Research Article

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PP2Acα positively regulates the termination of liver regeneration in mice through the AKT/GSK3β/Cyclin D1 pathway

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Background & Aims: Liver injury triggers a highly organized and ordered liver regeneration (LR) process. Once regeneration is complete, a stop signal ensures that the regenerated liver is an appropriate functional size. The inhibitors and stop signals that regulate LR are unknown, and only limited information is available about these mechanisms.

Methods: A 70% partial hepatectomy (PH) was performed in hepatocyte-specific PP2Acα-deleted (*PP2Acα*$^{-/-}$) and control (PP2Acα$^{+/+}$) mice. LR was estimated by liver weight, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and cell proliferation, and the related cellular signals were analyzed.

Results: We found that the catalytic subunit of PP2A was markedly upregulated during the late stage of LR. *PP2Acα*$^{-/-}$ mice showed prolonged LR termination, an increased liver size compared to the original mass and lower levels of serum ALT and AST compared with control mice. In these mice, cyclin D1 protein levels, but not mRNA levels, were increased. Mechanistically, AKT activated by the loss of PP2Acα inhibited glycogen synthase kinase 3β (GSK3β) activity, which led to the accumulation of cyclin D1 protein and accelerated hepatocyte proliferation at the termination stage. Treatment with the PI3K inhibitor wortmannin at the termination stage was sufficient to inhibit cyclin D1 accumulation and hepatocyte proliferation.

Conclusions: PP2Acα plays an essential role in the proper termination of LR via the AKT/GSK3β/Cyclin D1 pathway. Our findings enrich the understanding of the molecular mechanism that controls the termination of LR and provides a potential therapeutic target for treating liver injury.

Keywords: PP2A; Liver injury; Termination of liver regeneration; AKT; GSK3β.
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Abbreviations: LR, liver regeneration; PH, partial hepatectomy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GSK3β, Glycogen synthase kinase-3β; PP1, protein phosphatase1; NMR, nuclear magnetic resonance; NPCs, non-parenchymal cells.

Introduction

The liver has a remarkable capacity to regain its size, architecture, and function in response to loss of mass caused by a variety of injuries. This regenerative capacity provides assurances for the clinical outcome of patients after a serious hepatic injury, cancer resection, or living donor liver transplantation. Therefore, understanding the mechanisms that govern liver regeneration (LR) will shed light on the etiology of chronic and acute liver diseases, as well as hepatocarcinogenesis.

After partial hepatectomy (PH), hepatocytes exit their quiescent and highly differentiated state and rapidly re-enter the cell cycle, which causes enlargement of the residual lobe to compensate for the removed mass. The whole regenerative process includes initiation, proliferation and termination stages that involve the participation of numerous growth factors and cytokines, including HGF, EGF, TGF-β, and TNF-α [1]. After approximately two rounds of hepatocyte replication, the liver mass is reestablished, and the liver morphology is gradually rearranged and restored. Eventually, the regenerated liver mass is adjusted to the pre-hepatectomy size with a relatively high accuracy [2].

Although studies on LR have been conducted for many years, most have focused on the initiation stage, whereas few have concentrated on the termination stage. Until now, only limited information has been available about the inhibitory factors and stop signals, such as TGF-β1, that are involved in hepatic regeneration. Inhibiting type II TGF-β receptor activity with adenovirus or gene knockout markedly increases DNA synthesis in murine hepatocytes [3,4]. Other studies have shown that upregulation of SnoN, an inhibitor of TGF-β1, can also delay the termination of LR [5]. However, intact TGF-β1 signaling is not required for the termination of LR, unless the activin receptor has been inactivated [6]. In

addition, Apte and colleagues demonstrated that the ablation of integrin-linked kinase leads to enhanced liver proliferation, ultimately resulting in a liver that is 1.3-fold larger than the original [2]. A recent report suggested that cooperation among C/EBP family proteins and chromatin remodeling proteins is essential for LR termination [7].

Reversible phosphorylation regulated by phosphokinases and phosphatases is one of the most important mechanisms for regulating proliferation, migration, and tissue regeneration. Protein phosphatase 2 (PP2A) makes up 1% of all cellular proteins and, together with protein phosphatase 1 (PP1), accounts for over 90% of serine/threonine phosphatase activity in cells [8]. The PP2A holoenzyme complex consists of three distinct functional components: the catalytic subunit (PP2Ac), the structural subunit (PR65 or A subunit) and the regulatory subunit (B subunit). PP2A regulates many cellular processes, including signal transduction, cell cycle progression, DNA replication, gene transcription and protein translation [9–11]. PP2A has been proposed as a negative regulator of cellular growth. Inhibiting PP2A triggers premature meiosis and mitosis and the increased expression of several proto-oncogenes [9,12]. Although a number of studies have demonstrated that PP2A is critical for cell cycle control, the role of PP2Acα in regulating the mitogenic capacity of the regenerated liver remains unexplored. Herein, we performed PH on wild-type and *PP2Acα*$^{-/-}$ mice and analyzed the rate of LR and the activity of PP2A-regulated signaling pathways. We demonstrated that the induction of PP2Acα expression following PH is responsible for normal termination of regeneration. Mice with liver-specific ablation of PP2Acα exhibited impaired LR termination, resulting in a liver that was 30% larger than the pre-PH mass. The sustained cell proliferation and resulting abnormal regeneration size might be mediated by the AKT/GSK3β/Cyclin D1 pathway. To the best of our knowledge, this is the first time that PP2Acα has been characterized as a novel positive regulator of LR termination.

Materials and methods

Partial hepatectomy

All the animals used in the study were 8- to 10-week-old male mice on a mixed genetic background (129SV and C57/BL6). PH was performed between 8 and 12 am, and the mortality rate was <5%. Mice were sacrificed at the indicated time points after PH.

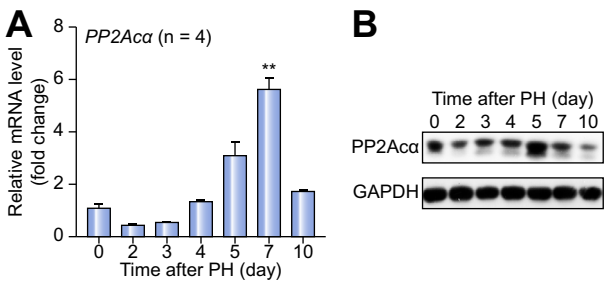

Fig. 1. PP2Acα expression pattern during LR progression. (A) Quantification of hepatic *PP2Acα* expression at the indicated time points after PH. $^{**}p$ <0.01. (B) Protein expression of PP2Acα during LR progression was analyzed via Western blot.

Histology

Liver tissues were fixed in 4% paraformaldehyde and embedded in paraffin. 5 μm paraffin liver sections were stained with hematoxylin and eosin for morphological examination and hepatocyte mitotic figures or used for routine immunofluorescent staining.

Protein extraction and Western blot

Whole liver protein was extracted from mice using RIPA buffer containing 1% SDS. 50 μg of total lysates was separated by 10–15% SDS-PAGE gel and transferred onto PVDF membranes, and blotted with appropriate antibodies.

Statistical analysis

Results are presented as means ± standard deviation. Statistical analyses were performed with Student's *t* test, and *p* values less than 0.05 were considered significant.

Detailed Materials and methods are provided in Supplementary data.

Results

PH induces a gradual and significant elevation of PP2Acα mRNA and protein in the regenerated liver

To explore the function of PP2Acα in LR, we first examined whether PH could induce PP2Acα in the regenerated liver. At the early stage of LR, there was no obvious change in the mRNA levels of *PP2Acα* (a small decrease on days 2 and 3) (Fig. 1A). *PP2Acα* expression gradually increased as regeneration advanced, increasing by approximately 3.1-fold and 5.6-fold on days 5 and 7 post-PH, respectively. However, there was a sharp decrease thereafter. PP2Acα protein levels also increased as the regeneration progressed and reached a peak on day 5 (Fig. 1B). According to Mohammed's schematic of the start and stop signals during LR [13], we postulated that the increase in PP2Acα expression after PH is specific to the late stage and might contribute to the termination of LR.

Generation of PP2Acα conditional knockout mice

Because the lack of the α isoform of the catalytic subunit results in loss of catalytic activity of the PP2A holoenzyme, we generated liver-specific *PP2Acα*-knockout mice by breeding PP2Acα-floxed mice [14] with albumin-Cre transgenic (Alb-Cre) mice (provided by Model Animal Research Center, Nanjing), which express Cre recombinase specifically in the liver under the control of the albumin promoter (Fig. 2A). We crossed *PP2Acα*$^{flox/flox}$ mice with Alb-Cre mice to generate Alb-Cre-*PP2Acα*$^{flox/+}$ mice (*PP2Acα*$^{+/-}$); *PP2Acα*$^{+/-}$ mice were crossed to *PP2Acα*$^{flox/flox}$ mice, yielding Alb-Cre-*PP2Acα*$^{flox/flox}$ mice (hereafter termed *PP2Acα*$^{-/-}$). *PP2Acα*$^{flox/flox}$ (*PP2Acα*$^{+/+}$) mice obtained from the same breeding were used as controls. As expected, *PP2Acα*$^{-/-}$ mice exhibited an 85% reduction in PP2Acα protein expression in the liver (Fig. 2B). Because the Alb promoter is active mainly in hepatocytes, it is likely that PP2Acα is expressed in other liver cells. Therefore, there was still significant expression of PP2Acα in *PP2Acα*$^{-/-}$ mice. *PP2Acα*$^{-/-}$ mice showed a similar liver structure and morphology to control mice (Fig. 2C). Although the body weight of *PP2Acα*$^{-/-}$ mice was not different from that of control mice, the liver weight and the liver/body weight ratio were slightly increased (Fig. 2D). Next, we compared the proliferation status