综述与专论

The New Face of Lipid Droplets

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Abstract Lipid droplets (LDs) were originally described in milk by van Leeuwenhoeck in 1674. Although over 300 years have passed since this original observation, many fundamental questions about LDs remain unaddressed. Until recently, very few of the surface proteins had been characterized and the functions of LDs, aside from their presumed role as neutral lipid storage depots, remain unknown. To better understand LDs, we and others have recently carried out several proteomic studies using a combination of purified LDs and mass spectrometry (MS) protein identification. Many of the proteins which have been newly identified by this approach can be divided into two functional categories: lipid synthetic/metabolic enzymes and membrane trafficking proteins. Importantly, different laboratories investigating a variety of different cell types have found similar results. These findings strongly suggest that LDs are a complex, metabolically active organelle that may participate in lipid synthesis and trafficking. This review will describe progress in identifying these proteins and will also discuss the potential implications for the function of animal LDs. For detailed information on other aspects of LD biology, please read the review from Denis Murphy[1].

Key words lipid droplets, proteomics, enzymes, membrane trafficking, cell organelles

1 Lipid droplets

Lipid droplets (LDs) are also termed adiposomes, lipid storage droplets, lipid bodies, lipid particles, fat bodies, or oil bodies. They are an intracellular organelle with a neutral lipid core surrounded by a phospholipid monolayer and are coated with proteins. LDs have been found in many species including bacteria[1], yeast[2], plants[1], insects[3], and animals[3]. The coat proteins include several PAT (Perilipin, ADRP or Adipophilin, TIP47) family members. In animal cells, the core lipids consist of triglycerides (TG), cholesterol esters (CE), retinol esters, cholesterol (CL), and fatty acids. The neutral lipid composition varies dramatically from tissue to tissue. For example, triglyerides predominate in adipocytes and cholesterol esters are abundant in adrenal cells. The size of LDs also varies, ranging from 0.05 to 200 μm [1]. Adipocytes, liver cells, and steroidogenic cells contain prominent LDs while they are small and inconspicuous in other cell types. Lipid droplets are known to function in energy balance and cholesterol homeostasis. Additionally, they may also be the site for eicosanoid production as suggested by the localization of cyclooxygenase and phospholipase A_2 on LDs of leukocytes[4].

The mechanism of LD biogenesis has not been well characterized. One current hypothesis suggests that LDs are generated on the surface of the endoplasmic reticulum (ER) and matured in cytosol[1, 5]. Briefly, neutral lipids are synthesized in ER, then inserted and accumulated between the phospholipid

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Brief bibliography of Dr. Liu: Pingsheng Liu, Ph.D., assistant professor at UT Southwestern Medical Center at Dallas. He was born in Shanghai, obtained his BS from Guizhou University in 1982 and MS from The Chinese Academy of Light Industry Science in 1985. He went to Clarkson University to study chemical biology in 1987 and received his Ph.D. in 1993. After 3 years of postdoctoral training and 2 years of instructor experience, he was promoted to assistant professor in 1999 at UT Southwestern Medical Center at Dallas. He has worked in three fields including the regulation of T cell development, signal transduction in caveolae, and lipid homeostasis. Recently, he is focusing on the molecular mechanism of lipid droplet biogenesis and lipolysis, which may provide critical information for the treatment of many metabolic diseases including obesity, type Ⅱ diabetes, cardiovascular diseases, and neutral lipid storage diseases. His contribution to the biological field includes 27 papers in major journals such as Nature Cell Biology, EMBO J, J Cell Biol, J Biol Chem, and Proc Natl Acad Sci USA, organizing a molecular page for Nature, reviewing manuscripts for several journals, and training students and postdoctors.

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Received: March 16, 2005 Accepted: March 28, 2005

monolayers of ER membrane. After reaching a critical size and forming a spherical shape, the LD buds off from ER membrane into the cytosol. The further maturation of the LD may take place in cytosol by homotypic fusion to increase its size and by exchanging or acquiring protein content to obtain specific functions. However, LDs were also recently found to be generated on plasma membrane caveolae after cells were fed with free fatty acid[6]. Although all cells appear to have the ability to form LDs, only a few types of cells have been studied. Table 1 presents the summary of these studies. Among them, adipocytes are the cells that have been best characterized.

Table 1 Lipid droplets in different animal cell types

Cell type	Surface protein	Major lipids	Possible functions	Reference
Adipocytes	Perilipins/ADRP	TG	Storage	[7, 8]
Hepatocytes	ADRP	CE/TG	Metabolism/Storage	[9]
Macrophages	ADRP	CE/CL	Storage	[10]
Other leukocytes	ADRP	TG?	Eicosanoid generation	[4]
Adrenal cells	Perilipins	CE	Steroidogenesis	[11]
Mammary cells	ADRP	TG	Storage	[12]

2 The PAT family proteins and caveolins

Previous work has identified some major LD proteins including adipose differentiation related protein (ADRP in murine or adipophilin in human), perilipins, caveolins, tail-interacting protein of 47 ku (TIP47), S3-12, and p160/p200[13]. Based on common structural motifs and their N-terminal sequence similarity, Perilipin, ADRP, TIP47 and S3-12 are classified as the PAT family[14]. ADRP is a ubiquitously expressed 52 ku protein first cloned from differentiated mouse 1 246 adipocytes by Jiang and Serrero[7]. It has been suggested that ADRP is involved in LD formation and fatty acid uptake[15, 16].

In contrast to ADRP, perilipins are only highly expressed in adipocytes and steroidogenic cells. In adipocytes, perilipins can make up 0.25% ~0.5% of total cellular protein[17]. Alternative splicing of a single gene gives rise to A, B, and C isoforms of perilipin. Since perilipins are very abundant and the major surface protein of LDs in adipocytes, it has been suggested that their function is to protect LDs from lipolysis. Animal studies with knockout mice lacking the perilipin protein showed that these mice are very lean and contain about 62% less white adipocytes than wild type animals[18]. Upon hormonal lipolytic stimulation both perilipin and hormone-sensitive lipase (HSL) are phosphorylated by protein kinase A (PKA). Phosphorylation of HSL activates the enzyme while it has been suggested that phosphorylation of perilipin leads to a conformation change that allows HSL to access the lipid core[17].

The functions of the other two PAT family members, TIP47 and S3-12, in context of LD biology are still unknown. In contrast to ADRP and perilipins, TIP47 and S3-12 are only recruited to LDs under specific metabolic states [19,20]. Moreover, the localization of TIP47 on LDs remains controversial [20,21]. Caveolins [9,22~24] and p160/p200 [13] have also been recently localized on LDs. Caveolins are integral membrane proteins located mainly in plasma membrane caveolae [25]. It is unknown how caveolins are translocated to LDs. However, the cholesterol and fatty acid binding capacity of the caveolins suggests that they may play a role in transporting lipids between membranes and LDs. The lean phenotype and insulin resistance of caveolin-1 null mice also imply that caveolins might be involved in lipid metabolism and storage[26, 27].

3 New proteins revealed by recent proteomics

During the past 20 years, research into pathologies of lipid metabolism has linked aberrations of lipid storage with the development and progression of several common metabolic diseases including obesity, type Ⅱ diabetes and cardiovascular diseases such as atherosclerosis. Despite the important role of lipid metabolism in human diseases, LDs have

received little attention. As a result, our knowledge of the associations between this organelle and lipid homeostasis and related diseases is limited. However, this neglect has begun to be addressed by several recent proteomic studies carried out by our and other groups. The first LD proteomic analysis in animal tissues was done by Wu and colleagues [28]. In their work, all 19 proteins identified from LDs of mammary epithelial cells are also found on the milk fat globule membrane. Four proteins were identified from liver LDs and only two of them, ADRP and fatty acid binding protein, are LD-related proteins. These observations suggest some possible interactions between the LD and the plasma membrane of mammary epithelial cells. However, due to technical limitations this work could provide only incomplete information regarding LD protein composition.

Using a newly developed LD purification method and a combined MS protein identification and immunoblot approach, we identified nearly 40 proteins from purified LDs of Chinese hamster ovary K2 cell (CHO K2) [29]. These proteins are classified into five groups: (1) LD coating proteins such as ADRP and S3-12; (2) enzymes involved in the synthesis, storage and metabolism of lipids and sterols; (3) membrane trafficking proteins including Rabs; (4) signaling molecules such as Ral A and B; and (5) proteins of unknown function. In addition, one protein identified in this study, CGI-58, is linked to increased numbers and size of LDs in Chanarin-Dorfman syndrome, a neutral lipid storage disease [30, 31]. The truly novel and most surprising result is the localization of numerous Rab proteins to LDs. Rabs are small GTP binding proteins and key players in membrane trafficking, targeting, docking, and fusion [32, 33]. We further showed that the physiological properties of the Rabs on LDs are similar to those on other membranes. For example, treatment of LDs with RabGDI (a protein involved in extracting Rab proteins in their GDP bound form from membranes and complexing them in the cytosol [34]) in the presence of GDP removed Rabs from LDs while addition of GTP led to recruitment of Rab proteins from cytosol to LDs (unpublished results).

Since our report, three more LD proteomic papers have been published. Even though three different cell types were investigated, the results were very similar to our findings with regard to the abundance of lipid synthesis/metabolic enzymes and membrane trafficking proteins. A comparison of the proteins identified in these studies can be found in Table 2. Only proteins reported in two or more studies (or in one study and found in our unpublished results) are included. Just after our report, Fujimoto and coworkers isolated LDs from the liver hepatocyte cell line HuH7 and identified 17 major proteins [9]. The most intriguing result is that the protein pattern of the HuH 7 LDs in SDS-PAGE ([9], Figure 3) is almost identical to that of CHO K2 ([29], Figure 2). Of the 17 proteins identified by Fujimoto *et al.* [9], only 4 were not found in our study, including TIP47, acyl-CoA synthetase 3 (ACS3), 3β-hydroxysteroid dehydrogenase, and Rap 1B. Instead of ACS3, we found ACS4 on LDs. Moreover, we also found Rap 1 in our sample by Western blot (data not shown in our report).

In additional work, Fujimoto *et al.* [9] demonstrated the localization of two important enzymes, 17 β-hydroxysteroid dehydrogenase 11 (17βHSD11) and ACS3, on LDs by immunofluorescence. Furthermore, a number of lipid metabolic enzymes have also been found on yeast LDs by proteomic analysis and localization of GFP-tagged proteins [35,36]. Taken together, these results demonstrate certain lipid metabolic enzymes are LD residents, suggesting that the organelle is likely to be an active site for lipid synthesis.

Using similar methods, Umlauf and colleagues recently identified about 33 proteins from LDs of A431 cells, including several lipid metabolic enzymes and 5 Rabs (Rab1, 6A, 7, 10, and 18) among other membrane trafficking proteins [38]. This work not only strengthens the observation of several Rab proteins localized on LDs, it further supports a possible role for LDs in cellular membrane trafficking events. These findings were also confirmed by another intriguing study performed by Brasaemle *et al.* [37] Comparing the proteomic profiles of adipocytes under basal and lipolytic conditions, the authors observed that Rabs are significantly recruited to LDs in hormonally stimulated lipolytic adipocytes. This suggests that Rabs may recruit some proteins required for lipolysis and function as transporters for products of lipolysis.

Although the results of these studies are very similar, there are some significant differences as shown in Table 2. These discrepancies may be explained by different cell types, sensitivity of protein identification, and purity of samples, as well as the metabolic conditions of the cells (e.g. basal/lipolytic). Indeed, the localization of some of these proteins on LDs are still needed to be verified by additional methods.

Furthermore, it should be pointed out that none of these analyses are complete since in every case

Table 2 Direct comparison of four proteomic studies

		CHO K2[29]	3T3 L1 (lipolytic)[37]	Hepatocyte (HuH 7)[9]	A431[38]
LD proteins	ADRP	+	+	+	+
	Perilipin	−	+	−	−
	S3-12	+	+	−	−
	TIP47	−	+	+	+
Caveolae	Caveolin-1	WB	+	−	−
	Caveolin-2	WB	−	−	−
Lipid synthesis/ metabolism	Acyl-CoA synthetase long-chain family member 1	−	+	−	−
	Acyl-CoA synthetase long-chain family member 3	−	+	+	−
	Acyl-CoA synthetase long-chain family member 4	+	+	+	−
	Aldehyde dehydrogenase ALDH3B1	−	+	−	−
	Hormone-sensitive lipase	−	+	−	−
	Lanosterol synthase	+	+	+	+
	NAD(P)-dependent steroid dehydrogenase-like	−	+	−	+
	Short-chain dehydrogenase/ reductase member 1	+	+	−	−
	3β-hydroxysteroid dehydrogenase	−	−	+	−
	NADH-cytochrome B5 reductase (Diaphorase)	+	+	+	+
	Acetyl-CoA Carboxylase	+	−	−	+
Membrane trafficking	Rab1	+	−	−	+
	Rab2	+	−	−	−
	Rab5	+	+	+	−
	Rab6	−	−	−	+
	Rab7	+	+	−	+
	Rab10	+	−	−	+
	Rab11	+	−	−	−
	Rab14	+	+	−	−
	Rab18	+	+	−	+
	Sec22	+	−	−	−
	α-SNAP	+	−	−	−
Signal transduction	Rap1	−	−	+	−
	RalA	+	−	−	−
	Rho GAP	+	−	−	−
ER proteins	BIP	+	+	−	+
	Calnexin	−	+	−	−
	p63	−	−	+	−
Miscellaneous	CGI-49	−	−	+	−
	CGI-58	+	+	−	+
	Vimentin	+	+	−	−
	Stomatin	−	−	−	+

specific bands were excised from stained SDS-PAGE gels for MS protein identification. A comprehensive knowledge of LD protein composition, including less abundant molecules, is critical to uncover LD function. Therefore, intensive proteomic approaches are necessary. Additionally, novel approaches to protein identification may extend our understanding. For example, Soni *et al*. [39] performed a functional proteomic study and identified triglyceride hydrolase (TGH) in adipocyte LDs.

In conclusion, the identification of lipid synthetic enzymes and membrane trafficking proteins on LDs suggests that LDs are involved in lipid synthesis and may have a role in delivering those lipids to target membranes. Therefore, aside from their role in lipid storage, the LDs may be a metabolically active cellular organelle involved in lipid biogenesis and trafficking.

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对脂肪滴的新认识

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摘要 早在 1674 年，van Leeuwenhoeck 就首次在牛奶里发现了脂肪滴. 从那以后，300 多年过去了，有关脂肪滴的许多根本问题仍然没有得到解决. 迄今，除有为数不多的几个脂肪滴表面蛋白被发现外，人类对脂肪滴的认识仍停留在其作为中性脂贮存器上. 为了更好地认识脂肪滴，我们以及其他几个研究小组分别从不同细胞中纯化了脂肪滴，然后使用质谱蛋白分析对这些脂肪滴的蛋白质进行了蛋白质组学研究，从中发现了两组非常有意义的功能蛋白. 一组是与脂肪合成及代谢有关的酶，另一组则是与膜转运有关的蛋白质. 尽管这些实验使用了不同的细胞，而且是由不同实验室分别完成的，但结果却非常相似. 这些发现表明，脂肪滴有可能是一种具有生理代谢活性的非常复杂的细胞器. 同时，它有可能参与细胞内的脂肪合成、代谢及转运. 这篇综述将重点介绍近年来的脂肪滴蛋白质组学研究进展，以及由此推测的脂肪滴的生理功能. 如果读者希望了解脂肪滴的其他方面内容，请阅读 Denis Murphy 发表于 2001 年的一篇非常完整的综述.

关键词 脂肪滴，蛋白质组学，酶，膜转运，细胞器

学科分类号 Q54

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收稿日期: 2005-03-16, 接受日期: 2005-03-28