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Hepatocyte-specific Pten-deficient mice as a novel model for nonalcoholic steatohepatitis and hepatocellular carcinoma

Sumio Watanabe [a,*], Yasuo Horie [a], Akira Suzuki [b]

[a] *Department of Gastroenterology, Akita University School of Medicine, 1-1-1, Hondo, Akita 010-8543, Japan*
[b] *Department of Molecular Biology, Akita University School of Medicine, 1-1-1, Hondo, Akita 010-8543, Japan*

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Abstract

Phosphatase and tensin homolog (PTEN) is a multifunctional phosphatase whose substrate is phosphatidylinositol-3,4,5-triphosphate (PIP3), and it is also a ubiquitously expressed tumor suppressor gene that down-regulates phosphatidylinositol-3-kinases (PI3Ks). Although there are a few reports about PTEN related to hepatocellular carcinoma, the role of PTEN in the liver remains unclear. Therefore, to clarify the role of PTEN in the liver, we generated and analyzed hepatocyte-specific Pten-deficient mice (Pten-deficient mice). The liver of 40-week-old Pten-deficient mice revealed macrovesicular steatosis, ballooning hepatocytes, lobular inflammatory cell infiltration, and perisinusoidal fibrosis that are characteristic of human nonalcoholic steatohepatitis (NASH). By 80 weeks of age, 100% of Pten-deficient livers showed adenomas and 66% had hepatocellular carcinomas. Thus, PTEN is important for the prevention of adipogenic and tumorigenic transformation, and Pten-deficient mice are a novel model for NASH and hepatocellular carcinoma. Our results suggest that the controlled blocking of molecules acting downstream of PI3K might provide significant therapeutic benefit to patients predisposed to NASH and hepatocellular carcinoma.

Keywords: PTEN; Nonalcoholic steatohepatitis (NASH); Hepatocellular carcinoma; Animal model

1. Introduction

Phosphatase and tensin homolog (PTEN) is a multifunctional phosphatase whose substrate is phosphatidylinositol-3,4,5-triphosphate (PIP3) [1] and is also a ubiquitously expressed tumor suppressor gene [2] that down-regulates phosphatidylinositol-3-kinases (PI3Ks). Thus, Pten deficiency induces cellular hyperproliferation, antiapoptosis, and oncogenesis via the activation of serine-threonine kinase protein kinase B (PKB/Akt) and its downstream signalings. It is well known that acquired and congenital mutation of *PTEN* induces many human sporadic cancers and tumorigenic hereditary disorders such as Cowden disease, respectively. Although there are a few reports about the PTEN/PI3Ks/Akt pathway related to hepatocellular carcinoma [3–5], the role of the PTEN in the liver remains unclear. Therefore, to clarify the role of PTEN/PI3K/Akt pathway in the liver, we generated and analyzed hepatocyte-specific Pten-deficient mice (Pten-deficient mice) [6]. In this paper, we review the Pten-deficient mice as a model for nonalcoholic steatohepatitis.

2. Steatosis, inflammation, and fibrosis in the livers of Pten-deficient mice

The livers of 10-week-old Pten-deficient mice were enlarged and light-colored (Fig. 1A). At 40 weeks, the livers were further enlarged and white in color (Fig. 1B). HE staining of the livers of 10-week-old Pten-deficient mice revealed that the cytoplasms of hepatocytes mainly around central veins contained micro- and macro-vesicular vacuoles (Fig. 1C, first and second rows right). Oil red O staining confirmed that these vacuoles contained lipids (Fig. 1C, third row right). No fibrotic changes, as determined by Azan staining, were evident in the livers of 10-week-old Pten-deficient

* Corresponding author. Tel.: +81 18 884 6099; fax: +81 18 836 2611.
E-mail address: sumio@doc.med.akita-u.ac.jp (S. Watanabe).

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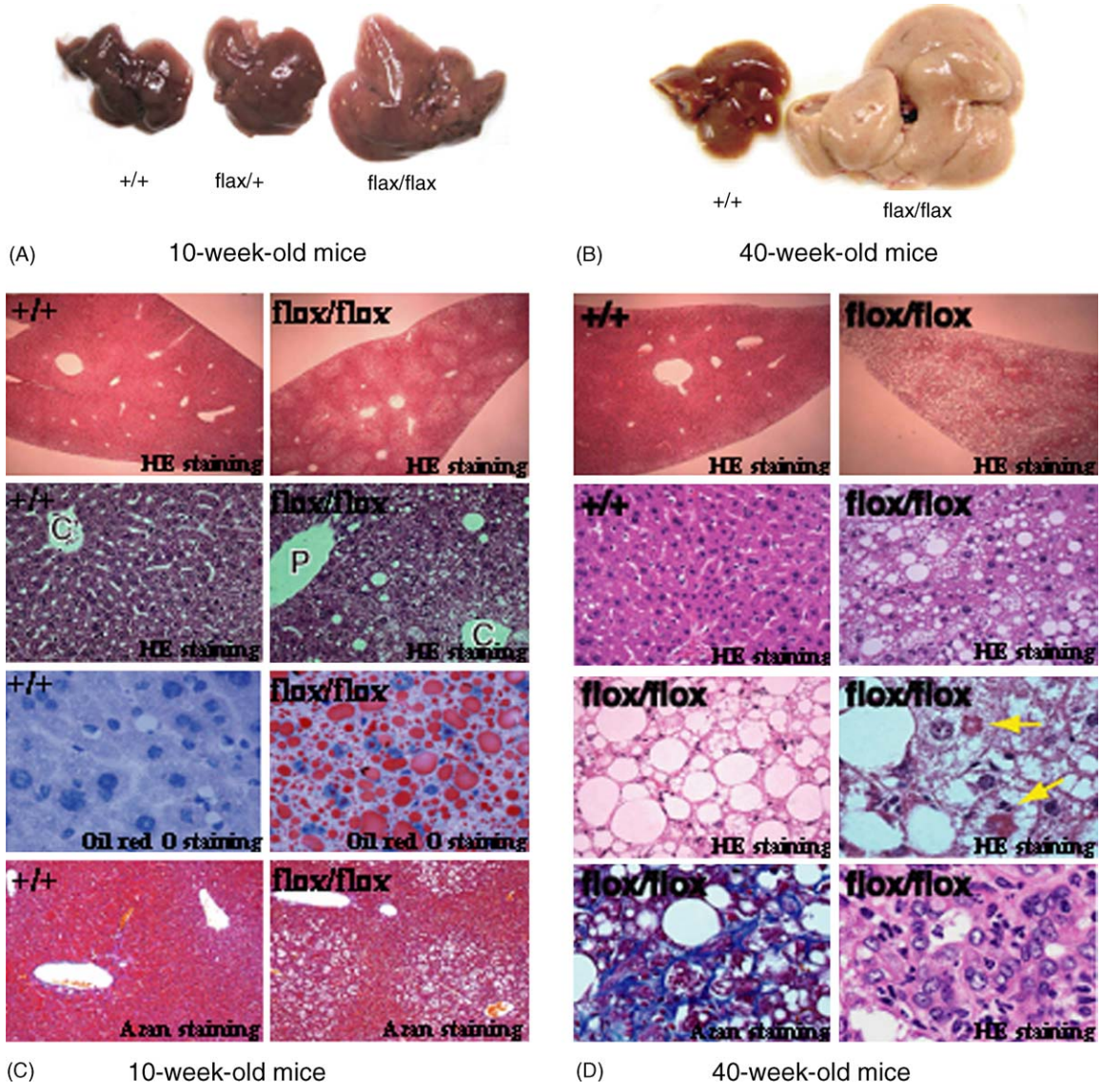

Fig. 1. Steatosis, inflammation, and fibrosis in the livers of Pten-deficient mice. (A and C) Macroscopic and microscopic findings of the livers from +/+, flox/+, and flox/flox mice sacrified at 10 weeks of age. (B and D) Macroscopic and microscopic findings of the livers from +/+ and fox/flox mice sacrified at 40 weeks of age. +/+: wild-ype mice, flox/+: hepatocyte-specific Pten heterozygous mutant mice, flox/flox: hepatocyte-specific Pten-deficient mice. C: central vein, P: portal vein, yellow arrow: Mallory bodies. [Figure reprinted with permission from [6].]

mice (Fig. 1C, fourth row right). By 40 weeks, the livers of Pten-deficient mice showed severe fatty changes throughout the hepatic lobules (Fig. 1D, first and second rows right). The vacuoles coalesced to form unilobular and macrovesicular vacuoles that displaced the nucleus to the periphery (Fig. 1D, third row left). Indeed, the histological picture of hepatocytes resembled that of adipocytes in fat tissues. Moreover, Mallory bodies, ballooning hepatocytes (Fig. 1D, third row right), sinusoidal fibrosis (Fig. 1D, fourth row left) and accumulation of lobular inflammatory cells (Fig. 1D, fourth row right) were also observed in the livers of 40-week-old Pten-deficient mice. These histological findings are strikingly similar to those reported for human nonalcoholic steatohepatitis (NASH) [7].

3. Background of steatosis and inflammation in the livers of Pten-deficient mice

To clarify the background of hepatic steatosis and inflammation, we analyzed the expression of adipogenic, lipogenic and β oxidation-related genes in the livers of Pten-deficient mice. RT-PCR analyses revealed a dramatic induction of PPARγ, a key transcriptional activator for adipogenesis and lipogenesis, in the livers of 10-week-old Pten-deficient mice (Fig. 2). Downstream target genes of PPARγ, such as the adipogenic genes adiponectin, adipsin, and aP2, were also induced in the livers (Fig. 2). Expression levels of PPARα, C/EBPα, and C/EBPδ important for adipocyte differentiation were normal, while expression of C/EBPβ was slightly