New tenants of the lipid droplet: MLX transcription factors

preview for "Mejhert et al. (2020) Partitioning of MLX-Family Transcription Factors to Lipid Droplets Regulates Metabolic Gene Expression. Molecular Cell, 77, 1251-1264."

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Introduction

Lipids are fundamental building blocks for biological membranes, reservoir for energy storage, and also signaling mediators. Therefore, the proper regulation of lipid storage and metabolism is directly related to cellular and organismal homeostasis. Lipids are preserved within the cells in the form of neutral lipids, such as triacylglycerides and sterol esters, and deposited in specialized organelles named lipid droplets (LDs). Universally found in almost all eukaryotes and some prokaryotes, lipid droplets are composed of a core of neutral lipids surrounded by a phospholipid monolayer membrane embedded with a variety of proteins. Dysregulation of lipid storage in LDs is central to the development of prevalent metabolic diseases, such as obesity and diabetes. The sequestration of lipids in lipid droplets also helps cells and tissues against lipotoxicity by mitigating overload of excess cholesterol as cholesterol esters (CEs) and fatty acid intermediates as triacylglycerol (TG). In addition, the role LDs is beyond lipid storage, because they are also dynamic structures that constantly interact with other organelles as endoplasmic reticulum (ER), peroxisome, mitochondria and lysosome, thus acting as vital role in modulating cellular metabolism.

Despite the progress in the biology of LDs, the basic mystery about cellular metabolic pathway adapt to and coordinate with lipid storage in LDs remains enigmatic. Among cell types, macrophages have been shown to be important in detoxifying lipid overload and harbor either CE- or TE-rich LDs.(Abdolmaleki et al., 2019; den Brok et al., 2018) In this study, Mejhert *et al.* conduct a systematic investigation of key regulators of lipid storage in human macrophages. With the combination of genome-wide RNAi and high throughput image processing, the authors identified ~550 genes as regulators of lipid storage in human macrophages. More intriguingly, MLX, a basic helix-loop-helix leucine-zipper transcription factor that regulates metabolic processes, and family members are enriched in the hits (Hunt et al., 2015; Ma et al., 2006). Further study shows the transcriptional activities of MLX are

regulated by binding to LD surfaces, which establish the correlation of the expression of metabolic genes with lipid storage.

Results

Human macrophages are important to the pathogenesis of metabolic diseases, and they store a variety of lipids. Thus, the authors built up a platform for systemically screening of human THP-1 macrophages, which contain TGs and CEs LDs when incubated with acetylated apolipo-protein B-containing lipoproteins (ac-Lipo). They developed an imaging strategy to measure LDs with BODIPY staining in cells, and validated the system by using RNAi to deplete genes that are known to affect lipid storage. They screened around 18,000 genes to find determinants of lipid storage, and identified 558 hits with altered LDs by 21 high-confidence image parameters. In the screen, they found many unknown genes that regulate lipid storage in LDs, and provided a comprehensive set for human macrophages. To begin analyzing the results of the screen, they categorized the hits into six major phenotypic classes based on similarity scores, from class1, small LDs, to class 6, large LDs. To identify genes that are directly involved in LD biology, they determined the genes encoding proteins that are enriched at LDs. They purified LD fractions from THP-1 cells and analyzed the total proteome and the LD-enriched fraction using high resolution mass-spectrometry-based proteomics. They identified 75 candidates by setting a threshold of 99% confidence for LD enrichment. Four proteins among the 75 candidates were associated with an LD phenotype in their RNAi screen. They then focus on the one of the proteins, MLX, because it was unexpected to be an LD protein.

MLX is a transcription factor, which forms a complex containing glucose-sensing domains, controlling the expression of multiple target genes upon glucose stimulation. MLX localizes to LDs not only in THP-1 cells, but also in other cell types. Interestingly, after oleate treating, MLX mRNA levels did not change, but cellular MLX protein levels are increased, which might be due to stabilization of the protein by binding to LDs. MLX targeted LDs using its 177-244 amino acids in C-terminal, which was verified by the truncated expression in SUM159 cells. The LD-targeting sequence of MLX contains potential amphipathic helices. The mutation in second helices impaired or abolished the binding, which showed that the C-terminal amphipathic helix of MLX is required for its binding to LDs. MLX, MLXIP, and MLXIPL shared similar sequence in C-terminal regions. The authors proved that they each have the capacity to target LDs independently of each

other without heterocomplex formation. Next, they found that MLX binding to LDs affects its transcriptional activity. The concentration of MLX is lower in the nucleus and higher on the LDs in the LDs presence. However, the regulation of MLX, MLXIP, and MLXIPL transcriptional activity does not depend only on cellular localization. RNA sequencing analyses showed that mRNA encoding the thioredoxin-interacting protein (TXNIP) increased 6-fold after the shift from glucose-free to glucose-containing medium. *TXNIP* is known as an MLX:MLXIP target gene. Re-expressing MLX in MLX knockout cells restored the glucose-dependent induction of *TXNIP* mRNA expression. To understand how LD binding impacts MLX:MLXIP transcriptional activity, the authors assayed for the transcriptional response to glucose in the presence or absence of LDs. Cells with abundant LDs has less MLX:MLXIP target expression. ChIP and qPCR results showed that cells depleted of LDs increased glucose-stimulated *TXNIP* promoter occupancy by MLX. *TXNIP* as a strongly glucose-regulated gene, whose induction was dependent on MLX. When LDs were induced by incubation with ac-Lipo, MLX-dependent genes reduced mRNA expression. MLX binding only occurred in TG-rich LDs. Consistent with the finding, formation of TG-rich LDs blunted the MLX occupancy on the *TXNIP* promoter and reduced *TXNIP* mRNA levels after glucose stimulation. The presence of TG-rich, but not CE-rich, LDs abolished the inhibition of glucose uptake as incubating cells with oleate abrogated the effect of glucose stimulation on 2-deoxyglucose uptake, whereas incubation with ac-LDL left the response intact. Taken together, these data indicate that TG-containing LDs, which bind to MLX, attenuate an MLX-regulated physiological response.

Perspectives

In this paper, Mejhert *et al*. performed a systematic genetic screen to identify genes whose knockdown alters LD biology in human macrophage cells. Their list of approximately 550 genes is an invaluable resource for the scientific community working on LD biology. Authors were specifically interested in one group of candidates in this list: glucose-sensing transcription factors - MLX, MLXIP and MLXIPL. The most important finding in this study is that MLX transcription factors can stably bind LDs, and be sequestered away from their real action site, nucleus.

The authors speculate that when the LDs are plenty, sequestering of glucose-sensing transcription factors can ensure that the cell utilizes fat deposits before taking any glucose in. However, there remains a big mystery in how MLX deficiency in macrophages, leading to

LDs that are fewer but larger in size and higher in fat content. The authors hypothesized that some of the MLX target genes could be mediating this phenotype, such as AQP3, BIN1 or CRABP2, deficiencies of which phenocopy MLX defect. Or could MLX on LDs be required to provide a feedback signal as a sensor of LD content and size, and perhaps in the absence of MLX, the cell keeps growing the LDs. Along the same line, it would be interesting to look at MLX localization in conditions that phenocopy MLX deficiency and investigate whether MLX localization on LDs is disrupted. In addition, future studies could examine the interaction between LDs and ER upon MLX deficiency to observe whether the LDs have any deficiency in budding. Another area to inspect for MLX presence is the LD-mitochondria contact sites, as MLX was previously shown to be bound to mitochondria.

Another key future study could be to investigate the homologs of MLX in model organisms and study its function in whole organism setup under physiological conditions - in endogenous LDs, without the need to induce LD formation. For example, in *C. elegans*, there is a single homolog of the human MLX genes, *mxl-2*, which was shown to regulate the lifespan of worm longevity models, including the germline removal, insulin and TOR signaling, and mitochondria mutants (Nakamura et al., 2016). Interestingly, these mutants also have altered lipid phenotypes, however the link between *mxl-2* and lipid levels in these mutants is unknown. The *Drosophila* homologs of MLX transcription factors are indeed enriched in the fat body and regulate fat storage, however their subcellular localization and how they are regulated is unclear (Sassu et al., 2012). The genetic power of these model organisms could enable screens for MLX mutants that constitutively reside on the LDs or locate to the nucleus and yield interesting clues for its function - such as how MLX is only targeted to TG containing LDs, and not CE containing LDs. Overall, this study by Mejhert *et al.* provides a plethora of novel genes involved in several aspects of LD biology and an interesting case study on one of the candidates, the MLX transcription factor that surprisingly resides on LDs.

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