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Original research

Whole-genome RNAi screen identifies methylation-related genes influencing lipid metabolism in *Caenorhabditis elegans*

Xiaotong Zhu [a, b], Yangli Liu [a, b], Hong Zhang [a, b], Pingsheng Liu [a, b, *]

[a] *National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, China*

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ABSTRACT

Lipid droplets (LDs) are highly conserved multifunctional cellular organelles and aberrant lipid storage in LDs can lead to many metabolic diseases. However, the molecular mechanisms governing lipid dynamic changes remain elusive, and the high-throughput screen of genes influencing LD morphology was limited by lacking specific LD marker proteins in the powerful genetic tool *Caenorhabditis elegans*. In this study, we established a new method to conduct whole-genome RNAi screen using LD resident protein DHS-3 as a LD marker, and identified 78 genes involved in significant LD morphologic changes. Among them, *mthf-1*, as well as a series of methylation-related genes, was found dramatically influencing lipid metabolism. SREBP-1 and SCD1 homologs in *C. elegans* were involved in the lipid metabolic change of *mthf-1*(RNAi) worms, and the regulation of ATGL-1 also contributed to it by decreasing triacylglycerol (TAG) hydrolysis. Overall, this study not only identified important genes involved in LD dynamics, but also provided a new tool for LD study using *C. elegans*, with implications for the study of lipid metabolic diseases.

1. Introduction

The ability to store energy rich in condensed hydrocarbons provides a distinct survival benefit and is highly conserved from bacteria to humans. However, disorders of lipid metabolism and storage are major contributors to the etiology of a large number of metabolic diseases such as obesity, atherosclerosis, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) (Greenberg et al., 2011; Krahmer et al., 2013). At present, the molecular mechanisms underlying the pathogeneses of these complicated diseases remain unclear, bringing about a prosperity in research activities on lipid metabolism and storage in the past decade.

Excess lipids can interfere with cellular metabolism and must be stored in inert forms to prevent lipid toxicity. Neutral lipids, such as triacylglycerol (TAG) and sterol esters, are extremely hydrophobic and packed efficiently by excluding water (Brasaemle and Wolins, 2012; Walther and Farese, 2012). In cells, neutral lipids mainly storage in the lipid droplet (LD), an organelle that contains a neutral lipid core composed of TAG, cholesterol ester, and ether lipids covered with a monolayer phospholipid membrane with associated proteins (Bartz et al., 2007; Thiele and Spandl, 2008; Fujimoto and Parton, 2011). Great progress of research on LDs has been made in the last two decades, including the identification of the proteomes of the organelle from many organisms. The LD-associated proteins can be categorized as LD resident/structure-like proteins, lipid synthetic and metabolic proteins, membrane traffic proteins, and cell signaling proteins (Liu et al., 2004). Remarkably, LDs and their associated proteins can be found from bacteria to humans, suggesting that LD is a conserved organelle of great importance (Murphy, 2012; Yang et al., 2012; Chen et al., 2014). On the other hand, LDs have been observed to closely associate with the endoplasmic reticulum (Martin et al., 2005; Ozeki et al., 2005), mitochondria (Pu et al., 2011), peroxisomes (Binns et al., 2006), early endosomes (Liu et al., 2007), and other cellular organelles (Zehmer et al., 2009), which implies that LDs are possibly involved in regulating intracellular trafficking and energy metabolism.

* Corresponding author. National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.
E-mail address: pliu@ibp.ac.cn (P. Liu).

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LD is an indispensable organelle in most cells and its connection to human metabolic disorders makes LD a great entry point in studying related diseases (Martin and Parton, 2006; Farese and Walther, 2009). Hence, the identification of genes regulating the dynamic changes in LDs could provide a strategy in deciphering the pathogenic mechanisms underlying lipid metabolism-related diseases. Many model systems including mice, mammalian cells, and *Drosophila*, have been used to study LDs. Mammalian model systems are often studied using biochemistry, molecular biology and cell biology, but are not easy to genetically manipulate for screening purposes. Genetic screen can be performed in *Drosophila* efficiently, but the LDs and fat content are difficult to visualize and quantify through the body of the fly (Al-Anzi and Zinn, 2011).

Caenorhabditis elegans is a highly genetically tractable organism with a short growth period, rapid propagation, and low consumption. The LDs in *C. elegans* are easy to observe and, importantly, its genome shares high homology with that of humans (Kamath and Ahringer, 2003). Further, *C. elegans* is a predominant genetic model in the linkage between energy homeostasis, lifespan, growth, and reproduction (Kenyon et al., 1993; McKay et al., 2003; Brock et al., 2007; Wang et al., 2008; Watts, 2009; Mak, 2012).

In order to make better use of this genetic model, fluorescent protein-tagged markers have been used widely in studying *C. elegans* subcellular structures like mitochondria and lysosomes (Boulin et al., 2006). Similarly, such fluorescent protein-tagged markers are also needed to label LDs in live worms to observe morphology and other dynamic changes in LDs more efficiently and accurately. In addition, fluorescent protein-tagged markers can overcome the limits of traditional techniques for observing LDs in *C. elegans*. For example, Nile red, BODIPY, and LipidTox staining can precisely label LDs only when worms are fixed (Na et al., 2015), while fixation may induce non-specific fluorescence background and easily damage the native LD morphology (Yen et al., 2010). In addition, the fluorescent protein-tagged LDs require no processing, simplifying the logistics of high-throughput screen. Recently, our laboratory has isolated the *C. elegans* LDs for proteomic analysis, resulting in the identification of several LD-associated proteins, such as DHS-3, MDT-28, and F22F7.1 (Zhang et al., 2012), which have been verified as LD residents (Na et al., 2015). Based on this finding, we generated fluorescent protein-tagged versions of these markers to facilitate a genetic screen for genes influencing fat storage in *C. elegans*.

Based on these tools, for the first time, whole-genome RNAi screen becomes possible using resident protein specifically locating on *C. elegans* LDs. We chose one LD resident protein, DHS-3, to conduct whole-genome RNAi screen in *C. elegans*. Through this approach, 78 genes were identified to have powerful influences on LD morphology. Compared with other RNAi screens in identifying genes that regulate LD morphological changes, our method, using a fluorescent protein-tagged marker from a *C. elegans* LD resident protein, allows whole-genome RNAi screen with direct visualization of LDs in living worms without fixation and LD marker staining.

Among genes identified from our screen, the one-carbon metabolism pathway-related genes (*mthf-1*, *metr-1* and *mel-32*) were identified to influence lipid storage and metabolism. This study not only identified important genes involved in LD dynamics, but also provided a new tool for LD study using *C. elegans*, with potential correlation to lipid metabolic diseases in humans.

2. Results

2.1. Whole-genome RNAi screen for LD dynamics using DHS-3::GFP as a marker

Previously we had identified DHS-3 as a LD resident protein in *C. elegans*, and constructed *dhs-3::GFP* transgenic worms for direct visualization of LD in live organisms (Zhang et al., 2012). To identify genes that regulate LD dynamics, we performed a whole-genome RNAi screen in *C. elegans* with a *dhs-3* single copy strain (*Pvha-6::dhs-3::GFP*) as a LD reporter. In addition, *rrf-3*, encoding an RNA-directed RNA polymerase homolog that inhibits somatic RNAi, was introduced into this strain to enhance the efficiency of RNAi. LDs in L4 stage worms of F_1 progenies were examined using fluorescence microscopy. *L4440* RNAi and *dhs-3* RNAi were used as negative and positive controls, respectively (Fig. 1A). The screen results from the *Pvha-6::dhs-3::GFP; rrf-3* transgenic worms were verified in worms with the LD resident protein MDT-28 as a fluorescent marker (MDT-28::mCherry) (Na et al., 2015) (Fig. 1A, e), as well as by Nile red staining on fixed worms (Fig. 1A, f).

Out of 16,749 genes screened by RNAi, we identified 78 genes that significantly affected the LD phenotype. After analyzing all screen results, we categorized the identified genes into 4 groups based on the LD phenotypes of their knockdowns: large size (32 genes), aggregation (22 genes), less number (24 genes), and small size (10 genes) (Fig. 1B). These results were verified respectively using MDT-28::mCherry, fixed Nile red staining, and fixed Oil red O staining methods (Fig. 1C).

The genes could be divided into 12 functional groups: lipid metabolism, mitochondrion, peroxisome assembly factor, other metabolism, trafficking and transport, histone, heat-shock protein, transcription, signal transduction, ribosome, molting, and unknown (Fig. 1D). Nearly half of these genes belong to the groups of mitochondrion, peroxisome assembly factor, and other metabolism. Of these, 8 genes are directly related to lipid metabolism and related functions, and 7 genes had not been reported before. Information related to all 78 genes is listed in Table S1.

These RNAi screen results were in agreement with previously published results on the regulation of *C. elegans* LDs. For example, *dhs-28* is a gene encoding an ortholog of human 17-β-hydroxysteroid dehydrogenase 4 (HSD17B4), the down-regulation of which is known to induce large LDs in *C. elegans* (Zhang et al., 2010). Our RNAi screen with the *Pvha-6::dhs-3::GFP; rrf-3* strain also confirmed this finding (Fig. S1B) and *dhs-28*(RNAi) was subsequently used as a positive control phenotype in our study. In addition, the gene *let-767* encodes a steroid dehydrogenase that is an ortholog to human 17-β-hydroxysteroid dehydrogenase 3 (HSD17B3). A previous study found that RNAi knockdown of this gene reduced fat content in *C. elegans*, which is consistent with our results (Fig. S1C) (Entchev et al., 2008). Moreover, knockdown of *C41D11.4* or *F52H2.6* dramatically induced LD aggregation, and this phenotype was also confirmed by our forward genetic screen using *Pvha-6::dhs-3::GFP* (Fig. S1D and E). Interestingly, mitochondrion- and peroxisome-related genes accounted for a large proportion in our results (Fig. S2; Tables S2 and S3). This observation demonstrates a strong connection between LDs and these organelles, suggesting that both organelles participate greatly in lipid metabolism processes in *C. elegans*.

2.2. mthf-1 RNAi induces large LD formation and enhances TAG accumulation

From a collection of RNAi screen candidates, we identified a gene named *mthf-1* whose RNAi phenotype showed dramatically enlarged LDs (Fig. 2A, a1, a2). Based on sequence comparison, *mthf-1* encodes a protein that is an ortholog to the human methylenetetrahydrofolate reductase (MTHFR), which catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF) in the folate cycle. 5-MTHF is a co-substrate for homocysteine re-methylation to methionine in the methionine cycle of human one-carbon metabolism pathway

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