PTEN in liver diseases and cancer

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Abstract
The phosphoinositide 3-kinase (PI3K)/phosphatase and tensin homolog (PTEN)/Akt axis is a key signal transduction node that regulates crucial cellular functions, including insulin and other growth factors signaling, lipid and glucose metabolism, as well as cell survival and apoptosis. In this pathway, PTEN acts as a phosphoinositide phosphatase, which terminates PI3K-propagated signaling by dephosphorylating PtdIns(3,4)P(2) and PtdIns(3,4,5)P(3). However, the role of PTEN does not appear to be restricted only to PI3K signaling antagonism, and new functions have been recently discovered for this protein. In addition to the well-established role of PTEN as a tumor suppressor, increasing evidence now suggests that a dysregulated PTEN expression and/or activity is also linked to the development of several hepatic pathologies. Dysregulated PTEN expression/activity is observed with obesity, insulin resistance, diabetes, hepatitis B virus/hepatitis C virus infections, and abusive alcohol consumption, whereas mutations/deletions have also been associated with the occurrence of hepatocellular carcinoma. Thus, it appears that [...]
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The phosphoinositide 3-kinase (PI3K)/phosphatase and TENSin homolog deleted on chromosome 10 (PTEN)/Akt axis is a key signal transduction node that regulates crucial cellular functions, including insulin and other growth factors signaling, lipid and glucose metabolism, as well as cell survival and apoptosis. In this pathway, PTEN acts as a phosphoinositide phosphatase, which terminates PI3K-propagated signaling by dephosphorylating PtdIns(3,4)P\(_2\) and PtdIns(3,4,5)P\(_3\). However, the role of PTEN does not appear to be restricted only to PI3K antagonism, and new functions have been recently discovered for this protein. In addition to the well-established role of PTEN as a tumor suppressor, increasing evidence now suggests that a dysregulated PTEN expression and/or activity is also linked to the development of several hepatic pathologies. Dysregulated PTEN expression/activity is observed with obesity, insulin resistance, diabetes, hepatitis B virus/hepatitis C virus infections, and abusive alcohol consumption, whereas mutations/deletions have also been associated with the occurrence of hepatocellular carcinoma. Thus, it appears that alterations of PTEN expression and activity in hepatocytes are common and recurrent molecular events associated with liver disorders of various etiologies. These recent findings suggest that PTEN might represent a potential common therapeutic target for a number of liver pathologies.

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Key words: Phosphatase and TENSin homolog deleted on chromosome 10; Obesity; Insulin resistance; Non-alcoholic fatty liver diseases; Steatosis; Steatohepatitis; Fibrosis; Hepatocellular carcinoma; Viral hepatitis; Alcohol

INTRODUCTION
Obesity, metabolic syndrome, hepatitis virus infections, and abusive alcohol consumption are major etiological factors contributing together, or independently, to the development of severe liver diseases\([1,2]\). Interestingly, the hepatic pathologies induced by these various factors are associated with common metabolic disorders, i.e. insulin resistance and dysregulated lipid metabolism, and encompass similar histological abnormalities, ranging from hepatic steatosis to steatohepatitis, fibrosis, and cirrhosis\([3]\). Hepatocellular adenoma (HCA) or hepatocellular carcinoma (HCC) might then occur as a likely end stage of these diseases\([4]\).
Deregulations of numerous signaling pathways leading to insulin resistance, steatosis, inflammation, fibrosis, aberrant cell proliferation, and resistance to cell death have been reported. Among these, abnormal signaling through the phosphoinositide 3-kinase (PI3K)/phosphatase and TENsin homolog deleted on chromosome 10 (PTEN)/Akt pathway critically contributes to the development of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic liver disease (ALD), viral hepatitis [hepatitis B virus (HBV) and hepatitis C virus (HCV)], and HCC. Of particular interest in this signaling pathway is the role of PTEN, an important tumor suppressor having a protein and phosphoinositidese phosphatase activity. Indeed, PTEN switches off signaling through the PI3K-Akt axis and by doing that, controls growth factor signaling, thereby acting as a potent tumor suppressor. In the context of hepatic metabolic disorders and cancer, increasing evidence now supports a crucial role of PTEN in the development of these diseases.

PTEN

The PTEN protein was first identified as a potent tumor suppressor that was frequently mutated or deleted in several human cancers, including HCC. The best-characterized function of PTEN is its phosphoinositide phosphatase activity, where it dephosphorylates the PtdIns(3,4,5)P₂ and PtdIns(3,4)P₂ second messengers on the 3′-position of the inositol ring. PTEN thus antagonizes PI3K activation and acts as a potent regulator of growth factor signaling, in particular insulin/insulin-like growth factor (IGF)-I signaling, in peripheral tissues such as the liver. In vitro studies also suggested that PTEN might have a protein phosphatase activity; however additional studies are required to confirm this enzymatic activity.

Compared to other classical tumor suppressor, PTEN represents a particular case, as loss of heterozygocity, or partial inhibition of its expression/activity, is sufficient to promote carcinogenesis, in addition to affecting critical cellular functions, such as glucose and lipid metabolism. Consistent with these observations, PTEN expression and activity appears to be regulated by numerous and complex mechanisms. Among these mechanisms, epigenetic silencing by hypermethylation of its promoter or histone deacetylase activity strongly affect PTEN expression. Several transcription factors, including Egfr, p53, peroxisome proliferator-activated receptor γ, Spry2, Arf2, and Myc, have been shown to directly bind the PTEN promoter and to upregulate PTEN transcription. On the other hand, transcription factors such as nuclear factor (NF)-κB, p300/CBP, Hes-1, Cbf-1, and c-Jun have been shown to negatively regulate PTEN transcription. Adding to the complexity of PTEN expression regulation, recent evidence also indicated that the PTEN mRNA undergoes post-transcriptional repression/degradation by specific microRNAs (miRNAs). Several miRNAs, including miR-21, miR-19a, miR-17-92, miR-214, miR-216a, and miR-217, have been shown to specifically modulate PTEN mRNA expression. Finally, additional processes, whereby the PTEN protein level and activity are modulated, occur post-translationally. These include modifications of the protein, such as phosphorylation, acetylation, ubiquitination, and the REDOX state, which affect PTEN stability, degradation and enzymatic activity. Similarly, PTEN sequestration in specific subcellular compartments or membranes through interactions with distinct proteins, i.e. FAK, MAST proteins, p53, NHERF1/2, and PICT1, likely represent additional mechanisms controlling PTEN stability and/or activity.

PTEN IN HEPATIC INSULIN RESISTANCE

Functional defects of key intracellular signaling proteins, in particular in the mammalian target of rapamycin (mTOR)/PI3K/PTEN/Akt pathways, are clearly associated with insulin unresponsiveness of peripheral tissues. Indeed, the imbalance between propagation and termination of signaling through mTOR/PI3K/PTEN/Akt represents basal molecular dysfunctions triggering the development of insulin resistance and type II diabetes. Given the enzymatic activity of PTEN, dysregulation of its activity/expression are potentially important mechanisms contributing to insulin resistance in tissues such as the liver.

Several in vivo studies, where PTEN expression was genetically altered in various organs of mice, support a crucial role for PTEN expression/activity in insulin sensitivity. PTEN haploinsufficiency and PTEN muscle-specific deletion were shown to improve skeletal muscle insulin sensitivity and to protect mice from insulin resistance and diabetes caused by high fat feeding, respectively. Deletion of PTEN in the adipose tissue prevented the development of streptozotocin-induced diabetes. Treatment of db/db mice with PTEN antisense oligonucleotides normalized plasma glucose levels in these animals.

Interestingly, liver-specific PTEN knockout mice also have an improved systemic insulin sensitivity and glucose tolerance. However, whether this is related to increased insulin sensitivity specifically in the liver, or to a complex in vivo systemic crosstalk between a PTEN-deficient liver and other peripheral tissues, remains unclear. In support of this latter hypothesis, PTEN deletion in the liver is accompanied by decreased circulating levels of leptin and body fat content. In addition, we observed that although constitutive Akt activity is increased in cultured hepatoma cells having downregulated PTEN, insulin signaling upstream of Akt, i.e. insulin receptor/IRS1 expression and phosphorylation, is impaired, as it has been already described in cancer cells. Consistent with these findings, we observed a lack of insulin responsiveness in terms of gene expression in PTEN deficient hepatocytes. These data raise the hypothesis that PTEN downregulation in hepatocytes might, paradoxically, cause insulin resistance despite an increased activation of specific insulin effectors, such as Akt. Further studies are needed to clarify whether
PTEN downregulation in hepatocytes is a causal factor for insulin resistance in this organ.

### PTEN IN NAFLD

Although liver-specific PTEN knockout mice have an overall improved systemic insulin sensitivity, they develop an important steatosis in the liver, suggesting that PTEN is required for an appropriate control of the hepatic lipid metabolism\[54,55\]. Consistent with these studies, hepatic PTEN expression is downregulated in obese and insulin resistant rat animal models and in humans having steatosis\[30\]. Further studies indicate that high levels of circulating free fatty acids, but not glucose or insulin, downregulate PTEN expression in hepatocytes\[39-41\]. PTEN downregulation by free fatty acids is triggered by an increase in miR-21, a miRNA targeting PTEN mRNA for degradation, through mTOR/NF-κB-dependent mechanisms\[80,86\]. In addition to an excess of circulating free fatty acids, a deregulated production of inflammatory cytokines by immune cells and/or of adipokines by the adipose tissue, as observed with NAFLD\[57\], can also independently, or synergistically with fatty acids, alter PTEN expression. Indeed, inflammatory cytokines, such as transforming growth factor-β\[59,58,60\], tumor necrosis factor (TNF)α\[29,61\], interleukin (IL)-6\[62\], IL-1β\[63\], or adipokines such as leptin, resistin and adiponectin\[64,65\], have been reported to either up- or downregulate PTEN expression or activity in various cells. Although most of these cytokines/adipokines are clearly involved in liver insulin sensitivity and steatosis/fibrosis/inflammation\[57,66\], it remains to be firmly established whether these factors modulate PTEN expression/activity in the liver and whether there is a causal relationship between potential PTEN alterations induced by these cytokines/adipokines and their beneficial/detrimental effects on the liver physiology.

PTEN loss of function in the liver leads to a progressive and step-wise development of steatohepatitis and fibrosis\[34,35,67\]. Consistent with studies using liver-specific PTEN knockout mice, decreased PTEN expression was also observed in the liver of rodents with hepatic fibrosis induced either by biliary stenosis or a choline-deficient diet\[68,69\]. Finally, PTEN depletion in hepatoma cells induces the expression of genes promoting inflammation, epithelial-to-mesenchymal transition, and fibrosis\[70\]. Taken together, these studies suggest that pathological dysregulation of PTEN expression/activity causing steatosis might also promote progression of this disorder towards different clinical stages of increasing severity. The molecular mechanisms by which PTEN deficiency triggers steatosis, inflammation, and fibrosis development in hepatocytes are still poorly defined. However, evidence indicates that de novo fatty acids synthesis is enhanced\[40,55\] in liver-specific PTEN knockout mice, whereas in cultured cells, accumulation of neutral lipids seems to rely mainly on increased fatty acids uptake and esterification\[54\]. These discrepant data might originate either from the different methodologies used to investigate fatty acid metabolism or, more likely, from the different extents of PTEN repression, i.e. complete deletion in knockout mice vs 40%-80% downregulation induced by fatty acids or silencing RNAs in cultured cells. Partial PTEN downregulation or total deletion can indeed mediate very different effects, as it was elegantly demonstrated in studies examining the role of PTEN in prostate tumor progression\[51,71\].

### PTEN IN LIVER CARCINOGENESIS

The first evidence supporting a critical role for PTEN in liver cancer came from genetic studies in mice, where heterozygous deletion of PTEN was shown to induce atypical adenomatous liver hyperplasia\[72\]. Additional studies then demonstrated that PTEN deficiency in the liver induces hepatomegaly, HCA, and HCC with ageing\[54,55\]. Weak expression or mutation/deletion of PTEN, as well as upregulation of miRNAs specifically targeting PTEN for degradation, are also frequently observed in human HCC\[11,13,16,34,73-76\]. However, the tumor suppressor activity of PTEN seems to principally involve its antagonistic effects on the anti-apoptotic, proliferative, and hypertrophic activity of PI3K\[77\]; recent studies demonstrated that PTEN also plays an essential role in the nucleus to maintain chromosomal stability and for DNA repair\[78\]. In addition, there is evidence indicating that PTEN can modulate cancer cell invasiveness by stabilizing E-cadherin/β-catenin adherens junctional complexes\[79,80\]. Finally, we demonstrated that fatty acids-mediated PTEN downregulation in hepatocytes promotes cell proliferation, migration, and invasiveness, in addition to modulating a set of genes involved in cell cycle regulation and HCC\[81\]. As inflammation, EMT and genomic alterations are typical features of HCC\[80,82\], impaired PTEN expression or activity can thus represent an important step in progression of NAFLD towards HCC. Further studies are however still needed to confirm the relevance of PTEN as a prognostic marker for the risk of HCA/HCC development.

### PTEN IN VIRAL HEPATITIS

Infections by HBV and HCV are major contributors to the high incidence of HCC, particularly in South-East Asia and Africa\[83,84\]. Similarly to NAFLD and NASH, HCV infection is strongly associated with liver insulin resistance and causes steatosis and fibrosis\[85,86\]. However, whether HBV infection causes similar liver disorders remains unclear\[87\].

Only a few studies have examined the involvement of PTEN in HBV/HCV-associated hepatocyte dysfunction. The HBV-X protein (HBx) was shown to trigger uncontrolled Akt activation by downregulating PTEN expression in Chang liver cells, thereby enhancing the invasive potential of these cells\[88,89\]. In accordance with these data, PTEN overexpression in Chang cells reversed pro-survival signaling and inhibited apoptosis induced by HBx\[89\]. In addition, PTEN was also shown to prevent HBx-mediated induction of IGF-II expression in hepa-
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Figure 1  Alterations of phosphatase and TENsin homolog deleted on chromosome 10 expression/activity in the liver by various etiological factors and associated liver disorders. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCA: Hepatocellular adenomas; CC: Cholangiocellular carcinomas; HCC: Hepatocellular carcinomas; PTEN: Phosphatase and TENsin homolog deleted on chromosome 10.

**PTEN IN ALD**

ALD also encompass a spectrum of histological and functional liver disorders, ranging from a relatively benign steatosis to alcoholic hepatitis, cirrhosis, and cancer. Given the outcomes of alcohol abuse in the liver, and the effects of PTEN deletion or downregulation for the liver physiology, it could be expected that ethanol induces alterations of PTEN expression/activity in the liver. Surprisingly, increased apoptosis and decreased insulin signaling in hepatocytes of Long-Evans rats chronically fed with ethanol (serum ethanol levels is about 50 mmol/L) was associated with increased levels of PTEN mRNA and protein, thus suggesting that ethanol upregulates PTEN expression in the liver. Increased hepatic PTEN expression was also observed in rats exposed to alcohol in utero. Consistent with these studies, chronic exposure of hepatoma HepG2E47 cells to ethanol increased PTEN expression and subsequently increased the sensitivity of cells to TNFα-induced cytotoxicity and apoptosis. In contrast, acute ethanol exposure did not affect PTEN expression in Huh-7 hepatoma cells. However, in these cells, ethanol increased the physical association between PTEN and the PI3K regulatory subunit p85α, which functionally resulted in a decreased Akt and downstream effectors activity.

Taken together, these studies indicated that, in contrast to the PTEN downregulation occurring with NAFLD, PTEN expression/activity is upregulated with ALD. This opposite regulation of a critical signaling effector strongly suggests that the mechanisms of insulin resistance and steatosis development in the context of NAFLD and ALD are distinct. In addition, PTEN might represent a differential diagnostic marker to distinguish between liver disorders with these different etiologies.

**CONCLUSION**

Accumulating evidence indicates that PTEN is a major dysregulated cellular factor contributing to the development of a broad spectrum of hepatic disorders, i.e. insulin resistance, steatosis, steatohepatitis, fibrosis, cirrhosis, and cancer (Figure 1). Indeed, hepatic PTEN expression/activity is altered in liver diseases associated with obesity, metabolic syndrome, viral infection, and alcohol consumption. Thus, it appears that dysregulation of PTEN expression/activity in hepatocytes represents an important and recurrent molecular mechanism contributing to the development of liver disorders with distinct etiologies.

Although hepatic steatosis is currently regarded as a benign disease, progression to inflammation, fibrosis, and cirrhosis can lead to liver failure and development of HCC. There are multiple molecular factors involved in the progression of hepatic steatosis towards more severe stages and among those, dysregulation of PTEN expres-
sion/activity, more than PTEN mutations or deletions, could be a critical step in the occurrence and development of these diseases. In addition, PTEN alterations induced by high levels of free fatty acids or inflammatory cytokines, provide an interesting link between insulin resistance and steatosis, which might also explain, at least in part, the high risk factor for HCA/HCC associated with diabetes and obesity.\[89,90\]. Given the tumor suppressor activity of PTEN, the role of the seostasis and steatohepatitis as preneoplastic states in the hepatocyte malignant transformation should also be re-evaluated. Additional studies are now required to carefully evaluate PTEN as a differential prognostic marker in liver pathologies with distinct etiologies and to assess the pertinence of future therapeutic interventions to restore physiological PTEN expression in the liver to prevent, or to alleviate, hepatic metabolic disorders and HCC.

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