Leydig tumor cells and mouse testes revealed LD associated proteins that are actively involved in modulating lipid homeostasis along with a number of steroidogenic enzymes. Microscopy analysis confirmed the localization of many of these proteins to LDs. These studies broaden the role of LDs to include being a platform for functional steroidogenic enzyme activity or as a port for transferring steroidogenic enzymes and/or steroid intermediates, in addition to being a storage depot for CEs.

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1. Introduction

Intracellular lipid droplets (LDs) are dynamic organelles that are composed of a neutral lipid core, a surface phospholipid monolayer and proteins that are embedded in or bound to the phospholipid layer. They are found in nearly all types of eukaryotic cells and are involved in multiple intracellular processes, such as membrane trafficking, lipid metabolism and cell signaling [1]. Depending on the tissue in which the LD accumulates and the metabolic function of the tissue, the size and the lipid content of the LDs are different. Mature adipocytes contain LDs that can be

Abbreviations: CE, cholesteryl ester; CIDE, cell death-inducing DFFA-like effector; ER, endoplasmic reticulum; ERAD, ER-associated degradation; FC, free cholesterol; HSL, hormone sensitive lipase; LD, lipid droplet; MOM, mitochondrial outer membrane; SNARE, soluble NSF attachment protein receptor; StAR, steroidogenic acute regulatory protein; TAG, triacylglycerol; SRM, selected reaction monitoring; TMT, tandem mass tags; VDAC, voltage-dependent anion-selective channel

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http://dx.doi.org/10.1016/j.yexcr.2015.11.024
0014-4827/Published by Elsevier Inc.

> 50 μm in size and occupy the entire cell volume and function as a long-term energy storage depot [2]. The LDs in other cell types are usually much smaller than those in adipocytes and they are thought to be involved in the temporal storage and effective utilization of lipids. Although both triacylglycerol (TAG) and cholesteryl esters (CE) accumulate in LDs, they seem to form distinct LD particles in some cells; the compositions of LDs can vary greatly in different tissues. While the intracellular LDs in adipocytes, liver, and muscle cells consist primarily of TAG and diacylglycerol (DAG), the LDs in steroidogenic cells, such as ovarian granulosa and adrenocortical cells, as well as testicular Leydig cells, primarily accumulate CE [3–5]. Over the past decade, the employment of genetic and proteomic approaches have allowed the identification of many proteins on the LD surface and have provided clues to their function in cellular and LD physiology [6–10]. Here we aim at providing a review of the findings related to LD function in steroidogenic cells.

1.1. General properties of LDs in steroidogenic tissues

Steroid hormones are synthesized *de novo* from cholesterol in mitochondria and the ER, and are secreted from specialized endocrine cells in the adrenal cortex, testes and ovaries. Unlike cells that produce polypeptide hormones, which can store large amounts of mature hormones for rapid release, there is very little steroid hormone storage in steroidogenic cells. Therefore, upon stimulation, there is a rapid response in steroidogenic cells to synthesize new steroids [11,12], a process that requires a constant supply of cholesterol as a precursor for conversion to steroids. Within steroidogenic tissue, cholesterol is stored in LDs in the form of cholesterol esters (CEs), and the mobilization of these stored CEs is the preferred source of cholesterol for steroidogenesis upon hormone stimulation. There are generally numerous quantities of cytosolic CE-enriched LDs in steroidogenic cells, which usually are small with a diameter of 0.5–1.5 μm [13]. When loaded with fatty acids, steroidogenic cells can also form LDs composed of TAG. In a study using specific fatty acid and cholesterol fluorescent probes in mouse Y1 adrenocortical tumor cells, Hsieh et al. [14] observed relatively limited mixing of lipid species in LDs when loaded simultaneously with fatty acids and cholesterol and noticed a distinctive separation of TAG-rich and CE-rich LDs in discrete LDs.

1.2. LD associated proteins in steroidogenic tissues

Since the initial identification of perilipin (now named PLIN1), which coats the surface of LDs, numerous proteins have been shown, based on genetic as well as proteomic analyses, to associate with LDs. Moreover, the protein composition of LDs changes under different physiological conditions.

1.2.1. PLIN, CIDE and lipase protein families

Two particular families of proteins intimately associated with LDs are the PLIN and CIDE protein families.

PLIN1 was the first protein identified to be associated with LDs [15]. The family has since expanded to include not only perilipin (PLIN1), but also PLIN2 (ADRP), PLIN3 (TIP47), PLIN4 (S3-14) and PLIN5 (OXPAT/LSDP5) [16–19]. PLIN family members display different tissue distributions and different preferences to associate with TAG-enriched or CE-enriched LDs [14]. A single *Peri* gene encodes 4 different alternatively spliced variants of PLIN1 a-d; PLIN1a and PLIN1b are expressed both in adipose and steroidogenic tissues, while PLIN1c and PLIN1d are limited to steroidogenic tissues [20]. In white adipocytes, PLIN1 is highly expressed and active, modulating LD enlargement as well as LD lipolysis through interactions with FSP27, HSL and CGI-58 [21–24]. PLIN2 is suggested to interact with LDs within the monolayer or at the monolayer surface and may play a role in increasing LD membrane size during LD expansion [25,26]. Down-regulation of PLIN2 was shown to decrease lipid accumulation in liver and attenuate the growth of tumor cells [27,28]. In contrast, higher levels of PLIN2 have been suggested to be protective against lipotoxicity and insulin resistance in skeletal muscle [29]. Most recently, a missense polymorphism of PLIN2, Ser251Pro, was shown to promote cellular lipid accumulation, increase the number of small LDs, decrease lipolysis, and decrease plasma triglyceride concentrations. This is the first functional variant of PLIN2 that is associated with plasma VLDL and triglyceride levels [30]. PLIN3 is widely expressed in hepatocytes, enterocytes, and macrophages, as well as in testicular cells [14], and has unique dual functions: involvement in mannose 6-phosphate receptor recycling and in lipid biogenesis [31]. The newest member of the family, PLIN5, was shown to be involved in regulation of fatty acid oxidation in various tissues including adipose, heart, liver and muscle [32].

PLIN1c, PLIN2, as well as PLIN3, have all been shown to be present in steroidogenic tissues, and expression of PLIN1c and PLIN4 was shown to be enhanced by cellular cholesterol loading [14]. In a recent study from our group, PLIN2 was shown to be the predominant PLIN in granulosa cells (adipose differentiation-related protein, ADRP) [10], which confirms a previous finding of ADRP in primate granulosa cells [33].

CIDE (cell death-inducing DFF45-like effector) protein family (CIDEA, CIDEB and CIDEC, also known as FSP27) has been shown to reside on LDs and to be found in the endoplasmic reticulum (ER). CIDE-A was identified as a regulator of energy homeostasis and thermogenesis in brown adipose tissue [34], whereas CIDE-B controls lipogenesis and fatty acid oxidation in the liver [35]. CIDE C (FSP27) was shown to interact with PLIN1 through its CIDE-N domain, leading to increased lipid transfer activity [22]. Meanwhile, we have shown that FSP27 interacts with nuclear factor of activated T cells 5 (NFAT5) at the LD surface and modulates the cellular response to osmotic stress by preventing NFAT5 from translocating to the nucleus and activating its down-stream targets [36]. Both CIDE A and CIDE B have been shown to be expressed in mouse ovary [37].

It is worth noting that while PLIN1, PLIN2, CIDE A and CIDE B have all been detected in the mouse ovary, the expression of PLIN2 was distinct from that of the other three LD proteins. PLIN2 was co-localized with LDs in oocytes [37].

For the lipid content of LDs to be utilized, lipases need to act upon the stored TAG, CE or various retinyl esters to release FA and glycerol from TAG, FA and cholesterol from CE, and FA and retinol from retinyl esters. Proteomic analyses have shown that the lipases and lipase activators, which are responsible for the catabolism of stored LDs to be utilized, reside on LDs in steroidogenic tissue and include: ATGL, HSL, CGI-58, Tgh/Ces3, MgII, Ldah, and LMf2 [38]. ATGL hydrolyzes TAG and is the primary enzyme responsible for the breakdown of TAG to DAG [39]. HSL can hydrolyze DAG and CE, as well as retinyl esters [40]. CGI-58 does not have lipase activity by itself, and upon hormone stimulation acts as an activator of ATGL hydrolytic activity. Most recently, ATGL and CGI-58 were reported to possibly be involved in the mobilization of retinoids in hepatic stellate cells [41,42]. MgII only hydrolyzes monoacylglycerol [43]. Tgh/Ces3 can hydrolyze TAG and DAG and is involved in VLDL assembly and LD maturation [44].

1.2.2. Other LD proteins in steroidogenic tissue

Recently, in order to better understand the proteins found on CE-enriched LDs in steroidogenic cells, we compared the LD proteins isolated from CE-enriched LDs to proteins found on TAG-enriched LDs in steroidogenic cells using tandem mass tags (TMT) for protein identification and quantification [10]. For these studies,

Please cite this article as: W.-J. Shen, et al., Lipid droplets and steroidogenic cells, Exp Cell Res (2015), http://dx.doi.org/10.1016/j.yexcr.2015.11.024