Review

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Seipin, adipogenesis and lipid droplets

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Seipin, the human Berardinelli–Seip congenital lipodystrophy 2 gene product, regulates adipocyte differentiation and lipid droplet (LD) formation. The molecular function of seipin, however, remains to be elucidated. Here we summarize recent advances in the investigation of congenital generalized lipodystrophies (CGLs) and the cellular dynamics of LDs. Increasing evidence suggests that phospholipids play a crucial role in some key forms of CGL and also in determining the size and distribution of LDs. We explore the hypothesis that seipin functions in the metabolism of phospholipids, and that seipin deficiency causes accumulation of lipid intermediates and/or alters membrane phospholipid profiles. These changes could lead to tissue-specific abnormalities upon seipin dysfunction, such as defective adipocyte development and clustered LDs in fibroblasts.

The role of seipin in lipid storage

The increasing prevalence of obesity and its related disorders in both developed and developing countries demands a better understanding of the molecular mechanisms underlying fat storage [1]. Storage neutral lipids, i.e. triacylglycerols (TAG) and sterol esters (SE), are stored in the form of lipid droplets (LDs) in almost all eukaryotic cells. LDs are dynamic subcellular organelles that not only govern the storage and turnover of lipids, but also function in membrane and lipid trafficking, protein storage and degradation, and even in the replication of hepatitis C virus [2]. All LDs comprise a core of storage neutral lipids which are wrapped by a monolayer of phospholipids with proteins embedded. LDs are believed to originate from the endoplasmic reticulum (ER), although the exact mechanism underlying their biogenesis remains to be determined.

At the whole-body level, animals develop a specialized tissue, the white adipose tissue, for efficient fat storage. This process involves the differentiation of mesenchymal stem cells to preadipocytes and then to mature adipocytes, which are characterized by the presence of a giant, unilocular lipid droplet that occupies over 90% of the cytoplasm [1,2]. Because obesity is characterized by the accumulation of mature adipocytes that are loaded with LDs, and by the appearance of prominent LDs in non-adipose tissues, it is important to elucidate the molecular mechanisms governing adipocyte differentiation (adipogenesis) as well as the cellular dynamics (biogenesis, size, distribution and interaction with other organelles) of the LDs.

Recent studies have found that seipin, the human Berardinelli–Seip congenital lipodystrophy 2 (*BSCL2*) gene product, appears to regulate both adipogenesis and cellular LD dynamics [3–8]. BSCL is an autosomal recessive disorder that is characterized by a near total loss of adipose tissue, severe insulin resistance, hypertriglyceridemia and fatty liver. One form of BSCL (BSCL2) is caused by loss-of-function mutations in the seipin/*BSCL2* gene. Interestingly, LDs of aberrant morphology have also been observed in seipin-deficient fibroblasts and yeast cells. Therefore, understanding the molecular function of seipin could provide important additional insights into adipogenesis and also into how the size and distribution of LDs are determined. This review aims to build upon recent advances in the investigations of LD dynamics, generalized lipodystrophies and seipin, and examines the hypothesis that altered phospholipid metabolism is the underlying cause of the tissue-specific abnormalities due to seipin deficiency.

Adipogenesis and PPARγ

Obesity is characterized by accumulation of fully-differentiated adipose cells (adipocytes) loaded with LDs. The excess of adipose tissue can be caused by both hypertrophy (increased lipid accumulation as LDs) and hyperplasia (increased proliferation and/or differentiation) of adipocytes [1]. Adipocytes are believed to derive from mesenchymal stem cells, and the differentiation of adipocytes can be divided into two phases [9]. In the determination phase, mesenchymal stem cells first develop into preadipocytes, which cannot be distinguished morphologically from progenitor cells but have lost the ability to differentiate into other cell types. In the terminal differentiation phase – from preadipocytes to mature adipocytes – adipogenic factors including insulin, cortisol and other unknown molecules initiate the adipogenic transcriptional cascade to activate two key regulators of terminal adipogenesis: PPARγ and C/EBPα [10]. Upon activation, PPARγ target genes promote lipid uptake (e.g. LPL, CD36, fatty acid transfer protein 1), glycerol uptake (aquaporin 7), and lipid storage (e.g. Fsp27, perilipin, S3-12) and inhibit lipolysis (e.g. GPR109, GPR81) [10]. It is also clear that a significant proportion of PPARγ target genes are coregulated by C/EBPα [11,12]. Of important medical relevance is the ligand-activated nuclear receptor PPARγ which was initially characterized as the master regulator for adipocyte differentiation and later recognized as a target of the thiazolidinedione (TZD) class of antidiabetic drugs [13,14]. Activation of PPARγ improves insulin sensitivity in mice and humans through better partitioning of lipid stores and the regulation of adipokines [10]. Not surprisingly, human subjects carrying dominant-negative mutations of PPARγ suffer from lipodystrophy and insulin resistance [15,16]. To date, many pro-adipogenic and

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anti-adipogenic pathways/factors have been identified that regulate the expression and activity of the core components of the adipogenic transcription cascade [9,10]. As an example, the sterol regulatory element binding protein 1c (SREBP1c) contributes to the production of PPARγ ligands [17]. Despite intensive studies on adipogenesis for the past 20 years, many fundamental questions remain. For instance, very little molecular information is available on how mesenchymal stem cells differentiate into preadipocytes. The endogenous agonists (presumably lipids) or possibly antagonists for the 'master' regulator of adipogenesis, PPARγ, have yet to be firmly established.

Seipin and congenital generalized lipodystrophy

Of particular relevance to adipogenesis is a rare human genetic disease: congenital generalized lipodystrophy (CGL, also known as BSCL and Berardinelli–Seip syndrome) [18]. Genome-wide linkage analyses have identified two loci for CGL: CGL type 1 [CGL1; OMIM (Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/omim) ID 608594] is caused by mutations in the 1-acylglycerol-3-phosphate-O-acyl transferase 2 (*AGPAT2*) gene and CGL2 (OMIM ID 269700) by mutations in the *BSCL2* gene which encodes seipin [19]. Recently, a homozygous nonsense mutation in caveolin-1 has been linked to CGL (CGL3; OMIM ID 612526) through a candidate-gene approach, and loss of cavin/polymerase I and transcript release factor (*PTRF*) can also cause CGL (CGL4; OMIM ID 613327) due to its effect on the caveolins [20–22]. Another well-known gene that is linked to severe lipodystrophy in mice is lipin-1 (*Lpin1*), which encodes a phosphatidate phosphatase [23,24]. AGPAT2, lipin-1 and caveolin-1/cavin have clear cellular functions: AGPAT2 catalyzes the formation of phosphatidic acid (PA) from 1-acylglycerol-3-phosphate (Figure 1); lipin-1 catalyzes the formation of diacylglycerol (DAG) by removing a phosphate group from PA (Figure 1); and caveolin-1/cavin is required for caveolae formation. By contrast, little is known about the molecular function of seipin, despite the fact that CGL2/BSCL2 patients suffer a more severe form of fat loss because both the mechanical and metabolically active adipose tissue depots are affected. By comparison, fat loss in CGL1 patients is restricted to metabolically active adipose tissue depots [18].

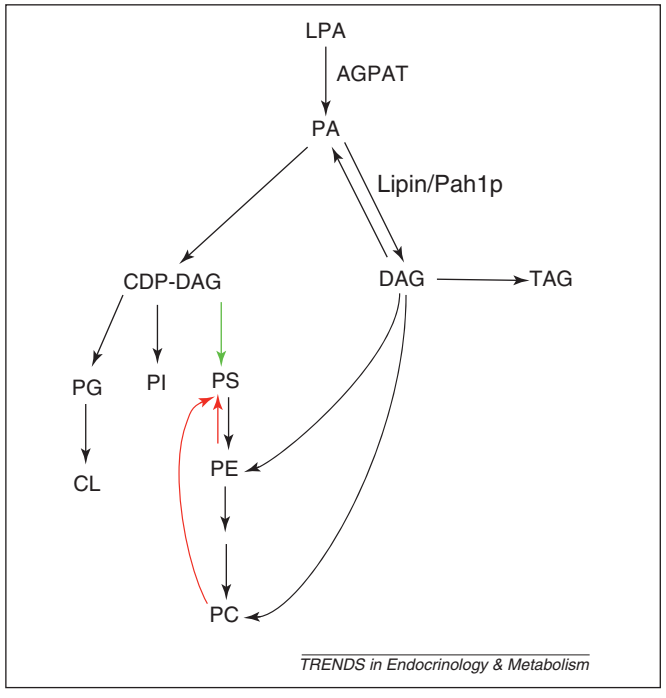

Figure 1. Pathways of glycerolipid biosynthesis. Pathways unique to mammals (red) and yeast (green) are marked. AGPAT: 1-acyl-sn-glycerol-3-phosphate acyltransferase; CL: cardiolipin; DAG: diacylglycerol; Lipin/Pah1p: mammalian (lipin) and yeast (Pah1p) phosphatidic acid phosphatase; LPA: lysophosphatidic acid; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol.

The human *BSCL2*/seipin gene has three transcripts: 1.6 kb, 1.8 kb and 2.2 kb [25]. The 1.8 kb mRNA is exclusively expressed in the brain and testis, whereas the other two transcripts are ubiquitously expressed [26]. It has been shown recently that seipin is highly expressed in adipose tissue and is strongly induced during adipocyte differentiation [5,6]. Using 3T3-L1 and C3H10T1/2 cells, Rochford and colleagues demonstrated that knocking-down seipin suppressed adipocyte differentiation and caused an early reduction in the expression of key genes in TAG synthesis, such as *SREBP-1c/SREBF1* [6]. Chan and colleagues showed that knocking down seipin in C3H10T1/2 cells did not affect bone morphogenetic protein-4-induced preadipocyte commitment. Instead, the differentiation of preadipocyte 3T3-L1 cells was greatly impaired by seipin knockdown, accompanied by an early suppression of PPARγ expression [5]. Interestingly, the defective differentiation due to seipin depletion can be reversed by addition of the PPARγ agonist, pioglitazone, suggesting that seipin functions upstream of PPARγ. These data confirm a crucial role of seipin in adipogenesis, but offer few clues to the molecular function of seipin and how seipin exerts its effects on the adipogenic program.

Seipin and the morphology of lipid droplets

LDs of diverse sizes have been observed in different tissues or within the same cell type under different (patho)physiological conditions [27]. A giant (up to 200 μm in diameter) unilocular LD often occupies the entire cytoplasm of white adipocytes, the energy reservoir of the body. By contrast, many much smaller LDs (usually less than 10 μm in diameter) are found in brown adipocytes, an energy-consumption organ. Lipid droplets are dynamic organelles whose number and size undergo constant changes in response to internal and external cues [28]. For example, liver cells usually contain only a few small lipid droplets; however, the size of liver LDs can increase dramatically in hepatic steatosis. Giant or 'supersized' LDs provide an efficient form of fat storage in terms of surface-to-volume ratio, and such increased lipid storage efficiency is necessary and beneficial under lipotoxic conditions and in certain cell types such as white adipocytes. By contrast, maintaining small LDs facilitates lipolysis by providing more surface area for lipases. In fact, it was recently reported that lipolysis is crucial for efficient cell-cycle progression, and therefore the size of LDs could also affect cell division [29–31]. However, the physiological significance of the differences in LD size has not been well recognized, and little is known about how the size difference is determined at the molecular level.