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Linkage between lipid droplet formation and nuclear deformation in HeLa human cervical cancer cells

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ABSTRACT

Because lipid droplets (LDs) and the nucleus are cellular organelles that regulate seemingly very different biochemical processes, very little attention has been focused on their possible interplay. Here, we report a correlation between nuclear morphology and cytoplasmic LD formation in HeLa human cervical cells. When the cells were treated with oleic acid (OA), LDs were formed in the cytoplasm, but not in the nucleoplasm. Interestingly, cells harboring OA-induced cytoplasmic LDs showed deformity of the nucleus, particularly at the nuclear rim. Conversely, when alteration from a single spherical nuclear shape to a multinucleated form was enforced by coadministration of paclitaxel and reversine, a significant amount of LDs was detected in the cytoplasm of the multinucleated cells. These two distinct pharmacological culture conditions not only allow analysis of the previously underappreciated organelle relationship, but also provide insights into the mutual affectability of LD formation and nuclear deformation.

1. Introduction

In eukaryotic cells, there are multiple discrete membrane-bound organelles including the nucleus, endoplasmic reticulum (ER), mitochondria, peroxisomes, lysosomes, and lipid droplets (LDs). Whereas these organelles allow separation of incompatible biochemical reactions, their activities and functions need to cooperate and be adjusted via direct or indirect contacts between them in response to physiological and pathological situations [1,2].

Among such cellular organelles, the nucleus and LDs regulate seemingly very different biochemical processes. The former maintains and controls genetic/epigenetic materials [3,4], and the later functions in lipid storage and transport [5,6]. So far, very little attention has been focused on the relationship between these two organelles. However, current studies suggest that LDs are usually detected in the cytoplasm, but sporadically merge in the nucleoplasm of some cell types [7–11], implying a previously underappreciated cell-type specific interaction of LDs with nuclear components. Although the existence of nuclear LDs might imply a direct role of LDs in regulating the nuclear structure and functions under certain cellular contexts, it remains to be clarified how the relationship between cytoplasmic LDs and the nucleus is guided in physiological and pathological situations. One of the reasons for the insufficiency of such research may be, at least in part, the lack of culture conditions to visualize and assess their relationship.

In this study, using confocal and electron microscopic techniques, we carefully observed cytoplasmic versus nucleoplasmic LDs in HeLa human cervical cancer cells, and investigated morphologically noticeable relationships between LDs and the nucleus under the two culture conditions. We first found that LDs were induced efficiently in the cytoplasm, but not in the nucleoplasm, when the cells were exposed to the fatty acid oleic acid (OA) premixed with ethanol. Importantly, in OA-exposed cells, a variation in circularity of the nuclear rim was detected as statistically distinguishable values. Second, we found that, when HeLa cells were co-treated with the microtubule inhibitor paclitaxel and

Abbreviations: LD, lipid droplet; OA, oleic acid; ER, endoplasmic reticulum; Per3, perilipin-3/TIP47; PBS, phosphate buffered saline; EM, electron microscopy.

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mitotic kinase inhibitor reversine, multinucleation was induced, which was concurrent with LD formation in the cytoplasm. These observations suggest a mechanism that excludes LDs from the nucleoplasm of HeLa cells and provide an insight into the cell type-specific interplay between LDs and the nucleus.

2. Materials and methods

2.1. Reagents and stock solutions

All reagents and stock solutions used in the experiments are listed in the Supplementary information.

2.2. Cell culture and drug treatments

Hela cells were grown in Dulbecco's modified Eagle's medium-F12 with 1% penicillin-streptomycin and 5% fetal bovine serum at 37 °C with 5% CO_2. To induce LDs, cells were treated with 0.2 mM OA for 16 h. To induce multinucleated cells, exponentially growing cells were treated with 1 μg/ml paclitaxel for 16 h to arrest cells at the mitotic phase. During the last 2 h of incubation with paclitaxel, 0.2 μg/ml reversine was added to the culture medium. Cells were either fixed immediately or incubated for up to 48 h.

2.3. Indirect immunofluorescence and LD staining

Cells cultured on coverslips in 12-well plates were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 min, followed by treatment with 0.01% digitonin in PBS for perilipin-3 (Per3/TIP47) detection or 0.1% Triton X-100 in PBS for detection of other proteins for 30 or 5 min, respectively. After blocking with 1% bovine serum albumin in PBS for 15 min, cells were labeled with primary and secondary antibodies. During secondary antibody incubation, samples were costained with Hoechst 33342 or Bodipy 493/503. All steps were carried out at room temperature. Samples were subjected to microscopic observation under an FV1200 laser scanning confocal microscope (Olympus, Tokyo, Japan) using Fluoview. For each image, six slices from the z-stack were captured using 1 μm as the step size. ImageJ was used for image processing accordingly.

2.4. Electron microscopy (EM)

EM was performed as described previously [10]. Briefly, cells cultured on coverslips were incubated in a fixation mixture for >2 h, post-fixed with a mixture of 1% osmium tetroxide and 0.1% potassium ferrocyanide in 0.1 M sodium cacodylate buffer, dehydrated, and then embedded in epoxy resin. Ultrathin sections were observed under a JEM1011 electron microscope (JEOL) operated at 100 kV. Images were collected using an Orius CCD camera SC200 (Gatan) and DigitalMicrograph software, version 1.83.842 (Gatan).

2.5. Imaging and statistical analyses

Fluorescent signals of the *anti*-lamin A/C antibody were measured as the nuclear rim shape and used to calculate the nuclear parameters by ImageJ software. Simply, all images were first converted to thresholded-binary images using the binary/fill holes, and then the particles were analyzed by fixing the scale of the size of particles to '100 μm^2-Infinity'. For definition of circularity ($4\pi \times$ area/perimeter^2), the scale of circularity was maintained between '0 and 1', in which '1' indicates a perfect circle and approaching '0' means that the shape becomes elliptical [12]. To examine the induction of LDs dependent on the microtubule (cell cycle) disruption following paclitaxel and reversine treatment, the number of cells with positive staining for Bodipy 493/503 and perilipin-3 was calculated manually. Data indicate the mean ± 95% confidence interval. Statistical significance was assessed by the unpaired, two-tailed Student's t-test. *P*-values of less than 0.01 were accepted as statistically significant.

3. Results and discussion

To assess the effect of LD assembly on nuclear architecture, we first established culture conditions to efficiently induce LD assembly in HeLa cells. Because Vanholder et al. reported that OA exposure with ethanol is effective to induce LDs in granulosa cells [13], we applied the conditions to HeLa cells. After OA was pre-mixed with ethanol, the mixture was added to the culture medium, and the cell culture was continued for various periods. We found many cells containing multiple Bodipy 493/503-positive spherical structures in the culture incubated for at least 2 h (Fig. S1A). In contrast, Bodipy 493/503-positive spherical structures were barely seen in cells exposed to ethanol alone, even for 16 h (Fig. 1A). Because Bodipy 493/503 is a fluorescent molecule used to stain LDs [14], these results implied that our experimental settings were suitable for induction of LDs in HeLa cells. Experiments with various times of OA exposure followed by Bodipy 493/503 staining revealed that the ideal incubation period of OA to obtain maximum Bodipy staining without any cytotoxicity was 16 h (Figs. 1A and S1A). Therefore, we used this culture condition unless stated otherwise.

To confirm whether Bodipy 493/503-positive structures represented mature LDs, we performed indirect immunostaining using an antibody against Per3/TIP47, a ubiquitously expressed membrane protein on mature LDs [15], as a primary antibody. The antibody visualized multiple dots that were merged with Bodipy 493/503 staining (Figs. 1A and S1B), suggesting that Bodipy 493/503-positive spherical structures under our experimental settings represented mature LD assemblies.

Next, to carefully investigate subcellular localization of LDs in OA-exposed HeLa cells, we stained cells with Hoechst 33342 in addition to Bodipy 493/503. Considering that Hoechst 33342 binds to DNA and emits fluorescence, its signals were considered to represent the nucleus. Confocal microscopic observations of the double-stained patterns in OA-exposed cells indicated that most Bodipy 493/503-positive signals were present in the Hoechst 33342-negative area, suggesting that LDs had preferentially formed in the cytoplasm, but not in the nucleoplasm. However, we also noticed that some Bodipy-493/503 signals were present in the Hoechst 33342-positive area (arrowheads in Fig. 1A and Fig. S1B). Therefore, we further investigated compartmentalization of LDs in OA-exposed HeLa cells by EM.

EM observation revealed that, in addition to LD assembly in the cytoplasmic area, there were LDs inside the shallow invagination of the nuclear envelope (Fig. 1B). Moreover, we frequently obtained images showing LDs that appeared to be located inside of the nucleoplasm but surrounded by a deeply invaginated nuclear envelope (Fig. 1C). These EM images suggested that the Bodipy signals detected in the Hoechst-positive area might represent such types of LDs and implied a mechanism that somehow excluded LD assembly in the nucleoplasm of HeLa cells, reinforcing previous results [10].

While there was no evidence indicating nuclear LD formation in OA-exposed HeLa cells, we speculated that observation of LDs in the shallow/deep invagination of nuclear envelope implied the potential for cytoplasmic LD assembly to facilitate alteration of the nuclear architecture, so called nuclear deformation. To detect quantitatively degree of nuclear deformation, we stained cells with an antibody against lamin A/C, a nuclear skeleton protein used as a marker to visualize the nuclear rim structure (Fig. 2A). In *anti*-lamin A/C antibody-stained cells, we measured several parameters,

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