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Liver-specific deletion of LASS2 delayed regeneration of mouse liver after partial hepatectomy

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ABSTRACT

The capacity of liver regeneration is critical for patients with liver diseases. However, cellular and molecular mechanisms of liver regeneration are still incompletely defined. Here, we assessed roles of LASS2 in liver regeneration following partial hepatectomy (PHx) in mice. Our results showed that protein level of LASS2 remarkably increased during liver regeneration after PHx in wildtype (WT) mice. Comparing to WT mice, liver regeneration index after PHx was significantly decreased from day 1 to day 5 in liver-specific LASS2 knockout (LASS2-LKO) mice. Interestingly, liver mass of LASS2-LKO mice could sufficiently recover at day 14 after PHx. Immunohistochemistry (IHC) and western blot analyses revealed that proliferation markers, such as PCNA and Ki67, were potently reduced during liver regeneration in LASS2-LKO mice. In addition, several cell cycle related molecules, such as cyclin A, CDK2 and p-Rb, were decreased in LASS2-LKO mice after PHx. Co-immunoprecipitation assay further revealed a decreased formation of CDK4/cyclin D1 complex after PHx in LASS2-LKO mice. However, phosphorylation of Akt was significantly activated from day 2 after PHx in LASS2-LKO mice when compared with that in WT mice, which may explain the recovery of liver mass at the late stage of liver regeneration in LASS2-LKO mice. Taken together, we conclude that LASS2 plays an important role in efficient liver regeneration in response to PHx.

1. Introduction

Liver is the only visceral organ that possesses unique capacity to regenerate. Although normally quiescent, hepatocytes can quickly enter the cell cycle and undergo division to restore liver size for proper functions after surgical removal or chemical injury [1,2]. Following injury, hepatocyte proliferation is efficient until the restoration of original liver mass and then precisely switched off. Clinically, liver regeneration is critical for patients of liver diseases with partial liver removal due to fibrosis or tumor. Insufficient regeneration of the remaining liver may be potentially fatal to these patients [3,4]. Therefore, elucidating underlying mechanisms and physiological features of liver regeneration potentially lead to great clinical benefits.

The technique of 2/3 partial hepatectomy (PHx) is a method that has been widely used to study liver regeneration in rodents [5]. Normally, liver mass is rapidly restored after PHx, and the original mass ratio of liver/body is recovered within a week in rodents. Experimental analyses using PHx models have identified many signals and molecular regulators, including cytokines, growth factors, intracellular signaling events, and transcription factors, that are essential for hepatocyte proliferation and normal liver

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regeneration [6–8]. However, detailed mechanisms involved in liver regeneration still remain largely unclear.

Homo sapiens longevity assurance homolog 2 of yeast LAG1 (LASS2), also known as TMSG1 (tumor metastasis suppressor gene 1) or CerS2 (ceramide synthase 2), is a gene firstly identified from a human liver cDNA library in our laboratory [9]. As the most abundantly expressed member of LASS family (LASS1–6), LASS2 has the broadest tissue distribution in mice, particularly in liver, kidney, and brain [10]. LASS2 has remarkable acyl-CoA specificity toward very long fatty acid residues (C22–C24), and is involved in sphingolipid metabolism [10,11]. The biological roles of LASS2 include protection from aging [12], hepatic insulin resistance [13], and hepatocellular carcinoma (HCC) progression [14,15]. We previously found that liver-specific knockout of LASS2 was associated with a high risk of spontaneous or diethylnitrosamine (DEN)-induced HCC in mouse models [16,17].

Herein, using mouse model of liver-specific LASS2 knockout, we investigated the role of LASS2 in liver regeneration.

2. Materials and methods

2.1. Animals

Mice with the *LASS2* gene flanked by loxP sites ($LASS2^{fl/fl}$) and mice that harbored the *Cre* transgene under control of the albumin promoter (Alb-Cre+) were used to generate liver-specific LASS2 knockout mice ($LASS2^{fl/fl}$-Cre+, LASS2-LKO) and wildtype mice ($LASS2^{fl/fl}$-Cre-, WT). The genotyping of LASS2 knockout mice and wildtype mice was performed by PCR on tail DNA using primer pairs for Cre (forward primer: 5′-GCGGTCTGGCAGTAAAAACTATC-3’; reverse primer: 5′-GTGAAACAGCATTGCTGTCACTT-3′) and for the loxP locus (forward primer: 5′-GGCTTCGTGTTGGTCTTCTGA-3’; reverse primer: 5′-ATTCCCTGGCATCCACCTTTC-3′). PCR products were run using a 2% agarose gel. The background of the transgenic mice was C57BL/6J. All animal procedures and euthanasia were conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985). All animal experiments detailed within the manuscript were approved by the Shanghai Medical Experimental Animal Care Commission.

2.2. Surgical procedure

All surgeries were carried out under isoflurane (Sigma-Aldrich, St. Louis, MO) anesthesia. PHx was performed on 8-10 week male mice according to the technique as described previously [5]. Briefly, the abdomen was opened with a midline incision, and about 2/3 liver mass was removed. After PHx, WT mice ($LASS2^{fl/fl}$-Cre-) and LASS2-LKO mice ($LASS2^{fl/fl}$-Cre+) were sacrificed at different time points: 0 h (sham-operation), 6 h, 1 day (D1), 2 days (D2), 3 days (D3), 5 days (D5), 7 days (D7), and 14 days (D14). Liver remnants were isolated and weighed. The wet weights were recorded to calculate the liver/body weight ratio [(liver weight/body weight) × 100]. Liver samples were snap-frozen in liquid nitrogen or processed for further studies.

2.3. Immunohistochemistry

Formalin-fixed liver tissues were embedded in paraffin and cut to 6-μm-thick sections. Immunohistochemistry (IHC) analysis was performed as described previously [18]. Hematoxylin and Eosin (H&E) staining was performed for structural evaluation. Briefly, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in a graded series of alcohol. Then, antigen retrieval was performed using citrate buffer. The sections were incubated with primary antibodies at 4 °C overnight, and with secondary antibodies conjugated with horse radish peroxidase (HRP) (1:500; Millipore) at room temperature for about 1 h. The information of antibodies was listed in Table 1. The tissue sections were finally lightly counterstained with Mayer's hematoxylin (Sigma Chemical Co). PCNA positive or Ki67 positive hepatocytes were counted by examination of at least three random high-power microscopic fields (magnification: 400X) in each tissue section.

2.4. Statistical analysis

Statistical analysis was performed with Student *t*-test. Data are presented as the mean ± standard error of the mean (SEM). Differences were considered to be statistically significant for $p < 0.05$.

3. Results

3.1. LASS2 level increased during liver regeneration and knockout of LASS2 delayed liver regeneration after PHx

We generated liver-specific *LASS2* knockout mice using Cre-loxP system. Mice homozygous for the loxP-flanked allele and positive for the Alb-Cre transgene were referred as LASS2-LKO ($LASS2^{fl/fl}$-Cre+) mice, while mice homozygous for the loxP-flanked allele and

Table 1
The antibodies used in IHC and Western blot analyses.

Protein	Assay	Origin	Dilution
LASS2	IHC	sc-65102, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:80
PCNA	IHC	sc-7907, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:100
Ki67	IHC	ab16667, Abcam Inc, Cambridge, MA	1:100
LASS2	WB	sc-65102, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:1500
PCNA	WB	sc-7907, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:2000
cyclin D1	WB	#2922, Cell Signaling Technology, Inc., Danvers, MA	1:2000
CDK4	WB	sc-260, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:2000
cyclin E	WB	#20808, Cell Signaling Technology, Inc., Danvers, MA	1:1000
cyclin A	WB	sc-751, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:1500
CDK2	WB	sc-748, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:2000
p-Rb	WB	#8516, Cell Signaling Technology, Inc., Danvers, MA	1:1500
p-Akt	WB	#4060, Cell Signaling Technology, Inc., Danvers, MA	1:1500
total-Akt	WB	#4685, Cell Signaling Technology, Inc., Danvers, MA	1:1500
p-Erk1/2	WB	#4370, Cell Signaling Technology, Inc., Danvers, MA	1:2000
total-Erk1/2	WB	#4695, Cell Signaling Technology, Inc., Danvers, MA	1:2000
p-p38	WB	#9211, Cell Signaling Technology, Inc., Danvers, MA	1:2000
total-p38	WB	#9212, Cell Signaling Technology, Inc., Danvers, MA	1:2000
β-actin	WB	sc-47778, Santa Cruz Biotechnology, Inc., Santa Cruz, CA	1:4000

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