Project number　54

Functional Analysis of Let-7 microRNA in Liver Metabolism

［1］Research group

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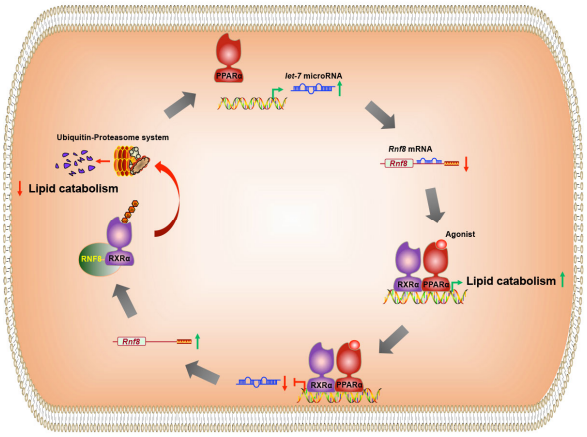
Expenditure report of research funds :
Consumables 150,000YEN

［2］Research setup

Let7 microRNA (miRNA) is a liver-enriched miRNA family consisting of Let7a, b, c, d, e, f, g, i and miR-98. Let-7 miRNA has roles in cell proliferation, carcinogenesis and glucose homeostasis by silencing target mRNAs. Our previous studies revealed that peroxisome proliferator-activated receptor alpha (PPARa) repressed transcription of the Let7 family miRNAs. PPARa is a ligand-dependent nuclear receptor abundantly expressed in liver that regulates lipid catabolism. To analyze the interactions between hepatic Let7 miRNA and PPARa signaling, Let7b/c2 liver-specific knockout (LKO) mice were generated. Intriguingly, these mice showed potent resistance of obesity upon 60% high-fat diet (HFD) feeding.

PPARa forms an obligate heterodimeric transcription complex with retinoid X receptor (RXRa). Our further analyses suggested Let-7 miRNA accelerate RXRa protein degradation via the inhibition of RNF8, E3 ubiquitin ligase for RXRa. However, RNF8 function in ubiquitin-proteasome system needs additional experiments.

The purpose of this international collaboration is to elucidate the PPARa-Let7-RNF8-RXRa axis in hepatic lipid metabolism. We prepared RXRa and RNF8 expression plasmids and antibodies for detecting each proteins and ubiquitin by Western blot. Also, Dr. Yagai prepared RXRa antagonist for analyzing if PPARa/RXRa inhibition resulted in inhibition of lipid accumulation in hepatocytes.



［3］Research outcomes

（3－1）Results

To determine whether RXRa activity has a role on lipid accumulation in hepatocytes, RXR antagonist (HX531) was added to cultured primary hepatocyte. The administration significantly reduced lipid accumulation or synthesis after 48 hours culture, suggesting that RXRa activity accelerated hepatic steatosis (Figure 1).

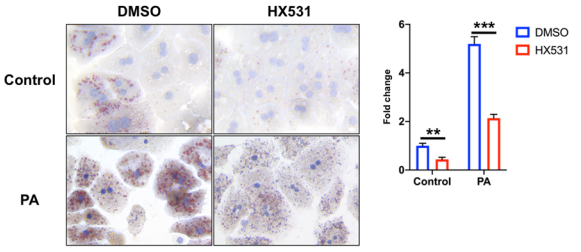


Figure 1. Oil red O staining and the morphometric analysis for palmitic acid and HX531 treated primary hepatocytes

let7 miRNA overexpression in mouse liver by AAV showed significant decrease of RNF8 protein, whereas the inhibition of let7 resulted in increase of RNF8, suggesting that let7 miRNA repressed the RNF8 protein stability (Figure 2).

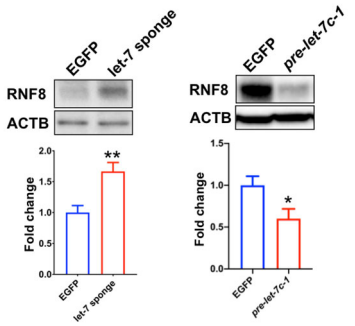


Figure 2. Exogenous regulation of let-7 miRNA changes RNF8 protein abundance alteration

To uncover the RXRa polyubiquitination by RNF8, ubiquitination assay in hepatic cells was performed. RNF8 and RXRa were overexpressed in the cells, and then RXRa was immunoprecipitated by the antibody. Immunoblot for the polyubiquitin demonstrated that RNF8 co-expression markedly increased the RXRa ubiquitination (Figure 3).

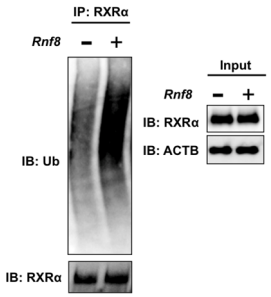


Figure 3. Ubiquitination assay for RXRα in RNF8 overexpressed hepatic cells.

Together with these data, PPARa - let7 - RNF8-RXRa axis was suggested as a novel regulatory pathway in lipid metabolism.

（3－2）Future perspectives

Based on the new findings above, further analyses for PPARa regulatory lipid metabolism network will be performed. We found that PPARa regulate target genes related with not only lipid metabolism but also inflammation. Our current study focusing on inflammatory factors would reveal additional links between inflammation and lipid metabolism via PPARa. Our study about these inflammatory mechanisms related with hepatic steatosis would provide new insight for therapeutic target for Non Alcoholic Steatohepatitis (NASH).

［4］List of Papers

Tomoki Yagai, Tingting Yan, Yuhong Luo, Shogo Takahashi, Daisuke Aibara, Donghwan Kim, Chad N. Brocker, Moshe Levi, Hozumi Motohashi and Frank J. Gonzalez, Feedback repression of PPARα signaling by *Let-7* microRNA, *Cell Reports*, **in revision**