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Prohibitin-1 deficiency promotes inflammation and increases sensitivity to liver injury

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ABSTRACT

Liver diseases are the fifth cause of mortality in Western countries, and as opposed to other major causes of mortality, their incidence is increasing. Understanding the molecular background contributing to the progression of liver ailments will surely open new perspectives for the better management of patients. The aim of this study is to elucidate mechanisms underlying the progression of liver injury associated with deficient prohibitin 1, an essential protein to maintain mitochondrial homeostasis and gene expression. *PHB1*+/– mice developed a more severe steatohepatitis than WT littermates when exposed to a choline and methionine deficient diet. The increased sensitivity was mediated by mitochondrial dysfunction and metabolic impairment in *PHB1*+/– livers, including inactivation of AMP kinase, measured under a non-restricted diet. Moreover, pro-inflammatory challenges induced higher mortality and liver injury in PHB+/– mice. The increased proliferative capacity of *PHB*+/– splenocytes, resulting from constitutive defects in central molecular pathways as stated by deregulation of GSK3β, Erk, Akt or SHP-1, and the concomitant overproduction of pro-inflammatory mediators in Phb1 deficient mice, might account for these effects. In light of these results it might be concluded that Phb1 deficiency is a potential driver of chronic liver diseases by inducing hepatocyte damage and inflammation.

1. Introduction

Liver disorders, afflicting more than 10% of the world population, are the fifth cause of mortality in the Western countries [1]. Great efforts have been devoted in recent years to identify driver mechanisms of liver disease progression aiming to improve the management of the afflicted patients. Independently of the aetiology, chronic liver injury and inflammation are increasingly recognized as pivotal factors in the advance of the pathogenic process [2]. Moreover, as one of the primordial targets of liver injury, mitochondrial dysfunction resulting in metabolic imbalance, apoptosis deregulation and oxidative

Abbreviations: Phb, prohibitin; MCDD, methionine and choline deficient diet; LPS, bacterial lipopolysaccharide; PP2A, protein phosphatase 2 A; GSK, glycogen synthase kinase; ETF, electron transfer flavoportein; ADH, aldehyde dehydrogenase; AMPK, AMP kinase; IPA, Ingenuity Pathway Analysis; IL, interleukin; TLR, toll like receptor; TNF, tumor necrosis factor; LIX, LPS induced chemokine; MIP, macrophage inflammatory protein; VCAM, vascular cell adhesion protein; PF-4, platelet factor 4; TPO, trombopoietin; DUSP, dual specificity phosphatase; F3, coagulation factor 3; HMOX, hemooxigenase; Shp1, Scr homology region 2 domain-containing phosphatase 1; Lck, lymphocyte specific protein tyrosine kinase.

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stress, is one of the principal factors compromising hepatocyte homeostasis [3]. In this regard, the identification of proteins regulating mitochondrial function, inflammation, and therefore hepatocyte fate, is of great interest since they may pave the way to the understanding of the molecular pathogenesis of chronic liver diseases.

Since prohibitin 1 (Phb1) was firstly cloned in 1991, evidence has been accumulated indicating its essential participation in cellular homeostasis. Phb1 is a ubiquitously expressed protein in eukaryotic cells [4] that exhibits a high degree of sequence homology among species. *PHB1* gene is located on chromosome 17q21 and encodes a 30 kDa protein that associates with Prohibitin 2 (Phb2) forming 16–20-mer ring-like structures with chaperone or scaffolding activities within the mitochondria [5]. The biological activity of Phb1 depends on the regulation of target proteins by direct interaction, which is modulated by posttranslational modifications [6], in different subcellular environments, most importantly mitochondria and nucleus [7]. Despite the increasing number of cellular protein intermediates which function have been reported to be controlled by Phb1, the definition of its molecular function based on an integrated envision of its interacting network, is still elusive. Phb1 has been involved in cell cycle control, differentiation, apoptosis and senescence, arising as a central node to determine cell fate. Alteration of Phb1 levels has been associated with pathologies including inflammation, obesity, autoimmunity or cancer [8–10]. Based on its antioxidant properties [11] Phb1 has been reported to prevent the inflammation-associated oxidative stress and injury [12] and has been proposed as a potential therapeutic target for the treatment of a variety of diseased states [13–15].

Recent correlation has been established between Phb1 deficiency and the progression of liver malignancies. Deregulation of Phb1 in the liver has been detected early in the development of NASH in mice [16,17] and also as a consequence of ethanol feeding [18], MCDD [19] or partial hepatectomy [20]. In agreement with these observations, it has been recently shown that complete loss of Phb1 in conditional liver-specific Phb1 KO mice rapidly promotes NASH and further HCC [21]. However, it is worth to consider that complete ablation of Phb1 is a lethal condition [22] and therefore a partial deficit might be more closely related to pathogenic conditions as it has been shown in obesity [16,17], condition at risk of liver disorders.

In the present study, we have examined for the first time the effect of a Phb1 deficiency in the progression of liver damage. Our results illustrate that partial deficiency of Phb1 leads to an increased sensitivity to liver insults and inflammatory aggressions due to mitochondrial defects in liver cells and a basal activation of lymphocytic cells leading to enhance inflammatory reactions.

2. Materials and methods

2.1. Animal experiments

PHB1+/–[22] and WT mice were housed in a temperature-controlled room with a 12 h light cycle and given fresh water and food ad libitum. Studies were approved by the University of Navarra Committee on Animal Care and satisfied National Institutes of Health guidelines for human treatment of animals.

For animal operations, male *PHB1*+/– and WT littermates were anaesthetised with ketamine chloride (Imalgène, Merial). Serum samples, obtained after centrifugation at 1500 ×*g* from 500 μl of blood collected by retro-orbital puncture, were analyzed in a Cobas analyzer (Roche). Mice were sacrificed for histological examination of the liver. Liver and spleen specimens were snap frozen in liquid nitrogen, and stored at −80 °C.

Five-month-old male WT and *PHB1*+/– mice were challenged with a MCDD (MP Biomedicals, LLC) either for 48 h or for 3 weeks followed by whole diet replacement for 48 h. A sub-lethal dose (15 mg/kg) of endotoxin (from *Salmonella typhimurium* LPS, Sigma) dissolved in sterile, pyrogen-free saline (n=5) was administered intraperitoneally, and survival was monitored up to 48 h post-treatment. Animals were treated with 22 mg/kg of Concanavalin A (L7647, Sigma) intravenously for 52 h. Liver sections were stained with hematoxylin and eosin for further histological examination.

2.2. AntiCD3 in vitro treatment of splenocytes

Splenocytes were isolated in 0.15 M ammonium chloride, 10 mM potassium bicarbonate, 0.1 mM disodium EDTA and exposed to 0.5, 0.25, 0.125 or 0.06 μg/ml antiCD3 (BD Pharmigen) during 72 h (n=6). Tritiated thymidine (0.5 μCi) was added for the last 16 h and proliferation was assessed as counts per minute (cpm) in a TopCount NXT (Perkin Elmer).

2.3. Microarray hybridization and data processing

Total RNA extraction, processing, labeling and hibridization on Mouse Gene 1.0 ST arrays (Affymetrix) were performed as previously described [23]. Bioinformatic analysis was done using RMA algorithm for data normalization and after filtering, LIMMA was used for the selection of differential features between WT and *PHB1*+/– conditions ($p<0.05$ and $|FC|=0.5$) [24].

2.4. Immunoblotting analysis

Fifteen micrograms of protein was resolved in 12.5% SDS-PAGE gels. Protein electrophoresis and blotting were done as described [25]. Primary antibodies were: Phb1 (Calbiochem); Phb2 (Upstate); SHP-1 (ECM Biosciences); β-Actin, IL-1α, pPP2A Y307 (Abcam); pErk Thr202/Tyr204, AMPKα, pAkt Ser473, Akt, Lck, PP2A sub A, pGSK3β S9 (Cell Signaling). After incubation with the appropriate horseradish peroxidase-conjugated secondary antibody (1:5000), the immunoreactivity was visualized by enhanced chemiluminiscence (Perkin Elmer). Equal loading of the gels was assessed by Ponceau staining and by β-actin detection with a specific antibody.

2.5. Cytokine protein array

A dot-blot protein array was used for cytokine profiling (RayBiotech). Briefly, membranes with 62 cytokine antibodies were blocked with the manufacturer's BSA buffer and incubated for 2 h with 150–200 μl of undiluted serum samples from 8 month WT and *PHB1*+/– mice (n=4). After washing, a biotinylated anti-cytokine antibody mixture was added to the membranes followed by incubation with HRP-conjugated streptavidin and then exposed to the manufacturer's peroxidase