Distinct Roles of Two Types of Lipid Droplets inside the Nucleus in Liver Diseases

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Article
Abstract

Aim

Lipid droplets have been found in the nuclei of hepatocytes, however, their role in liver is not clearly understood. The purpose of this study was to explore the pathophysiological roles of intranuclear lipid droplets in liver diseases.

Method

This study included 80 patients who underwent liver biopsies. A part of the liver biopsy specimen was dissected and fixed for electron microscopic observation. Lipid droplets in the nuclei were distinguished into two types based on the presence of adjacent cytoplasmic invagination of the nuclear membrane: nuclear lipid droplet (nLD) and cytoplasmic lipid droplet invagination with nucleoplasmic reticulum (cLD in NR).

Results

nLDs were found in 69% of the liver biopsy samples, and cLDs in NR were found in 32%. A significant positive correlation was observed between the frequencies of nLD and cLD in NR. Although nLD was frequently found in hepatocytes of patients with nonalcoholic steatohepatitis, there was no correlation between the frequency of nLD and hepatic steatosis, indicating that nLD does not directly reflect cytoplasmic lipid accumulation. Significant positive correlations were found between the frequencies of nLD and endoplasmic reticulum (ER) expansion or liver enzymes, suggesting that nLD is formed in the nucleus under ER stress. Conversely, cLD in NR showed a significant negative correlation with hepatic steatosis, implying that cLD in NR is formed in lipid-restricted hepatocytes. Moreover, no correlations were observed between the frequency of cLD in NR and ER expansion.

Conclusion

This study revealed two distinct pathophysiological roles of lipid droplets in liver diseases.

Introduction

The nucleus is the center of all biological phenomena, where the chromatin encoding genetic information is organized and folded. It is separated from the cytoplasm by a phospholipid bilayer called the nuclear envelope (NE). The NE consists of two layers: the outer nuclear membrane (ONM) facing the cytoplasm and the inner nuclear membrane (INM) facing the nuclear matrix. Besides the luminal space between the outer and inner nuclear membranes, the ONM is continuous with the endoplasmic reticulum (ER) membrane, thus forming a sequential luminal space between the ER and the NE. The NE can form a
complex branched network within the nucleoplasm called the nucleoplasmic reticulum (NR)\(^1\). Moreover, two types of nuclear membrane invagination exist: type 1 NR (the extension of INM) and type 2 NR (the invagination of INM and ONM coupled with cytoplasmic structures)\(^2\). The link between these nuclear structures and possible pathological roles is largely unknown.

Most animal cells contain intracellular lipid droplets consisting of a phospholipid monolayer and a core of neutral lipids (triacylglycerols and cholesteryl esters). Lipid droplets are believed to be formed when neutral lipids synthesized in the lumen of the phospholipid bilayer of the ER grow in size and are released into the cytoplasm using the ER membrane as an outer shell\(^3\). Lipid droplets in the cytoplasm have been shown to play a plethora of physiological functions, including energy storage, heat production, and proteolysis, depending on their cellular localization\(^4\). Since a half-century ago, lipid droplets have also been reported in the nuclei of animal hepatocytes, intestinal cells, plants, and yeasts\(^5\). Recently, hepatocytes and hepatocellular carcinoma cells have been found to contain abundant lipid droplets in their nuclei, and the mechanisms underlying their formation and functions have attracted attention\(^6,7\).

However, the pathophysiological significance and the frequency of formation of intranuclear lipid droplets in hepatocytes in liver diseases remains unknown. In this study, we aimed to analyze the frequency and morphology of intranuclear lipid droplets in hepatocytes and elucidate the underlying mechanisms toward their pathophysiological significance in various liver diseases. The results presented in this study will serve as a compass in emerging trends of nuclear biology research.

**Materials And Methods**

**Human Liver Biopsy Sample**

Eighty patients who underwent liver biopsies for liver diseases at Nagoya University Hospital (Nagoya, Aichi, Japan) were included in this study (Fig. 1A). Liver biopsy procedures were performed percutaneously using an ultrasound imaging guide. Briefly, disinfectant was applied below the right rib cage, a small incision was made under the influence of a local anesthetic. A biopsy needle (16G Bard Monopty Biopsy Gun, Bard Peripheral Vascular, Inc. Tempe, AZ, USA) was then inserted into the right lobe of the liver. A part of the liver biopsy specimen (about 1 mm square) was dissected using a scalpel and fixed in 0.1 M cacodylate buffer containing 2.5% glutaraldehyde. In cases of biopsies aiming at liver tumor diagnosis, a portion of surrounding normal liver tissue was also collected for electron microscopy. A mixture of 1% osmium tetroxide and 0.1% potassium ferrocyanide in 0.1 M sodium cacodylate buffer was added post-fixation. Further, samples were embedded in epoxy resin, and ultrathin sections were observed using a JEOL JEM-1400PLUS electron microscope (JEOL Ltd. Tokyo, Japan) operated at 100 kV. Digital images were captured using an EM-14661FLASH camera (JEOL Ltd. Tokyo, Japan). Lipid droplets in the nucleus were distinguished into two types depending on the presence of adjacent cytoplasmic invagination with nuclear membrane, nuclear lipid droplet (nLD) and cytoplasmic lipid droplet invagination with NR (cLD in NR) (Fig. 1B)\(^2\).
Histological Assessment

Histological assessment of hepatocyte ultra-structures was performed independently in the absence of clinical information. The frequencies of nLD and cLD in NR were determined by counting more than 100 nuclei per specimen. ER enlargement was blindly assessed based on the typical images (Suppl. Figure 1). Written informed consent about sample collection for electron microscopy was obtained before the biopsy. This study was carried out under the Declaration of Helsinki. This study was approved by the Bioethics Review Committee of Nagoya University Hospital (2020 – 0229).

Clinical Tests

Blood samples were collected a day before liver biopsies. All laboratory tests were performed at Nagoya University Hospital. Liver biopsy samples were fixed and embedded into paraffin for hematoxylin and eosin (H&E) staining. Histological diagnosis was made by certificated pathologists.

Statistical analysis

Correlations were analyzed using the simple linear regression method. Student’s t-test was used to compare the differences in blood parameters for liver biopsies with and without lipid droplets in the nucleus. Statistical significance was defined at $P < 0.05$. All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

Results

Distinct nuclear lipid droplet characterized in liver biopsies

The patient characteristics are summarized in Table 1. Out of 80 patients, 35 were males and 45 females. Their mean age was 58, ranging from 24 to 89 years old. Liver biopsy-proven liver diseases included nonalcoholic steatohepatitis (NASH) in 12 patients, drug-induced liver injury (DILI) in 11 patients, malignant tumor in 22 patients, autoimmune hepatitis (AIH) in 7 patients, and others in 28 patients. According to the METAVIR classifications of liver fibrosis, the scores were F0 in 39 patients, F1 in 26 patients, F2 in 3 patients, and F3 in 5 patients, respectively. Liver steatosis assessed by H&E staining was less than 5% in 46 patients, 5–33% in 21 patients, 33–66% in 4 patients, and more than 66% in 2 patients. Blood profiling of the patients is summarized in Table 2. Although the levels of liver enzymes, alkaline phosphatase, and gamma-glutamyl transpeptidase were elevated, that of plasma lipids were within normal range.

Electron microscopy observations of liver biopsy samples were conducted independently from clinical diagnosis by light microscopy. Six patients were excluded from further analysis because the electron microscopy sample did not contain the corresponding background liver specimen. Surprisingly, 76% of the liver biopsy specimen presented lipid droplets in the hepatocyte nucleus.
Case assessment toward liver diseases and patient profile

Three representative cases are as shown in Figure 1C–E. The first case is a female in her 70s showing mild increase in aminotransferase levels (aspartate aminotransferase; AST 125 U/L and alanine aminotransferase; ALT 106 U/L), who was diagnosed with NASH. In her liver biopsy, hepatocyte ballooning and mild inflammation were observed with 40% steatosis and F2 fibrosis liver tissue (Figure 1C). nLDs were found in 5.6% of nuclei whereas cLD in NR was not found in nuclei (Figure 1C). The second case is a male in his 20s with elevated AST (211 U/L) and ALT (546 U/L) levels, who was diagnosed with DILI. Mild inflammation in the portal area was observed with 0% steatosis and F0 fibrosis liver tissue (Figure 1D). nLDs were found in 4.2% of the nuclei and cLDs in NR were found in 1.2% of the nuclei in the liver biopsy sample (Figure 1D). The third case is a male in his 40s with mild elevations in AST (134 U/L) and ALT (250 U/L), who was diagnosed with AIH. Severe inflammation in the portal area was observed with 0% steatosis and F1 fibrosis liver tissue (Figure 1E). nLDs were found in 5.7% of nuclei and cLDs in NR were found in 5.7% of nuclei of the liver biopsy sample (Figure 1E). Overall, nLDs were found in 69% of liver biopsy samples and cLDs in NR were found in 32%, less frequent than nLDs (Figure 2A, B). Notably, there was a significant positive correlation between the frequencies of nLD and cLD in NR (Figure 2C). Although both nLD and cLD in NR are most frequently detected in AIH (Table 1), no disease-specific distributions were observed in their frequencies (Figure 2D). These results revealed that two types of nuclear lipid droplets are found in the human liver.

Correlation analysis of lipid droplets and clinical parameters

Next, we studied the correlations between these two types of lipid droplets in the nucleus and their clinical characteristics. Although patients who presented with nLD showed higher plasma liver enzymes than those who without nLD, there were no significant differences in tests depending on the presence of nLD (Table 3). Conversely, patients who presented cLD in NR showed significantly lower levels of total cholesterol and low-density lipoprotein (LDL) cholesterol compared to patients without cLD in NR (Table 3). Thus, the two types of lipid droplets in the nucleus were clinically different. Further, nLD was frequently found in hepatocytes of NASH, however, there was no correlation between nLD and hepatic steatosis, indicating that nLD does not reflect only cytoplasmic lipid accumulation (Figure 3A). Moreover, positive correlations were found between nLD and ER expansion or liver enzymes (Figure 3B, C), suggesting nLD formation in the nucleus under ER stress or liver damage. In contrast, cLD in NR showed a negative correlation with steatosis, implying that it is formed in lipid-restricted circumstances (Figure 3D). Furthermore, no correlations were found between cLD in NR and ER expansion or liver enzyme (Figure 3E, F). Unlike our expectations, no correlation was found between these two types of nuclear lipid droplets and plasma lipids (Suppl Figure 2A–F). These results unveiled two distinctly possible pathophysiological roles of nLD and cLD in NR in liver diseases (Figure 4).

Discussion
The results of this study revealed that two types of nuclear lipid droplets existed and showed correlations with distinct pathophysiological roles in human liver disease. To date, there is only one case report on nuclear lipid droplets in the human liver in a patient with hepatitis C virus infection. However, only cLD invagination with NR was detected; therefore, this report is the first to show the existence of “pure” nucleoplasmic lipid droplets in human hepatocytes in vivo.

Our approaches using electron microscopy shed a light on ultrastructural components in the nuclei of hepatocytes and unveiled previously unknown roles of nuclear lipid droplets based on the presence of adjacent cytoplasmic invagination of the nuclear membrane. Importantly, only electron microscopy enables us to distinguish these two types of nuclear lipid droplets.

Under excessive lipoprotein synthesis in hepatocytes or suppressed secretion from the ER, lipoprotein precursors stagnate in the lumen of the ER and are released into the nucleus through the invagination of NR, forming intranuclear lipid droplets. Lipid transfer from cLD to ER occurs during very low-density lipoprotein (VLDL) synthesis. Based on the result that cLD in NR was higher when LDL levels were lower, it may imply that the cLD trapped in type 2 NR is isolated from the ER, leading to no lipid transfer, thereby, reducing lipoprotein synthesis. Taken together, the significantly lower levels of total and LDL cholesterol in patients with cLD in NR suggests that these lipid droplets may regulate the VLDL secretion machinery in hepatocytes.

Intranuclear lipid droplets also activate CDP-choline diacylglycerol phosphotransferase α (CCTα), a rate-limiting enzyme of the novel phosphatidylcholine (PC) synthesis pathway, in their surface layer, thereby enhancing PC synthesis and reducing ER stress. In this study, we found that hepatocytes with minimal hepatic damage do not present intranuclear lipid droplets, and 5–10% of hepatocytes show intranuclear lipid droplets in most cases of ER stress involving its enlargement. These results suggested that nLD plays a role in enhancing PC synthesis to reduce ER stress in various liver diseases.

Even with a limited number of studied cases, nLDs were highly found in NASH and DILI, and cLDs in NR were found in DILI and AIH. Although nLDs were frequently found in hepatocytes of patients with NASH, there was no cLD in NR observed. Further studies are warranted to elucidate the pathophysiological significance of nLD and cLD in NR in each disease condition. The possible correlations of nLD or cLD in NR and liver disorders may lead to a new pathophysiological diagnosis of hepatitis of unknown cause.

Together, our presented results unveiled the presence of intranuclear lipid droplets in humans and displayed their functional correlation with the nuclear ultrastructure in causing pathological conditions.

Declarations

Conflict of Interest: The authors declare no conflict of interest.

Authors’ contributions: Concept and study design: N. Imai and Y. Ohsaki, Acquisition of data: N. Imai, J. Cheng, J. Zhang, F. Mizuno, K. Yamamoto, T. Ito, Y. Ishizu, T. Honda, M. Ishigami, H. Kawashima, H. Wake,
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Availability of Data and Materials: The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

References


Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures
Figure 1

Representative cases of nuclear lipid droplets and cytoplasmic lipid droplets in the nucleoplasmic reticulum.

(A) Schematic depicting the collection of liver biopsy samples and their processing for both hematoxylin and eosin staining observed using light microscopy and osmium staining for electron microscopy. (B)
Schematic illustration of nuclear lipid droplet (nLD) and cytoplasmic lipid droplet invagination with nucleoplasmic reticulum (cLD in NR). Biopsy section from respective patients with (C) nonalcoholic steatohepatitis (NASH) showing nLDs in hepatocytes, and (D) drug-induced liver injury (DILI) and (E) autoimmune hepatitis (AIH) showing both nLD and cLD in NR.

Figure 2

Frequencies and correlations of nLD and cLD in NR.

(A) Frequency of nLD in liver biopsy samples. (B) Frequency of cLD in NR in liver biopsy samples. (C, D) Scatter plot showing a correlation between nLD and cLD in NR frequencies. The black line depicting linear regression. DILI; drug-induced liver injury, NASH; nonalcoholic steatohepatitis, AIH; autoimmune hepatitis.
Figure 3

Correlation of nLD and cLD in NR with clinical parameters.

(A–C) Scatter plots showing the correlation between frequencies of nLD and hepatic steatosis, endoplasmic reticulum expansion, and plasma level of alanine aminotransferase (ALT). The black line depicts linear regression and the dotted line shows 95% confidential intervals. (D–F) Scatter plots showing the correlation between frequencies of cLD in NR and hepatic steatosis, endoplasmic reticulum expansion, and plasma level of ALT. The black line depicts linear regression and the dotted line shows 95% confidential intervals.
Figure 4

Two distinct roles of lipid droplets inside the nucleus in liver diseases.

Schematic presentation of proposed roles of lipid droplets in the nucleus. nLD plays a role in enhancing PC synthesis to reduce ER stress in various liver diseases. On the other hand, cLD in NR regulates the VLDL secretion machinery in hepatocytes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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- nLDfigS2.jpg
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