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Deficiency of Calcium-independent Phospholipase A2Beta In Vivo Causes Apoptosis, Autoinflammation, Fibrosis, and Hepatocellular Carcinoma

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BACKGROUND Chronic liver disease is very common worldwide, which is associated with lots of different causes. Among these causes, abnormal lipid metabolism is considered to be one important factor, despite the fact that the mechanisms involved into these pathology processes are far from understanding. Calcium-independent phospholipaseA2beta (iPLA2beta), also named iPLA2β, is one of the patatin-like phospholipase domain-containing genes which has multifaceted functions in lipid synthesis and metabolism. iPLA2β catalyzes the hydrolysis of fatty acyl bond at the sn-2 position of phospholipids, as well as lysophospholipase activity and lysophospholipid transacylase activity which are important for cell membrane remodeling. One main product of iPLA2beta is Arachidonic Acid (AA) which acts as a signalling molecule in the eicosanoid pathway, and iPLA2beta also produces lysophosphadidylcholine as a 'find me signal' for phagocytes to remove apoptotic cells. We herein hypothesize that deficiency of iPLA2beta leads a dysregulation of liver homeostasis, which leads to a disorder of immune system and an accumulation of apoptotic cells, and subsequently results in a chronic inflammation. Inflammatory pressure induced the generation of proliferative hepatocytes which may lead to hepatocellular carcinoma (HCC) or any other malignant lesions.
AIMS Our aim was to clarify whether iPLA2β deficiency can induce chronic hepatitis and exacerbate tumorgenesis in livers of male mutant mice, and further to explore the possible mechanism.
METHODS We selected iPLA2β-null (PLA2β-/-) C57BL/6 mice as experimental group and iPLA2β+/+ C57BL/6 mice as wild- type controls. Whole body iPLA2□-/- (with the deletion of the catalytic domain exon 9) and wild-type (WT) male mice were allowed to age to 20 months old. Serum transaminases, serum and liver lipids were determined using diagnostic kits. Formalin-fixed and optimal cutting temperature compound embedded liver sections were stained with hematoxylin-eosin and Sirius Red, respectively. Immunohistological chemistry (IHC) processes were performed following the antibodies' instructions separately. Hepatic gene expression to target some inflammatory and tumor markers was analyzed by quantitative TaqMan RT-PCR.
RESULTS Compared with WT, iPLA2β-/- mice had significant whole body and organs weight loss. Unlike the wild-type ones, iPLA2β deficient mice revealed a relatively decrease in metabolic parameter including some liver enzyme and serum lipid components. Histology data revealed that mutant mice exhibited decreased

hepatic steatosis. Hepatic steatosis was observed in aged WT, but not in mutant livers. Livers of mutant mice exhibited periportal inflammation, necrosis, and immune cell infiltration with a 47%, 19%, 17% incidence rate, respectively. The mutant livers showed significant fibrosis as seen by Sirius red and α-smooth muscle actin staining. Mutant mice also showed immune abnormalities with a significant increase in the number of B cell, T cell and Kupffer cells and an increase in apoptosis in liver. Among 36 aged mutant mice, 9 nodular HCC was observed showing structural destruction with decreased E-cadherin expression, and increased proliferation Ki67 marker. Loss of cytokeratin 19 in mutant nodular HCC indicated the destruction of bile duct architecture. Moreover, mutation in exon 7 of iPLA2beta gene did not produce any fibrosis or HCC in mutant livers. By IHC, we found that iPLA2beta protein was expressed in normal human livers with or without steatosis, while being completely absent in human HCC livers without steatosis.

CONCLUSION In an absence of steatosis, the deficiency of exon 9 of iPLA2beta caused systemic inflammation which led to chronic aggressive hepatitis, liver fibrosis and exacerbates hepatocarcinogenesis. Thus, in the presence of suppressed hepatic steatosis and low body weight, iPLA2beta regulation on immune function in liver may be involved in the development of HCC, and this gene may be used as a therapeutic target.