博士論文(要約)

Molecular Genetics of Kinesin Superfamily Protein 12 (KIF12) in the Liver

(肝臓におけるキネシンスーパーファミリータンパク質 12（KIF12）の分子遺伝学的研究)

アシエ・エテマド・ファシュカチェ

Kinesin superfamily proteins (KIFs) generally serve as microtubule motor proteins that play fundamental roles in the survival and functions of cells. Human mutations in the *KIF12* gene have been linked to some congenital impairments. KIF12 was reported to be a key regulator in β cell lipotoxicity by increased oxidative stress. KIF12 was also found to be involved in the pathogenesis of polycystic kidney. Two recent studies pointed to the role of KIF12 in the liver, which shared a similar degree of liver cirrhosis among patients with KIF12 congenital mutations. However, the molecular pathway in liver pathogenesis involving KIF12 dysfunction is still unknown.

In this study, I performed initial characterization of KIF12 relevance in the liver, using histological analyses of *Kif12* knockout mice, identification of a responsible KIF12 domain using the human hepatocyte cell line HepG2 cells, and in vitro and in vivo transfection of the responsible domain to the disease models; to reveal the functional relevance of a C-terminal domain of KIF12 against liver lipidosis.

For histological analyses, more than 3 pairs of knockout (KO) and wild-type (WT) mouse livers were fixed by perfusion at the age of 6 and 12 months, and then processed for cryo- and paraffin-sections. First, I performed Oil Red O staining of cryosections, to label the neutral lipid particles within hepatocytes. The KO tissue exclusively presented progressive lipidosis compared with the WT tissue. Next, I performed Hematoxylin & Eosin staining of paraffin sections. Although the overall cytoarchitecture in low magnification remained unaltered, the KO tissue more frequently revealed immune cell infiltration and cellular ballooning than the WT tissue in high magnification. Finally, I performed Sirius Red staining of paraffin sections. The KO tissue revealed a mild level of fibrosis.

These pathological features in KO mouse livers were consistent with those of human non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which are common metabolic syndromes in industrial countries. The NAFLD etiology is almost 30% in Japan, and several percent of the NAFLD patients progress to NASH, hepatic cirrhosis, and hepatocellular carcinoma. Since the precise molecular pathogenesis of NAFLD/NASH is still unclear, I further performed initial analyses of KIF12-knockdown hepatocytes using knockdown-rescue studies.

First, I transduced the human hepatocyte cell line HepG2 cells with an adenoviral *Kif12* knockdown miRNA expression vector (KD) and a scrambled control vector (SC), and stained neutral lipids using BODIPY 493/503 or HCS LipidTOX™ Deep Red dyes. Intriguingly, both dyes revealed significant lipidosis in KD cells compared with SC cells, suggesting that the liver lipidosis in *Kif12*$^{-/-}$ mice is largely hepatocyte-intrinsic rather than a secondary phenotype due to diabetic changes.

Then, I performed a knockdown-rescue assay against the in vitro lipidosis using the full-length and domain-specific constructs of KIF12. A mCitrine-tagged full-length KIF12 protein nicely rescued the lipidosis, supporting that the lipidosis phenotype was truly derived from KIF12 protein deficiency. I transduced a series of deletion mutants of KIF12 and further conducted the knockdown-rescue assay. Accordingly, constructs with a C-terminal non-motor domain were specifically capable to rescue the lipidosis. This domain likely regulates the lipid metabolism in the liver in a motor-independent manner, consistent with the results of my in-silico analyses suggesting that the KIF12 isoform expressed in the liver occasionally have a large deletion in the motor domain.

I further extended this rescue study into the following three aspects: [1] Not only expressing the C-terminal domain in parallel to the knockdown vector transduction, but also expressing it following to the knockdown vector transduction, was effective. [2] In vivo transfection of the C-terminal domain by intraperitoneal injection of a DNA-lipid transfection complex could significantly resolved the liver lipidosis in vivo. [3] Transduction of the C-terminal domain into a well-documented cellular model of lipidosis in HepG2 cells, undergoing MAPK inhibition and oleic acid treatment, could successfully reduce the degree of lipidosis. These results suggested that this C-terminal domain can have a preventive and therapeutic potential against liver lipidosis in general.

Mammalian liver balances the lipid contents within a narrow range, through uptake, esterification, oxidation, and secretion of fatty acids, any of which disturbance could facilitate the development of metabolic syndrome. The present study newly provided genetic evidence that KIF12 knockout mice could serve as a good model for NASH/NAFLD pathogenesis, and that a C-terminal domain of KIF12 is essential and sufficient for preventing or ameliorating liver lipidosis. Because this activity is considered to rely on a new non-motor scaffolding role of KIF12 in regulating lipid metabolism, my study will provide a new therapeutic potential of KIF12-related pathways against liver lipidosis. Future identification of the metabolic process regulated by this unique non-motor kinesin activity will provide new insights on therapeutic approach of NASH/NAFLD as well as how kinesins can play fundamental roles in smaller nonneuronal cells where simple diffusion appears sufficient for cellular logistics.