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Expression of hypoxia inducible factor-1α and its correlation with phosphoenolpyruvate carboxykinase after portal vein ligation in rats☆

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ABSTRACT

Aims: Portal vein ligation (PVL) has been used to effectively increase future liver remnant (FLR) in hepatectomy for treatment of liver cancer. However, the underlying molecular mechanisms have not been well characterized. The present study aimed to determine expression of hypoxia inducible factor-1alpha (HIF-1α) in response to PVL and assess the correlation of HIF-1α and phosphoenolpyruvate carboxykinase (PEPCK).

Main methods: Male Sprague–Dawley rats underwent PVL and were sacrificed at different time points (12, 24, 48, 72, and 168 h) after surgery. Hepatic HIF-1α expression in the regenerating liver was assessed by Western blot and immunohistochemistry, in parallel; PEPCK levels were quantified by ELISA.

Key findings: We found that the ligated liver lobes diminished progressively, whereas the unligated lobes underwent compensatory regeneration after 70% ligation of portal vein branches in the PVL group. Hepatic HIF-1α and PEPCK levels in the unligated liver lobes were significantly increased in the PVL group compared to the hepatic artery ligation (HAL) and the sham (SH) operation groups. Pearson's correlation analysis revealed positive correlation between HIF-1α expression and PEPCK levels in the unligated lobes after PVL. Further analysis indicated that higher levels of HIF-1α and PEPCK in response to liver regeneration were paralleled by an increase in the ratio of the mass and volume of unligated lobes to the whole liver.

Significance: HIF-1α was up-regulated and positively correlated with PEPCK during liver regeneration after PVL in rats, suggesting that HIF-1α may modulate hepatic gluconeogenesis through PEPCK, which may ensure the energy supply required for liver regeneration.

1. Introduction

Liver resection has been the most effective approach to curative treatment for primary and secondary liver cancers. Acute liver failure (ALF) can be a fatal complication after hepatectomy if postoperative residual liver volume and functional reserve is insufficient. Indeed, patients who underwent major hepatectomy were at an increased risk of developing ALF if the normal liver postoperative future liver remnant (FLR)/total liver volume (TLV) was < 20%, or if the pathologic liver (i.e. chronic hepatitis, liver cirrhosis, liver received chemotherapy) was < 40% [1]. To date, a number of effective methods to increase FLR and reserve liver function prior to hepatectomy have been demonstrated to improve clinical outcomes of patients. In particular, portal vein ligation (PVL) or portal vein embolization (PVE) has been widely used to effectively treat liver cancer. However, the underlying molecular mechanisms are not well understood [2–5].

It has been well documented that PVL or PVE can lead to hypertrophy in the unligated and future remnant lobes and atrophy in the ligated lobes [6,7]. Liver regeneration triggered by PVL or PVE is a multifactorial, complex process [8,9], in which tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), nuclear factor kappa B (NF-κB), and signal transducer and activator of transcription 3 (STAT3) are important signal transduction factors in the induction of liver regeneration. In addition, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and other important growth factors have been reported to play roles in the proliferation of hepatocytes during liver regeneration [10]. Recently it has been shown that hypoxia inducible factor-1α (HIF-1α) was markedly increased in the early stage of liver regeneration [11], implicating HIF-1α as a likely factor in liver regeneration after PVL. However, little is known about how hepatic HIF-1α acts during liver regeneration triggered by PVL.

HIF-1, which is a dimer composed of alpha subunit (HIF-1α) and

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beta subunit (HIF-1β), functions as a key transcription factor in response to cell hypoxia. HIF-1α is the oxygen adjustable subunit that determines HIF-1 activity. Under constant oxygen, HIF-1α is unstable, with a half-life of < 5 min, mainly due to the rapid breakdown of the HIF-1α protein via ubiquitination. Under hypoxic conditions or presence of other stimuli (i.e. growth factors and cytokines), HIF-1α binds to HIF-1β, up-regulates HIF-1, and promotes downstream gene transcription [12].

In the present study, we aimed to determine the role of HIF-1α in liver regeneration following PVL in a rat model. Liver structure in the experimental rats was evaluated. Hepatic HIF-1α expression in the regenerating liver following 70% PVL was assessed by Western blot and immunohistochemistry, and levels of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme in hepatic gluconeogenesis, were quantified by ELISA. We further analyzed if there was a correlation between hepatic expression of HIF-1α and levels of PEPCK. These findings may advance our knowledge about the molecular mechanisms underlying PVL-induced liver regeneration, and may support the application of PVL prior to major hepatectomy for treatment of liver cancer.

2. Materials and methods

2.1. Animals

A total of 90 Male Sprague–Dawley rats weighing between 250 and 300 g used in this study were purchased from the Central Laboratory of Shengjing Hospital of China Medical University (Shenyang, Liaoning, China). All animal experiments were conducted in accordance with the guidelines for the care and use of laboratory animals and approved by the Institute of Experimental Animals of China Medical University. The rats were maintained in temperature-controlled rooms under a 12-h dark/light cycle and had access to water and food, or so called standard laboratory chow, ad libitum.

2.2. Operative procedures

The rats were randomly divided into three groups (n = 30 per group): portal vein ligation group (PVL group), hepatic artery ligation group (HAL group), and the sham operation group (SH group). In each group, six rats were used per each time point (12, 24, 48, 72, and 168 h) following surgery. Under anesthesia with chloral hydrate, 70% PVL was performed in the PVL group with double ligation of the head branch of the portal vein, which supplies blood to the left lateral lobe, left inner lobe and middle lobe. As a result, the ligated liver accounted for 70% of liver weight in the PVL group. Double ligation of the head branch of the hepatic artery was conducted in the HAL group, while the portal vein and hepatic artery were isolated only in the SH group. All surgeries were performed between 08:00 am and 12:00 pm, and the animals had access to food and water ad libitum during the postoperative period.

The rats were sacrificed at indicated time points (12, 24, 48, 72, and 168 h) after PVL, HAL, or SH. The volume and weight of the ligated and unligated lobes were measured, and changes in liver structure were evaluated. For immunohistochemistry, liver tissue samples were fixed, processed, and embedded. For other subsequent experiments, a portion of the ligated and unligated lobes in the PVL, HAL, and SH groups at the indicated time points were immediately collected and stored at − 80 °C.

2.3. HIF-1α immunohistochemistry

The paraffin-embedded liver tissues in the PVL, HAL, and SH groups at the different time points were sectioned and mounted on slides. The slides were rinsed with phosphate buffered saline (PBS) and immunostained with an anti-HIF-1α primary monoclonal antibody (Santa Cruz, CA, USA) at a 1:50 dilution at 4 °C overnight using an Immunodetection Kit (Zhongshanjinqiao, Beijing, China). PBS was used as a negative control. The positive expression of HIF-1α protein was stained brown in the cytoplasm and the nucleus, and the levels of HIF-1α protein was quantified by analyzing the mean optical density in the unligated lobes following SH, HAL, and PVL procedures at the indicated time points.

2.4. Western blot analysis

For Western blot analysis, total proteins were prepared from liver tissue samples collected after PVL, HAL, and SH at the all time points. The proteins were separated on 8% SDS/polyacrylamide gels, and transferred onto polyvinylidene difluoride overnight in buffer containing 10 mmol/l Tris, 100 mmol/l glycine and 10% methanol. Membranes were quenched using 20% fat-free dried milk in buffer. The membranes were incubated with a rabbit anti-HIF-1α polyclonal antibody (Santa Cruz, CA, USA) at a 1:500 dilution overnight at 4 °C. After the membranes were incubated with the primary antibody, a goat anti-rabbit IgG secondary antibody (Zhongshanjinqiao, Beijing, China) was used at a 1:2000 dilution for 2 h at room temperature. The membranes were washed, developed with a chemiluminescence system, and targeted proteins were measured. The protein levels of glyceraldehyde phosphate dehydrogenase (GAPDH) were used as an invariant control, and the relative levels of HIF-1a protein were normalized to GAPDH. The representative images of Western blot were from six individual rats in each time point in each group.

2.5. Enzyme-linked immunosorbent assay (ELISA)

PEPCK levels in the unligated lobes were measured using a sensitive ELISA kit (R & D, USA) according to the manufacturer's instructions.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0). Statistical comparisons for significance were made with the one way ANOVA and LSD. Correlation analysis was performed with Pearson. Data were presented as means ± SE (standard error). Statistical significance was defined as a P value < 0.05.

3. Results

3.1. Changes in liver structure after surgery

Changes in liver structure were examined in a total of 90 rats following PVL, HAL, or SH. We observed that the ligated liver lobes became darker than the unligated lobes in the PVL group which displayed reddish brown coloring. There were no significant alterations in color, atrophy, or hypertrophy in both lobes of the liver in the HAL and SH groups. Furthermore, the unligated lobes showed regenerating liver structure, whereas the ligated lobes atrophied 12 h after surgery in the PVL group (Fig. 1). The weight and volume ratios of the unligated lobes compared to the whole liver in the PVL group at the different time points were as follows: 35.813 ± 1.810%:36.917 ± 3.013% (12 h after surgery), 41.392 ± 1.682%:42.305 ± 2.004% (24 h after surgery), 53.985 ± 3.849%:54.078 ± 3.675% (48 h after surgery), 61.898 ± 1.756%:63.152 ± 2.137% (72 h after surgery), and 87.913 ± 2.491%:89.243 ± 3.436% (168 h after surgery). All ratios were significantly increased in the PVL groups compared to the SH and HAL groups (P < 0.01 at 12, 24, 48, 72 and 168 h after surgery).

3.2. HIF-1α protein expression in unligated lobes following surgery

We next measured HIF-1α protein expression in the regenerating liver by immunohistochemistry and Western blot analysis following PVL. As shown in Fig. 2, HIF-1α was localized both in the cytoplasmic and nuclear compartments (× 400 magnification) and positive

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