Progress in Understanding the Pathogenesis of Nonalcoholic Fatty Liver Disease

Sahai A, Malladi P, Melin-Aldana H, Green RM, Whitington PF. Upregulation of osteopontin expression is involved in the development of nonalcoholic steatohepatitis in a dietary murine model. Am J Physiol Gastrointest Liver Physiol 2004;287:G264-G273. (Reprinted with permission of American Physiological Society)

Abstract

The pathogenesis of nonalcoholic steatohepatitis (NASH) is poorly defined. Feeding mice a diet deficient in methionine and choline (MCD diet) induces experimental NASH. Osteopontin (OPN) is a Th1 cytokine that plays an important role in several fibroinflammatory diseases. We examined the role of OPN in the development of experimental NASH. A/J mice were fed MCD or control diet for up to 12 wk, and serum alanine aminotransferase (ALT), liver histology, oxidative stress, and the expressions of OPN, TNF-α, and collagen I were assessed at various time points. MCD diet-fed mice developed hepatic steatosis starting after 1 wk and inflammation by 2 wk; serum ALT increased from *day 3.* Hepatic collagen I mRNA expression increased during 1-4 wk, and fibrosis appeared at 8 wk. OPN protein expression was markedly increased on *day 1* of MCD diet and persisted up to 8 wk, whereas OPN mRNA expression was increased at *week 4.* TNF-α expression was increased from *day 3* to 2 wk, and evidence of oxidative stress did not appear until 8 wk. Increased expression of OPN was predominantly localized in hepatocytes. Hepatocytes in culture also produced OPN, which was stimulated by transforming growth factor-β and TNF-α. Moreover, MCD diet-induced increases in serum ALT levels, hepatic inflammation, and fibrosis were markedly reduced in OPN$^{-/-}$ mice when compared to OPN$^{+/+}$ mice. In conclusion, our results demonstrate an upregulation of OPN expression early in the development of steatohepatitis and suggest an important role for OPN in signaling the onset of liver injury and fibrosis in experimental NASH.

Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, et al. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. J Clin Invest 2004;113:1774-1783. (Reproduced with permission of the Journal of Clinical Investigation.)

Abstract

PTEN is a tumor suppressor gene mutated in many human cancers, and its expression is reduced or absent in almost half of hepatoma patients. We used the Cre-*loxP* system to generate a hepatocyte-specific null mutation of *Pten* in mice (*AlbCrePten$^{flox/flox}$* mice). *AlbCrePten$^{flox/flox}$* mice showed massive hepatomegaly and steatohepatitis with triglyceride accumulation, a phenotype similar to human nonalcoholic steatohepatitis. Adipocyte-specific genes were induced in mutant hepatocytes, implying adipogenic-like transformation of these cells. Genes involved in lipogenesis and β-oxidation were also induced, possibly as a result of elevated levels of the trans-activating factors PPARγ and SREBP1c. Importantly, the loss of Pten function in the liver led to tumorigenesis, with 47% of *AlbCrePten$^{flox/flox}$* livers developing liver cell adenomas by 44 weeks of age. By 74-78 weeks of age, 100% of *AlbCrePten$^{flox/flox}$* livers showed adenomas and 66% had hepatocellular carcinomas. *AlbCrePten$^{flox/flox}$* mice also showed insulin hypersensitivity. In vitro, *AlbCrePten$^{flox/flox}$* hepatocytes were hyperproliferative and showed increased hyperoxidation with abnormal activation of protein kinase B and MAPK. Pten is thus an important regulator of lipogenesis, glucose metabolism, hepatocyte homeostasis, and tumorigenesis in the liver.

Comments

Nonalcoholic fatty liver disease (NAFLD), representing a spectrum of disorders including fatty liver alone, nonalcoholic steatohepatitis (NASH), and cryptogenic cirrhosis, is a major cause of morbidity and mortality worldwide. Based on analysis of data from the Third National Health and Nutrition Examination Survey (NHANES III), performed from 1988-1994, the prevalence of elevated serum transaminases was 7.9% in the United States, with the majority of instances unexplained and attributed to NAFLD.[1] Since obesity has been established as a major risk factor, albeit not a prerequisite, for NAFLD[2] and obesity is reaching epidemic proportions worldwide, the population at risk for developing chronic liver disease and its complications from NAFLD most likely far exceeds these prevalence estimates and will increase over time. As a result, there is a critical need to better understand the pathogenesis and natural history of NAFLD and develop treatment strategies for these patients.

A "two-hit" model has been proposed to explain the natural history of NAFLD from steatosis to steatohepatitis.[3] According to this model, hepatic steatosis represents the "first hit" in the pathogenesis of NAFLD. Once steatosis develops, hepatocytes are vulnerable to a "second hit" that induces oxidative stress and eventual steatohepatitis that may progress to fibrosis. Much of the progress in understanding the pathogenesis of NAFLD has been possible with the use of several animal models, in particular genetically obese ob/ob mice, lipoatrophic mice, and normal mice and rats fed methionine-restricted, choline-deficient (MCD) diets.[4] Steatohepatitis, but not fibrosis, develops in the leptin-deficient ob/ob mouse model, consistent with an essential role for leptin in liver fibrosis in animal models of NAFLD.[5] By contrast, rats fed an MCD diet eventually develop cirrhosis.[6]

Sahai et al.[7] use this model in which oxidative stress is induced by the depletion of hepatic antioxidants, such as reduced glutathione and *S*-adenosylmethionine, to examine the role of osteopontin (OPN) in NAFLD. OPN is a Th1 cytokine that derives its name from its high expression in bone, although it is also expressed by various cell types, including macrophages, endothelial cells, smooth muscle cells, and epithelial cells.[8] OPN plays a proinflammatory role in several fibrotic disorders and is upregulated in vascular smooth muscle and kidney in diabetes.[9] In liver, OPN is important in experimental models of injury[10] and in the formation of epithelioid granulomas and bile duct injury in primary biliary cirrhosis,[11] and plasma OPN levels are elevated in fulminant hepatitis.[12] OPN has also been implicated in cardiac and lung fibrosis as well as several types of cancers, including hepatocellular carcinoma.[13–15] However, its ability to inhibit production of the proinflammatory mediators nitric oxide and prostaglandin E_2[16] suggests it has a dual role in the regulation of inflammation that might not make it an obvious candidate as a major mediator in NAFLD. Nevertheless, OPN expression markedly increased in mice fed an MCD diet and this increased expression occurred at the posttranscriptional level and preceded increases in tumor necrosis factor α (TNF-α) and collagen I expression and oxidative stress, as measured by hepatic thiobarbituric acid–reactive substance concentration. The predominant location of the increased expression of OPN was hepatocytes, and hepatocytes in culture could be stimulated to produce OPN by TNF-α and tumor growth factor β (TGF-β), cytokines known to play a role in liver disease. Most importantly, although macrovesicular steatosis developed and hepatic triglyceride content increased to a similar degree in OPN$^{-/-}$ mice and OPN$^{+/+}$ mice on an MCD diet, serum ALT levels and hepatic lobular and portal inflammation were significantly decreased in OPN$^{-/-}$ mice compared with OPN$^{+/-}$ mice. In addition, collagen I mRNA expression and perivenular, pericellular, and portal fibrosis were markedly reduced in OPN$^{-/-}$ mice on the MCD diet. Although the mechanism by which hepatocyte-derived OPN delivers the "second hit" to induce injury and fibrosis after fat accumulates in hepatocytes is not addressed by these studies, a heretofore relatively overlooked cytokine has now been given a potential starring role as a signal in the progression of disease in this experimental model of NAFLD.

PTEN (phosphatase and tension homolog deleted on chromosome 10) is a tumor suppressor gene that encodes a lipid phosphatase, whose major substrate is the lipid second-messenger, phosphatidylinositol-3,4,5-triphosphate (PIP3).[17] As a result, PTEN acts as a negative regulator of the signaling pathway for a range of stimuli, including insulin, various cytokines, leptin, and estrogen, that activate phosphatidylinositol 3-kinase (PI3K) and its downstream target, the
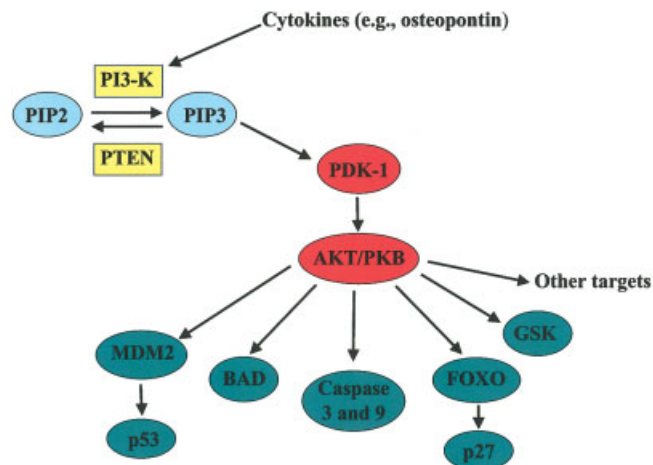

Fig. 1. Schematic representation of factors and signaling pathways involved in the pathogenesis of NAFLD. The second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP-3) is produced by phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP-2), mediated by phosphatidylinositol-3-kinase (PI3-K) in response to a broad range of signals, including cytokines. By dephosphorylating PIP-3, PTEN inhibits a number of downstream signaling molecules that affect cell growth, differentiation, migration, and death, among which the serine/threonine kinase, AKT/PKB, is the best characterized. FOXO, forkhead transcription factor family; GSK, glycogen synthase kinase.

serine-threonine kinase protein kinase B (PKB/Akt), which is involved in antiapoptosis, proliferation, and tumorigenesis (Fig. 1).[17] PTEN has been previously shown to play a role in the pathogenesis of hepatocellular carcinoma, where reduced PTEN expression correlates with tumor progression, elevated alpha-fetoprotein levels, p53 overexpression, and poor prognosis.[18] Hepatocellular carcinoma is recognized as a late complication of NAFLD-related cirrhosis[19] and hepatocyte-specific deletion of *Pten* was recently found to result in hepatomegaly and hepatic steatosis.[20] Horie et al.[21] expand the use of this animal model to examine hepatic tumorigenesis in NAFLD. At 40 weeks of age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and, strikingly, alkaline phosphatase levels were markedly increased in *Alb-CrePten^{flox/flox}* mice, and their livers exhibited histological findings of NASH. Triglyceride and cholesterol ester levels in the livers of mutant mice increased over time and analysis of total fatty acid composition revealed disruption of several aspects of hepatic fat metabolism. RT-PCR analysis demonstrated induction of several adipogenic, lipogenic, and lipid β-oxidation–related genes in hepatocytes of *AlbCrePten^{flox/flox}* mice, including PPARγ, a critical gene in adipocyte differentiation, the adipocyte-specific genes, adipsin, adiponectin, and aP2, and SREBP-1c, a transcription factor that regulates lipid synthesis, and its target genes. Immunohistochemistry confirmed that hepatocytes from mutant mice had acquired expression of the adipocyte-specific marker, adiponectin. By 40 to 44 weeks of age, 66% of male and 30% of female

AlbCrePten^flox/flox mice had developed macroscopic tumors, and by 74 to 78 weeks of age all *AlbCrePten^flox/flox* mice had developed macroscopic tumors, most (8 of 12) demonstrating hepatocellular carcinoma developing within liver adenomas. Consistent with an antiapoptotic and proliferative effect of Pten deficiency, phosphorylation of PKB/Akt, its downstream substrate Foxo1, and MAPK, another major signaling molecule activated downstream of PIP3, was significantly increased in *AlbCrePten^flox/flox* hepatocytes. Therefore, Pten deficiency in mice leads not only to steatohepatitis that resembles that observed in humans, but the dysregulation of signaling pathways that results from loss of this key negative regulator leads to increased hepatic tumor formation.

Insulin resistance, recognized as a proinflammatory condition, with increased oxidative stress, production of cytokines, in particular TNF-α, and activation of cellular stress signaling pathways, is considered to be a major factor in the pathogenesis of NAFLD.[22] However, *AlbCrePten^flox/flox* mice exhibited insulin hypersensitivity, not insulin resistance. In addition, insulin resistance is not the mechanism for the fibrosing steatohepatitis in the MCD dietary model.[23] Yet the lack of insulin resistance in these animal models does not make them any less useful in determining the pathogenesis of and therapeutic targets in NAFLD. As previously noted, insulin resistance may play a role in the development of hepatic steatosis[23] (*i.e.*, the "first hit"), but less of a role, if any, in the progression to inflammation and fibrosis (*i.e.*, the "second hit"). As a result, the recent findings that a PPARα agonist can reverse fibrosis and steatohepatitis in mice fed the MCD diet[24] and the development of selective agents that specifically target the PTEN/PI3K/Akt pathway[25] hold the promise that as we gain more insight into the pathogenesis of NAFLD, new agents may become available to treat this all-too-common liver disease.

RICHARD H. MOSELEY, M.D.
Division of Gastroenterology
Department of Internal Medicine
Ann Arbor VA Health Care System and
University of Michigan Medical School
Ann Arbor, MI

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