博士論文（要約）

Role of insulin signaling in the development of metabolic dysfunction-associated fatty liver disease

（代謝関連脂肪肝疾患形成におけるインスリンシグナルの役割）

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Metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed as a new nomenclature to encompass the full spectrum of fatty liver disease. This updated term is defined as the presence of hepatic steatosis combined with one of the following three conditions including overweight/obesity, type 2 diabetes, and metabolic derangements. Recently, the rapidly growing prevalence has made hepatic steatosis the most frequent chronic liver disease in many parts of the world. This raises the significance to gain more comprehension of the molecular basis underlying the pathogenesis of hepatic steatosis. Numbers of studies have revealed that hyperinsulinemia, insulin resistance and elevated PPARγ activity in the liver are the common findings of hepatic steatosis. However, the mechanistic relationship between those features towards the development of hepatic steatosis remains largely unidentified. The present study aims to explore the potential mechanistic relationship of hyperinsulinemia, insulin resistance, and the elevation of PPARγ underlying the development of hepatic steatosis, which is the essential component of metabolic dysfunction-associated fatty liver disease.

The high-fat diet (HFD)-fed, *db/db*, and liver-specific insulin receptor substrate 1 and 2 knockout (LIRS1/2DKO) mice were prepared and analyzed. The mouse primary hepatocytes were used for in vitro experiments. Furthermore, the adeno-associated virus (AAV)-mediated vectors were applied for gene-silencing study. The enzyme-linked immunosorbent assay

(ELISA), immunoblotting, Real-Time quantitative reverse transcription PCR (qRT-PCR), and Oil Red O staining were used as analytical methods.

The results showed that, in parallel to the development of hepatic steatosis, both HFD-fed and *db/db* mice exhibited excessive accumulation of hepatic cAMP. This was due to the insulin-induced repression of the cAMP transporter, multidrug resistance-associated protein 4 (MRP4). The overaccumulation of cAMP levels led to the augmentation of PPARγ mRNA levels in the liver, which was mediated by the endoplasmic reticulum (ER) stress-induced FoxO6 upregulation. Consequently, the triglyceride (TG) accumulation in the liver was increased. In contrast, the inhibition of ER stress or the ablation of FoxO6 substantially mitigated cAMP-induced PPARγ upregulation, leading to the lowered hepatic TG concentration. On the other hand, in mice-lacking hepatic insulin signaling (LIRS1/2DKO mice), which did not develop hepatic steatosis, the Mrp4-mediated cAMP extrusion remined intact. Additionally, the ER stress response was remarkably low in LIRS1/2DKO mice, coupled with decreased FoxO6 and PPARγ expression levels.

Taken together, these results manifest that the overabundance of hepatic cAMP, caused by the reduction of MRP4, plays an indispensable role in the pathogenesis of hepatic steatosis under the hyperinsulinemic and insulin-resistant condition, whereby the steatohepatic mice displayed the unrestraint ER stress response, accompanied with increased expression levels of FoxO6 and PPARγ. Therefore, approaches that enhance the MRP4-

mediated cAMP egress in the liver could possibly serve as a novel therapeutic strategy for the treatment of hepatic steatosis, which would also provide the therapeutic benefits to MAFLD.