論文の内容の要旨

論文題目　Analysis of a novel lipolytic enzyme that links phospholipids to epigenetic regulation
(リン脂質とエピゲノムをつなぐ新規脂質分解酵素の解析)
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Choline is recognized as an essential dietary nutrient for normal body function due to its broad range of functions as a precursor of the neurotransmitter acetylcholine, as a structural component of membrane phospholipids, and as a source of methyl group metabolism. Choline supplies methyl groups for regeneration of methionine and the universal methyl donor *S*-adenosylmethionine (SAM) in the liver. Although phosphatidylcholine (PC) in cell membrane is the main cellular reservoir for choline, the biological importance of PC catabolism that liberates free endogenous choline from PC remained obscure. PNPLA7, a member of the phospholipase A_2 (PLA_2) family, acts as a lysophospholipase that hydrolyzes lysophosphatidylcholine to give rise to glycerophosphocholine (GPC), a precursor of endogenous choline. Our recent study using *Pnpla7*-null mice has revealed an unexplored link of the PNPLA7-driven phospholipid-catabolic pathway to hepatic choline/methionine metabolism, where the methyl groups of choline are transferred to methionine via betaine and then to S-adenosyl-methionine (SAM). *Pnpla7*-deficient mice show marked decreases in hepatic GPC, choline, and several metabolites related to the methionine cycle including SAM, accompanied by various signs of methionine insufficiency such as growth retardation, leanness, impaired triglyceride secretion, hypoglycemia, increased energy expenditure, reduced fat mass with adipocyte browning, and premature death. As the methyl group of SAM is transferred by various methyltransferases to numerous substrates, the cellular levels of SAM can influence the epigenetic regulation of gene expression. As such, the aims of the present study are to examine 1) whether the

expression of PNPLA7 could be nutritionally regulated by certain choline/methionine metabolites in cultured liver cells, 2) whether the reduced flux of SAM as a result of PNPLA7 deficiency could affect the methylation status of histones and DNA in mouse liver, and 3) whether the expression of PNPLA7 and related enzymes would be affected in human liver cancer.

1) Methionine availability controls PNPLA7 expression

We found that the expression of PNPLA7, rather than other PNPLA members, was preferentially induced in human hepatocarcinoma HepG2 cells following depletion of methionine, but not choline, and conversely downregulated by methionine re-supplementation, suggesting that PNPLA7 acts as a checkpoint to monitor methionine availability. In a methionine-rich environment, PNPLA7 expression was downregulated through hypermethylation of the *PNPLA7* promoter. Methionine depletion decreased intracellular SAM level and thereby SAM-dependent methylation of the *PNPLA7* promoter, allowing upregulation of its expression. Then, the increased PNPLA7 replenished GPC and choline through facilitating PC catabolism. As such, the endogenous choline thus produced may be utilized for supply of methyl groups to the methionine cycle to overcome methionine insufficiency and also for *de novo* PC synthesis to maintain membrane homeostasis as adaptive responses.

2) PNPLA7-driven SAM is coupled with epigenetic regulation

Pnpla7$^{-/-}$ mice had a decreased hepatic SAM level due to impaired PC catabolism and methionine cycle. In accordance with this, methylation levels of several even if not all histones were noticeably reduced in the liver of *Pnpla7*$^{-/-}$ mice relative to *Pnpla7*$^{+/+}$ mice. Moreover, genome-wide DNA methylation analysis, in combination with microarray gene profiling, revealed that, of more than 10,000 genes, 12 genes displayed lower

promoter methylation and higher gene expression in *Pnpla7*$^{-/-}$ mice than in *Pnpla7*$^{+/+}$ mice. Thus, the PNPLA7-driven SAM pool is coupled with specific histone and DNA methylation events, uncovering a previously unrecognized association of phospholipid metabolism with epigenetic regulation.

3) PNPLA7 is downregulated in human liver cancer

It has been reported that SAM supplementation inhibits the growth, transformation, and invasion of liver cancer. We found that the expression levels of *PNPLA7* and *PNPLA8*, two enzymes participating in the PC-catabolic pathway in the liver, but not their respective homologs *PNPLA6* and *PNPLA9*, were significantly lower in tumor tissues than in non-tumor tissues. The reduced expression of *PNPLA7* and *PNPLA8* in human hepatocellular carcinoma was not related to the grades of hepatic fibrosis nor to the etiology, suggesting that oncogenic transformation leads to downregulation of *PNPLA7* and *PNPLA8* regardless of the cause of liver cancer. Additionally, the expression levels of the enzymes in the methionine cycle, such as *BHMT*, *MAT1A* and *PEMT*, were also significantly lower in the tumor samples than in the non-tumor samples Collectively, multiple enzymes in the PC catabolism and methionine cycle were concomitantly downregulated in human hepatocellular carcinoma, suggesting a decreased metabolic flux through this pathway. Taken together, concomitant reduction of a series of enzymes involved in the PC catabolism and methionine cycle, particularly *PNPLA7* whose expression level is dramatically attenuated in most hepatocellular carcinoma patients, could be a novel diagnostic marker of this life-threatening disease. Furthermore, controlling SAM levels by manipulating the expression or activity of PNPLA7 would be a novel therapeutic strategy for treatment of liver cancer and possibly other cancers.