PTEN down-regulation by unsaturated fatty acids triggers hepatic steatosis via an NF-kappaBp65/mTOR-dependent mechanism

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Abstract

BACKGROUND & AIMS: Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a tumor suppressor and a regulator of insulin sensitivity in peripheral tissues. In the liver, PTEN deletion increases insulin sensitivity, but induces steatosis, steatohepatitis, and hepatocellular carcinoma. Here, we investigated the pathophysiologic mechanisms regulating PTEN expression in the liver and the development of steatosis. METHODS: PTEN expression was evaluated in the liver of rats and human beings having metabolic syndrome. Signaling pathways regulating PTEN expression and lipid accumulation in hepatocytes were examined in vitro. RESULTS: PTEN expression is down-regulated in the liver of rats having steatosis and high plasma levels of fatty acids, as well as in steatotic human livers. Unsaturated fatty acids inhibited PTEN expression in HepG2 cells via activation of a signaling complex formed by the mammalian target of rapamycin (mTOR) and nuclear factor-kappaB (NF-kappaB). Down-regulation of PTEN expression induced steatosis by affecting import, esterification, and extracellular release of fatty acids. CONCLUSIONS: [...]
SUPPLEMENTAL METHOD I

Lentiviral transduction of PTEN siRNAs in HepG2 cells.

A PTEN-specific 19-nucleotide sequence, corresponding to the 1065-1083 region (relative to the first nucleotide of the ATG) in the human PTEN transcript (i.e. TCCAGAGGCTAGCAGTTCA and the complementary strand separated by a TCTCTTGAA spacer), was inserted in the mammalian expression vector pSuper.basic (OligoEngine, UK) to obtain pSUPER-ShPten. H1-ShPten cassette from pSUPER-ShPten was then subcloned in pLVTHM vector (kindly provided by D. Trono, EPFL, Switzerland) by replacing the H1 promoter cassette in pLVTHM by the H1-ShPten cassette excised from pSUPER-shPten using standard cloning procedures. All constructs were confirmed by DNA sequencing. Recombinant lentiviruses were produced by transient transfection in 293T cells according to standard protocols. Viral titers ranged between $1 \times 10^6$ and $5 \times 10^6$ cell-transducing U per ml as determined by flow cytometric analysis of GFP expression in HeLa cells. HepG2 cells were then transduced with lentiviral vectors at a multiplicity of infection of 20 for 16 h (more than 90% of HepG2 cells were transduced).