主論文の要旨

Nuclear lipid droplets derive from a lipoprotein precursor and regulate phosphatidylcholine synthesis

（核内脂肪滴はリポプロテイン前駆体から形成され、
ホスファチジルコリン合成を制御する）

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【Introduction】

Lipid droplets (LDs), confined to the cytoplasm, exist widely in eukaryotic cells. But in some cell types, LDs exist also inside the nucleus. Yet, the origin and significance of LDs in the nucleus has not been known. The abundance of nuclear LDs in hepatocytes led us to hypothesize that they may be related to the synthesis of very low-density lipoprotein (VLDL). In VLDL synthesis, two kinds of lumenal LDs are generated in the ER by microsome triglyceride transfer protein (MTP). They are primordial apolipoprotein B100 (ApoB)-containing particles and ApoB-free lumenal LDs, which give rise to mature VLDL in post-ER compartments.

We find that ApoB-free lumenal LDs accumulate under ER stress and generate LDs in the type I NR (nucleoplasmic reticulum; an invagination of inner nuclear membrane) lumen, which then relocate to the nucleoplasm through defects in the NR membrane. That is, LDs in the nucleoplasm are derived from a VLDL precursor in the ER lumen. Nucleoplasmic LDs recruit CCTα, the rate-limiting enzyme for phosphatidylcholine (PC) synthesis, and increase de novo PC synthesis. Perilipin-3 competes with CCTα in binding to nucleoplasmic LDs. Thus, knockdown of perilipin-3 upregulates PC synthesis by increasing nucleoplasmic LD-bound CCTα, whereas overexpression of perilipin-3 decreases CCTα in nucleoplasmic LDs and suppresses PC synthesis.

【Results】

MTP activity is essential for nuclear LD formation

OA increases nuclear and cytoplasmic LDs in Huh7, a human hepatocarcinoma cell line. We found that MTP inhibitors (MTPi) suppressed the OA-induced increase of nuclear LDs, but not that of cytoplasmic LDs (Fig. 1a). Knockdown of MTP also suppressed the OA-induced increase of nuclear LDs, but not that of cytoplasmic LDs (Fig. 1b) Co-transfection of MTP (cDNA) canceled the effect of RNAi (Fig. 1b). Moreover, MTP overexpression increased nuclear LDs (Fig. 1b), suggesting that a disproportionate increase of MTP, which enhances the production of ApoB-free lumenal LDs, may enhance nuclear LD formation. A similar imbalance between MTP and ApoB is known to occur under ER stress. By OA and tunicamycin (OA/TM) nuclear LDs increased (Fig. 1c). The increase of nuclear LDs caused by OA/TM was suppressed by MTPi (Fig. 1c). The results indicated that the nuclear LD formation may be correlated with ApoB-free lumenal LDs generated by MTP activity.

LDs are present in the type I NR lumen

Electron microscopy (EM) showed Huh7 treated with OA/TM harbors lumenal LDs not only in the ER, but also in the nuclear envelope and the type I NR (Fig. 2a). NR-lumenal LDs were delineated with DAB precipitates in cells expressing HRP-KDEL (Fig. 2b). Lumenal LDs also occurred in mouse hepatocytes after high-fat diet feeding and TM

administration. The total nuclear LDs also increased in mouse hepatocytes in vivo (Fig. 2c). Consistent with the EM, the microsome of the OA/TM-treated cells accumulate triglycerides and cholesterol esters (Fig. 2d). Much less ApoB was in the OA/TM-treated microsome than in the control, indicating that NR-lumenal LDs in the OA/TM-treated cell are largely ApoB-free lumenal LDs (Fig. 2d). This means that nuclear LDs observed by fluorescence microscopy contain nucleoplasmic LDs, NR-lumenal LDs, and cytoplasmic LDs within the type II NR (an invagination of inner and outer nuclear membrane) (Fig. 2e). Using lamin B1 receptor (LBR), LDs outside of LBR rings can be judged as nucleoplasmic LDs (Fig. 2f). LDs within LBR rings may be NR-lumenal LDs or cytoplasmic LDs in the type II NR, but EM revealed the latter to be scarce (Fig. 2g). We confirmed that NR-lumenal LDs and nucleoplasmic LDs increase in cells treated with OA or OA/TM, and that the increase of both LDs is suppressed by MTPi (Fig. 2f).

NR-lumenal LDs are converted to nucleoplasmic LDs

The above result suggested that NR-lumenal LDs may become nucleoplasmic LDs. Consistent with this, ApoE and ApoCIII, were found in nucleoplasmic LDs (Fig. 3a). Moreover, CCTα and perilipin-3, which bind to nucleoplasmic LDs, showed colocalization with ApoE (Fig. 3b). Live imaging revealed that the LBR ring around the NR-lumenal LD was disintegrated to relocate LD to the nucleoplasm (Fig. 3c). Defects in the NR membrane around the NR-lumenal LD were also observed by EM (Fig. 3d). Moreover, the contents of NR-lumenal and nucleoplasmic LDs exhibited continuity through the NR membrane defect (Fig. 3e). To study how NR-lumenal LDs and nucleoplasmic LDs grow we did FRAP experiment. Fluorescence recovery was observed in both kinds of LDs (Fig. 3f). Triacsin C retarded fluorescence recovery in both, whereas MTPi affected only NR-lumenal LDs (Fig. 3f). Based on FRAP and previous data we concluded that NR-lumenal LDs grow continuously through MTP-dependent lipid transfer, whereas nucleoplasmic LDs grow largely by a MTP-independent mechanism.

CCTα recruited to nucleoplasmic LDs activates PC synthesis

Most nucleoplasmic LDs were labeled for either CCTα or perilipin-3 and rarely for both (Fig. 4a). Perilipin-3 knockdown increased CCTα-positive nucleoplasmic LDs (Fig. 4b). Moreover, perilipin-3 knockdown increased PC synthesis (Fig. 4c). The result indicated that an increase in CCTα recruitment to nucleoplasmic LDs activates PC synthesis. Overexpression of the wild-type perilipin-3 significantly decreased CCTα in nucleoplasmic LDs, but expression of perilipin-3-NES (nuclear export signal) had no effect (Fig. 4d). The result indicated that CCTα in nucleoplasmic LDs is directly affected by perilipin-3 binding to nucleoplasmic LDs. PC synthesis was found to decrease in cells expressing wild-type perilipin-3, but not in those expressing perilipin-3-NES (Fig. 4e). These results corroborated

that nucleoplasmic LDs are a major site of CCTα activation and that perilipin-3 downregulates PC synthesis by displacing CCTα from nucleoplasmic LDs.

【Discussion】

As reported in enterocytes, when ApoB is deficient while MTP remains active, ApoB-free lumenal LDs accumulate in the ER lumen because their export to post-ER compartments is suppressed. A similar condition occurs in hepatocytes under ER stress, in which ApoB is decreased through degradation, whereas the expression of MTP is maintained. ER stress enhances MTP activity, because the IRE1α- XBP1 pathway increases expression of protein disulfide isomerase, the obligate cofactor of MTP. These changes combine and can cause accumulation of lumenal LDs. Lumenal LDs grown in the type I NR relocate to the nucleoplasm. This movement is made possible by disintegration of the NR membrane surrounding NR-lumenal LDs. The nuclear envelope rupture is known to occur in other conditions, but they involve rupture of both INM and ONM. The present finding indicates that disruption of the INM alone occurs, not as a result of cellular damage, but in a regulated manner to maintain cellular homeostasis. Upregulation of PC synthesis in ER stress occurs by activation of CCTα on the increased nucleoplasmic LDs. Active PC synthesis is necessary for VLDL secretion. An increased supply of fatty acids stimulates ApoB synthesis, but also elicits ER stress, through the increase of ApoB in the ER, making VLDL secretion less than maximal. Here, PC is required for VLDL assembly and synthesis of ApoB and PC needs to be coordinated to maintain an optimal condition. Our result indicates that nucleoplasmic LDs constitute a feedback mechanism to regulate PC synthesis in accordance with the level of ER stress.