Autophagy Contributes to Liver Regeneration After Portal Vein Ligation in Rats

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: This study investigated the effect of 70% portal vein ligation (PVL), a widely used procedure for inducing rapid liver regeneration, on the expression of autophagy-related proteins in non-ligated liver lobes in rats.

Material/Methods: Rats were subjected to either sham (n=30, major portal vein branches were exposed but kept intact) or PVL (n=30, major portal vein branches were double-ligated) operations. Liver samples were collected 12, 24, 48, 72, and 168 h after the operation. Liver volume, liver color, non-ligated liver percentage, and the expressions of light chain (LC) 3, beclin 1, and cyclin D1 in the non-ligated liver lobes were determined.

Results: When compared to sham rats, increased (P<0.001) growth of the non-ligated liver lobes was observed in PVL rats as early as 12 h after surgery; an increased (P≤0.001) LC3 II/I ratio was observed in the non-ligated lobes of PVL rats as early as 24 h after surgery. Increased expressions of beclin 1 (P≤0.001) and cyclin D1 (P<0.001) were observed in the non-ligated lobes of PVL rats from 12 to 72 h after surgery and from 12 to 168 h after surgery, respectively, when compared to sham rats. In the non-ligated lobes, the expressions of beclin 1 and cyclin D1 were linearly and positively correlated with the LC3 II/I ratio.

Conclusions: Autophagy is activated in the non-ligated liver after PVL. Both beclin 1 and cyclin D1 are linearly and positively correlated with autophagy activity in the PVL-induced rapid liver regeneration model.

MeSH Keywords: Autophagy • Liver Regeneration • Tetracycline

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Background

The use of portal vein ligation/embolization (PVL/PVE) to reduce the risk hepatic insufficiency after partial hepatectomy was documented as early as the 1980s [1,2]. Preoperative PVL/PVE induces regeneration of the non-ligated/non-embolized liver and subsequently increases the size and function of the future liver remnant (FLR), which is positively correlated to the outcome of partial hepatectomy patients [3]. Effective (rapid and high-quality) regeneration of the non-ligated/non-embolized liver is the major goal of PVL/PVE in clinical practice, especially for patients receiving large-volume liver resection [4]. Better understanding of the rapid regeneration mechanism of the non-ligated/non-embolized liver after PVL/PVE would help to improve clinical efficacy and safety.

The hemodynamic changes and the cellular and molecular events after PVL/PVE have been extensively studied in recent decades [5]. Several humoral mediators (e.g., inflammatory cytokines like circulating interleukin-6 and tumor necrosis factor-α; growth factors like hepatocyte growth factor, transforming growth factor-α, and epidermal growth factor; and eicosanoids, vasoregulators, and various hormones) appear to play critical roles in the initialization and progression of PVL/PVE-induced rapid liver regeneration [3]. Interestingly, recent reports indicate that macroautophagy (hereafter referred to as autophagy) – an intracellular lysosomal pathway in which cytosolic components are degraded for the purposes of maintaining cell function, survival, and regeneration – may also be a key player in this process [3].

In autophagy, cytosolic components are surrounded by autophagosomes, which are double-membrane structures that fuse with lysosomes [6,7]. The cytosolic components are thereafter degraded by lysosomal enzymes, and the breakdown products are released back into the cytosol. Alterations of autophagy have been repeatedly identified in the literature as one of the major underlying mechanisms of many hepatic diseases such as liver injury (e.g., toxin, drug, and ischemia/reperfusion), viral hepatitis, fatty liver, and hepatocellular carcinoma [8–10].

Moreover, the potential role of autophagy in liver regeneration has also been investigated. Lin et al. reported that autophagy was activated in the initialization of rapid liver regeneration following 70% partial hepatectomy in mice [11]. In the same study, amiodarone (an autophagy promoter) enhanced liver growth and hepatocyte proliferation after 70% partial hepatectomy. These results suggest that autophagy is a potential target for controlling liver regeneration quality after partial hepatectomy. Since PVL/PVE- and partial hepatectomy-induced rapid liver regeneration share similar mechanisms [5], it would be reasonable to hypothesize that autophagy is also essential for rapid non-ligated/non-embolized liver regeneration

after PVL/PVE. Therefore, this study investigated the function of autophagy in PVL.

Material and Methods

All of the protocols used in this study were approved by the Animal Care and Use Committee of Shengjing Hospital (2019PS381K) and complied with guidelines issued and implemented by the Government of China on the proper use of laboratory animals.

Animals

A total of 60 male, 5-week-old, SD rats (each weighing 250–300 g) were obtained from the Central Laboratory of Shengjing Hospital, China Medical University (Shenyang, Liaoning Province, China). Rats were housed in a room with controlled temperature (24±1°C) and relative humidity (RH, 60±5%) with a 12-h light: 12-h dark photoperiod and fed a standard corn-soybean meal-based diet (Beijing HFK Biotech Co., Beijing, China). Food and water were supplied *ad libitum*.

Rat PVL model

After the rats were acclimatized for 1 week, the PVL procedure was conducted as described by Chojkier and Groszmann (1981) [12]. Briefly, prior to PVL, the rats (n=30) were food-fasted for 12 h and water-fasted for 4 h. We intraperitoneally injected 300 mg/kg chloral hydrate (10%; Sinopharm Chemical Reagent Co., Beijing, China) to anesthetize the rats. The abdominal cavity was then carefully opened through a 3–4 cm midline incision under sterile conditions. The intestine was lifted out of the abdominal cavity and kept moist. Then, the hepatic artery and bile duct were carefully separated and kept intact, and the major portal vein branches of the left lateral lobe, left inner lobe, and middle lobe were exposed and double-ligated with 4-0 silk suture. Next, the abdominal viscera were replaced into the abdomen, and the abdominal cavity was carefully closed in layers (this is a 70% ligation model since the portal vein branches of the quadrate and caudate lobes were kept intact). Another 30 rats (sham group) underwent the same procedure as PVL rats, except the major portal vein branches were exposed but not ligated.

Sample collection

The postoperative status of sham and PVL rats was recorded twice a day. All 60 rats used in this study were in good condition after PVL. Six rats from each group were randomly selected and sacrificed for liver sample collection at each of the following postoperative time points: 12 h, 24 h, 48 h, 72 h, and 168 h. The whole liver weight was recorded. The ligated and

non-ligated lobes were then identified, divided, and weighed (for sham rats, the left lateral lobe, left inner lobe, and middle lobe were identified as the ligated lobes; and the right lobe, quadrate lobe, and caudate lobe were identified as non-ligated lobes). The color and volume of the ligated and non-ligated lobes were recorded with photographs. Tissues (0.1 g) from the non-ligated lobe were rapidly collected and frozen in liquid nitrogen and then transferred to a –80°C freezer for further Western blot analyses. Another $1.0 \times 1.0 \times 0.4$ cm^3 sample was randomly selected and cut from the non-ligated lobe and fixed in 4% paraformaldehyde for future immunohistochemical analysis.

Western blot analysis

Western blot analysis was conducted using the method modified by Zhu et al. (2006) [13]. Briefly, the non-ligated liver tissues were powdered in liquid nitrogen and homogenized in lysis buffer (RIPA lysis buffer; Beyotime Biotechnology, Shanghai, China) and pretreated with protease inhibitor PMSF (1 mM; Beyotime Biotechnology, Shanghai, China). The tissue-RIPA mix (kept on ice) was exposed to ultrasound for 5 min. The lysate was centrifuged at 14 000×g and 4°C for 15 min. Part of the supernatant was collected for the determination of protein concentration (BCA Protein Assay Kit; Beyotime Biotechnology, Shanghai, China). The remaining supernatant was diluted using phosphate-buffered saline (PBS, pH=7) and then mixed with SDS-PAGE protein loading buffer (Beyotime Biotechnology, Shanghai, China; the mixture contained 4 parts of sample and 1 part of SDS-PAGE protein loading buffer). The mixture was then electrophoresed on 5–12% SDS-polyacrylamide gels (PAGE) and transferred electrophoretically to PVDF-immobilon membranes. The membranes were incubated overnight with the primary antibody at 4°C. The secondary antibody (horseradish peroxidase-conjugated) was then applied (2 h, at room temperature). The membranes were then washed and probed using BeyoECL Plus **(**Beyotime Biotechnology, Shanghai, China**)** and autoradiographed using the Gel Imaging System (Syngene, MD, USA). Polyclonal antibodies against LC3 A/B (rabbit, No. 4108) and beclin 1 (rabbit, No. 3738) were purchased from CST Inc. (Beverly, MA, USA) and diluted to 1: 1000. Antibody against GAPDH (mouse, KC-5G5; Shanghai Kangchen Biotech, Shanghai, China) was provided by the Central Laboratory of Shengjing Hospital, China Medical University. The goat anti-rabbit (No. ZB2308) and rabbit anti-mouse (No. ZDR5109) secondary antibodies were purchased from Zhongshanjinqiao Biotech Co. (Beijing, China) and diluted to 1: 2000. The band density was quantified using Quantify One 1-D analysis software (version 4.6.9; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and then normalized to GAPDH content, because the levels of GAPDH did not differ between experimental groups.

Immunohistochemistry

The paraformaldehyde-fixed liver tissues were transferred to 70% ethanol and paraffin-sectioned. Then, the slides were placed in an oven at 60°C for at least 1 h (1 to 3 h) to melt the paraffin wax from the slides. After that, the slides were deparaffinized in 3 changes of xylene for 10 min each, incubated in absolute ethanol, 95% ethanol, 70% ethanol, 50% ethanol (for 1 min each), and then incubated in 3 changes of PBS (pH=7) for 3 min each. Slides were then placed in a citric-acid buffer solution (0.01 M, pH=6) and boiled in a microwave oven (350 W) for 5 min. Slides were kept at room temperature for 5 min and boiled again in a microwave oven for 10 min. When cooled to room temperature, the slides were washed in 3 changes of PBS (pH=7) for 3 min each and incubated at 37°C with fetal bovine serum (50 ul; Zhongshanjinqiao Biotech Co., Beijing, China) for 30 min. Antibody to cyclin D1 (rabbit; Zhongshanjinqiao Biotech Co., Beijing, China) was diluted to 1: 50 in normal antibody diluent, and (50 ul) applied on the slides overnight (4°C). For, negative control slides, PBS (pH=7) and cyclin D1 antibody were applied. After washing with 3 changes (3 min each) of PBS buffer, the slides were incubated with 50 ul biotin-labeled secondary antibody (diluted to 1: 2000; Zhongshanjinqiao Biotech Co., Beijing, China) for 30 min (37°C). Avidin-biotin-peroxidase (50 ul) was then applied and incubated at 37°C for 15 min. After washing with 3 changes (3 min each) of PBS buffer, the slices on the slides were incubated with a DAB buffer (Zhongshanjinqiao Biotech Co., Beijing, China), counterstained with hematoxylin (2 min), and dehydrated before coverslipping. The slides were visualized at transmission light on a Nikon E800 imaging system (Nikon, Tokyo, Japan).

Statistical analysis

An independent-samples *t* test (SPSS v17.0, SPSS, Inc., Chicago, IL, USA) was used to determine the differences between sham rats and PVL rats within each sample time point. One-way ANOVA was performed to determine the changes over time of LC3 II/I, beclin 1, and cyclin D1 expressions in the non-ligated liver after PVL. Dunnett's T3 (where equal variances were not assumed) or LSD (equal variances assumed) post hoc test was conducted when the one-way ANOVA showed significant changes. Linear correlation analysis was applied to demonstrate the correlation between LC3 II/I and beclin 1, and the correlation between LC3 II/I and cyclin D1 (in PVL rats only). Data are presented as the mean±SD. P<0.05 was regarded as a statistically significant difference.
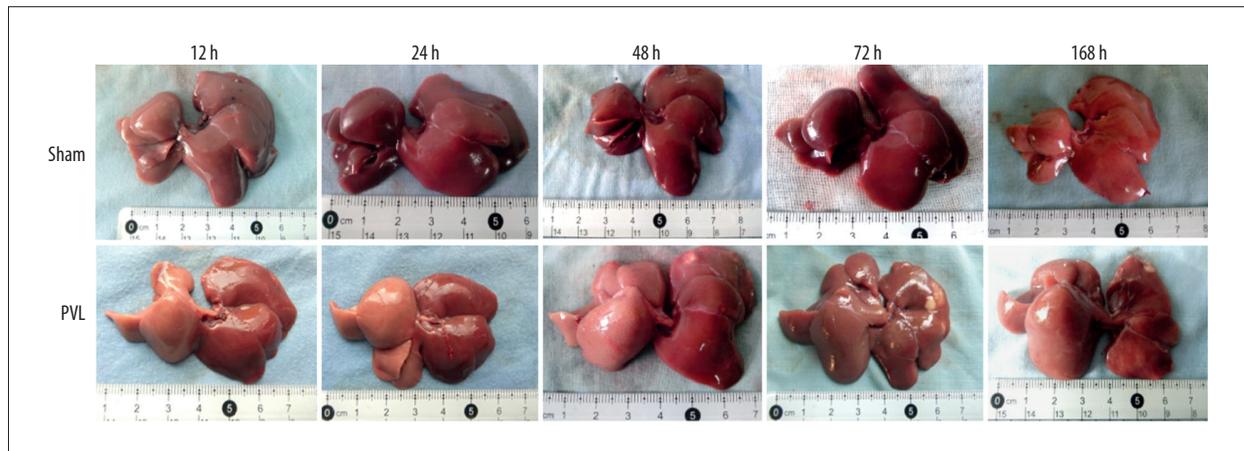
Figure 1. Liver volume and color after portal vein ligation (PVL). Livers were collected and photographically recorded 12, 24, 48, 72, and 168 h after PVL. In each image, the ligated liver (left and right lobes) was placed on the right side and the non-ligated liver (quadrate and caudate liver lobes) was placed on the left side.

Table 1. Non-ligated liver weight percentage in total liver weight[1].

Time after PVL[2]	Sham (%)	PVL (%)	P-value[3]
12 h	29.8±1.3	33.5±1.6[e]	0.970
24 h	32.3±0.9	42.2±1.0[d,*]	<0.001
48 h	32.9±2.9	53.7±1.3[c,*]	<0.001
72 h	31.7±1.6	63.3±2.9[b,*]	<0.001
168 h	30.3±1.9	88.3±3.0[a,*]	<0.001
P-value[4]	0.205	<0.001	

* $P<0.001$ when compared to the Sham group within each time point. [a,b,c,d,e] Different superscripts in a row indicate differ significantly ($P<0.05$). [1] Data are shown as the mean ±SD. PVL – portal vein ligation. The PVL model used in the current study is a 70% ligation model since the portal vein branches of the quadrate and caudate liver lobes were kept intact in all rats. The hepatic artery and bile duct were kept intact in all rats. [2] In PVL rats, the major portal vein branches of both left and right liver lobes were exposed and double ligated with 4-0 silk suture. In sham rats, the major portal vein branches of both left and right liver lobes were exposed but kept intact. [3] Two-tailed independent Student's t-test (n=6) within each time point. [4] One-way ANOVA (n=6) within each group.

Results

Liver growth

As shown in Figure 1, the sham operation caused no change in liver volume or liver color. After the PVL operation, the colors of the ligated and non-ligated liver lobes were different and the ligated liver lobes turned darker. In PVL rats, the volume of the non-ligated liver lobes was visibly increased and the volume of the ligated liver lobes was visibly decreased 48, 72, and 168 h after the PVL operation. Table 1 demonstrates the percentage of non-ligated lobes (in the whole liver) that did not change (P=0.205) after the sham operation. However, in PVL rats, the percentage of non-ligated lobes increased (P<0.001) over time and reached a maximum 168 h after the PVL operation. In addition, the percentage of non-ligated lobes in PVL rats was higher (P<0.001) than in the sham rats at 24, 48, 72, and 168 h after the PVL operation.

LC3 II/I expression

In sham rats, the LC3 II/I ratio was unchanged (P=0.087) in the non-ligated lobes after surgery (Figure 2A; see Supplementary Table 1 for the data). However, the LC3 II/I ratio increased in the PVL rats (P<0.05) 24, 48, and 72 h after surgery (compared to 12 and 168 h after surgery). The LC3 II/I ratio of non-ligated lobes was higher (P≤0.001) in PVL rats than in sham rats at 24, 48, 72, and 168 h after surgery.

Beclin 1 expression

In sham rats, increased (P<0.05, Figure 2B, see Supplementary Table 2 for specific numbers) beclin 1 expression was observed in the non-ligated lobes at 24, 48, 72, and 168 h after the operation (when compared to 12 h after the operation). In PVL rats, increased (P<0.05) beclin 1 expression was observed in

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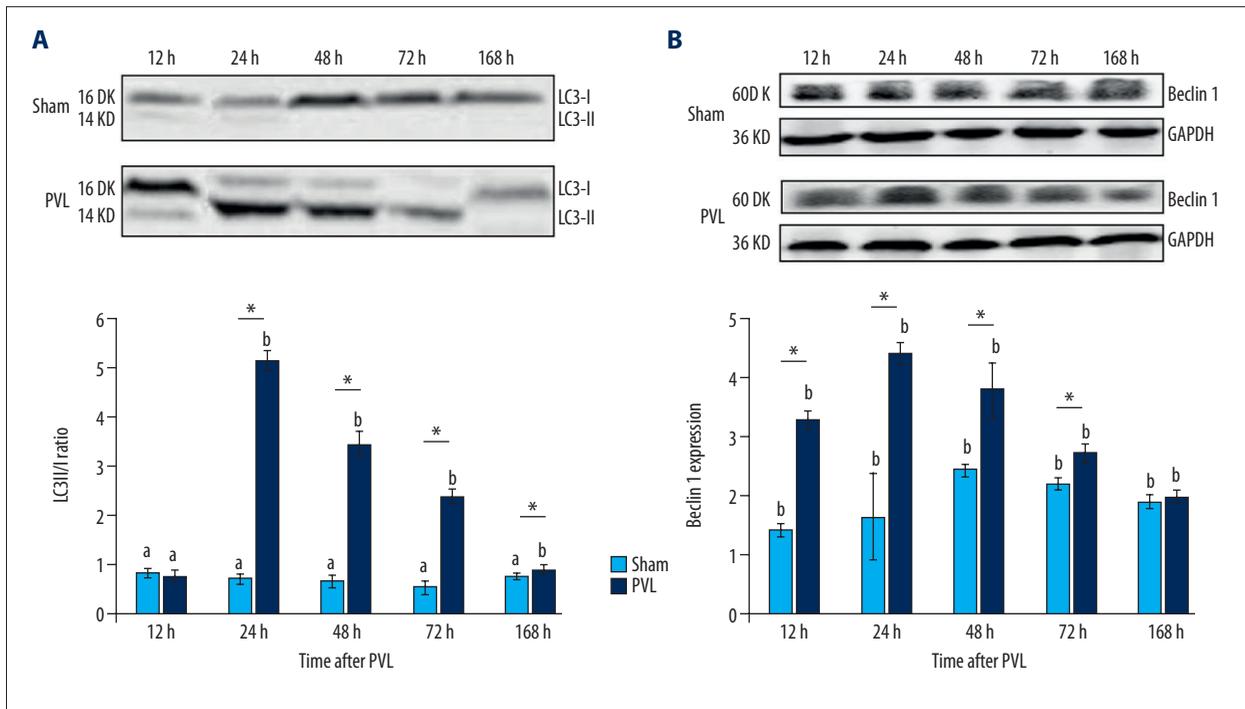
Figure 2. Expressions of LC3 II/I and beclin 1 in the non-ligated liver lobe of sham and PVL rats. Non-ligated liver samples were collected 12, 24, 48, 72, and 168 h after PVL. (**A**) Western blot analysis of hepatic LC3 II/I protein expressions in the non-ligated liver lobes 12, 24, 48, 72, and 168 h after PVL. (**B**) Western blot analysis of hepatic beclin 1 protein expression in the non-ligated lobes 12, 24, 48, 72, and 168 h after PVL. * P<0.001.

the non-ligated lobes 12, 24, and 48 h after the operation (compared to 72 and 168 h after the operation). The beclin 1 expression in the non-ligated lobes was higher (P≤0.001) in PVL rats than sham rats at the following sample time points: 12, 24, 48, and 72 h after surgery.

Cyclin D1 expression

Cyclin D1 is a 37-kDa nuclear cell cycle protein which promotes proliferation by mediating the transition from the G1 to the S phase [14,15]. In sham rats, cyclin D1 expression was unchanged (P=0.129) in the non-ligated lobes after surgery (Figure 3, see Supplementary Table 3 for specific numbers). In PVL rats, increased (P<0.05) cyclin D1 expression was observed in the non-ligated lobes 24 h after surgery (when compared to the other sample time point). The cyclin D1 expression in the non-ligated lobes was higher (P≤0.001) in PVL rats than sham rats at all of the sample time points.

Correlations between LC3 II/I and beclin 1/cyclin D1

Figure 4 shows the correlations between the LC3 II/I ratio and the expression of beclin 1 or cyclin D1. In PVL rats, in non-ligated liver lobes, the LC3 II/I ratio was linearly and positively correlated with the expressions of beclin 1 (R^2=0.639, P<0.05) and cyclin D1 (R^2=0.778, P<0.05).

Discussion

In the present study, the expression levels of autophagy-related proteins LC3, beclin 1, and cyclin D1 were investigated in the non-ligated livers of PVL rats. The data showed that LC3 II/I ratio was increased as early as 24 h after surgery, while expression levels of beclin 1 and cyclin D1 were increased in the PVL rats from 12 to 72 h after surgery and from 12 to 168 h after surgery, respectively, when compared to sham rats. Moreover, the expression levels of beclin 1 and cyclin D1 were linearly and positively correlated with the LC3 II/I ratio. Our results indicate cellular autophagy was significantly activated and contributed to PVL-induced rapid liver regeneration.

Autophagy is a multi-stage process (initiation, nucleation, and expansion/elongation) and involves many evolutionarily-conserved autophagy-related genes (ATGs) [7,16]. Autophagy initiates with the activation of the ATG1 complex, which activates a class III PI3K complex involving beclin 1. Then, the ATG5-ATG12 complex conjugates with ATG16 to expand the autophagosome membrane, during which microtubule-associated protein LC3 (a protein that has been identified as a mammalian homolog of yeast Atg8) and other proteins are conjugated to the lipid phosphatidylethanolamine and recruited to the membrane, where LC3-I is converted to LC3-II and inserted into autophagosome membranes [17]. Therefore, LC3-I is commonly

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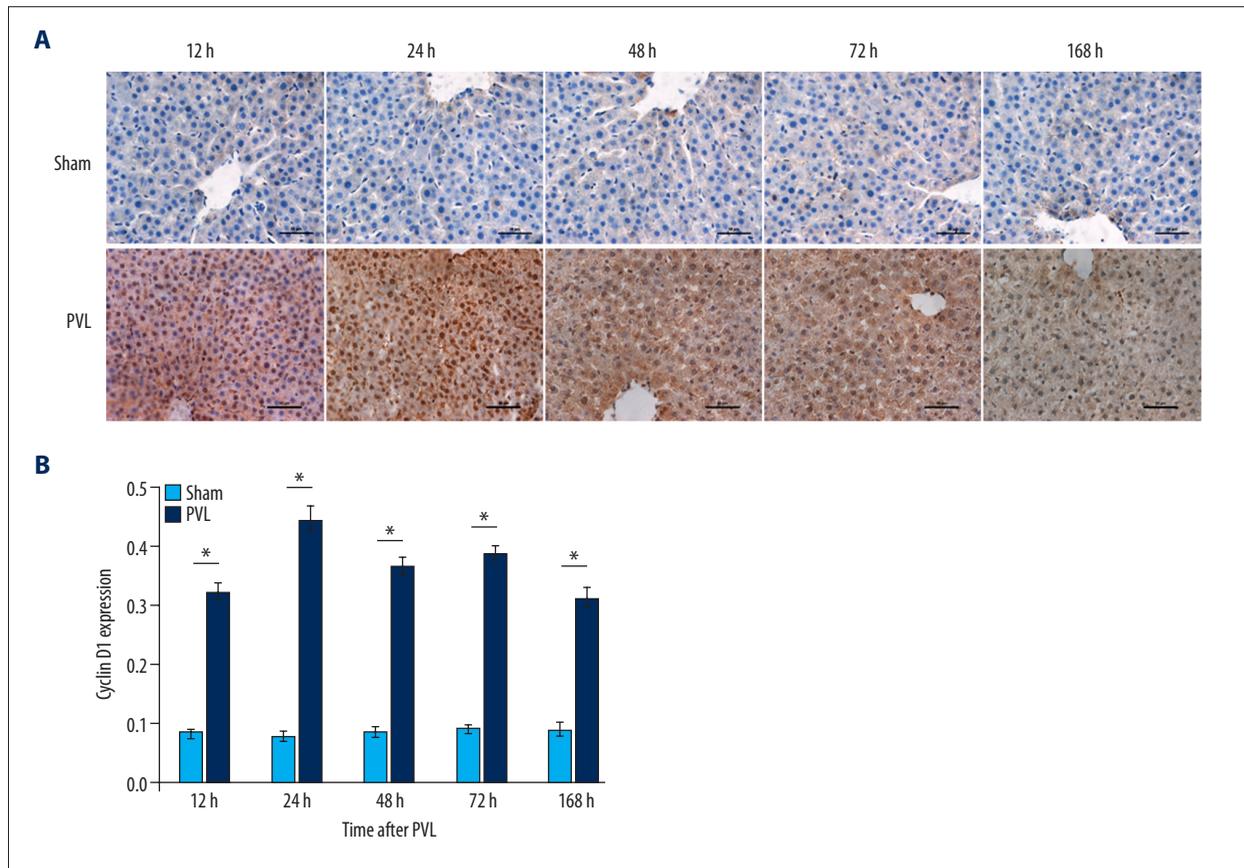
Figure 3. (**A, B**) Expression of cyclin D1 in the non-ligated liver lobe of sham and PVL rats. Non-ligated liver samples were collected 12, 24, 48, 72, and 168 h after PVL. Hepatic cyclin D1 protein expression in the non-ligated liver lobes was detected using immunohistochemical staining 12, 24, 48, 72, and 168 h after PVL. * P<0.001.
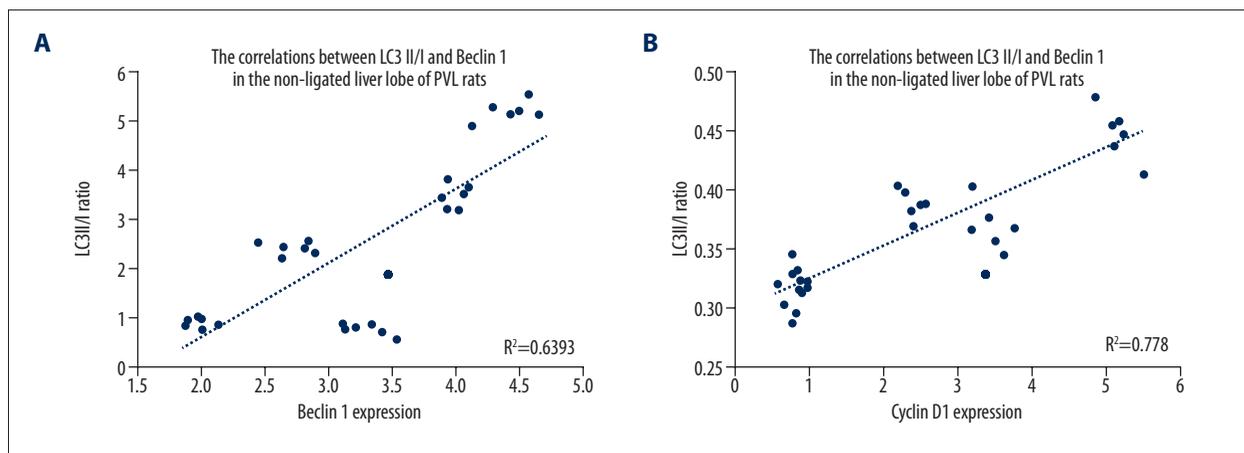
Figure 4. Correlations (**A**) between LC3 II/I beclin 1, and (**B**) between LC3 II/I and cyclin D1 in PVL rats. Each point depicts data from an individual mouse.

used as an autophagosome marker, and the conversion of LC3-I to LC3-II (i.e., the LC3 II/I ratio) could be used to indicate autophagic activity.

In this study, as expected, an obvious increase was observed in the volume of the non-ligated liver lobes as early as 48 h after PVL. Interestingly, in PVL rats, the LC3 II/I ratio significantly increased 24 h after PVL surgery and remained high until 72 h after surgery. These results indicate autophagy was activated

in the early stage of liver regeneration following PVL. Indeed, the role of autophagy in rapid liver regeneration has long been proposed in the literature. In mice, the suppression of autophagy during liver regeneration was linked to decreased energy charge and hepatocyte senescence [18]. Lin et al. reported that autophagy was increased during rapid liver regeneration after 70% partial hepatectomy in mice [11]. The involvement of autophagy in rapid liver regeneration after PVL or partial hepatectomy could help develop new therapy strategies for patients awaiting hepatectomy or liver donation. Specifically, the understanding that autophagy is activated after liver ligation or section could help develop methods to control the speed and quality of rapid liver regeneration. Lin reported that in 70% partial hepatectomy rats, amiodarone, a well-tolerated anti-arrhythmic drug which can decrease calcium permeability and induce autophagy, increased autophagy status, hepatocyte proliferation, and liver growth; decreased liver injury; and delayed the termination of rapid liver regeneration [11]. Notably, in the same study, amiodarone improved liver regeneration, survival, and liver injury, even after 90% partial hepatectomy [11]. Thus, pharmacological approaches using medications such as amiodarone may also be used to increase the speed and quality of ligation-induced rapid liver regeneration because PVL- and partial hepatectomy-induced rapid liver regeneration share the similar mechanism [5]. The present results also suggest that ligation is a useful model for studying rapid liver regeneration and liver autophagy.

In the non-ligated liver lobes of PVL rats, the expression of beclin 1 (a functional homolog of yeast Apg6/Vps30) was increased as early as 12 h after PVL surgery and remained at a high level until 72 h after PVL surgery. In particular, the expression of beclin 1 was linearly and positively correlated to LC3 II/I. Liang et al. reported that beclin 1 plays important roles in the initiation and progression of nitrogen deprivation-induced autophagy [19]. Unlike LC3-II, which presents with the final formation of autophagosomes, beclin 1 is involved in the very early stages of autophagy (including the nucleation of the autophagic vesicle and recruiting cytosolic proteins) [20,21]. In mammalian cells, suppression of beclin 1 expression decreases autophagy and sensitizes cells to starvation-induced cell death [22]. While we had insufficient direct evidence to support the fact that increased autophagy in non-ligated liver lobes was induced or partially induced by beclin 1 in this study, Toshima et al. found the same results [18]. In their study, both LC3-II and beclin 1 were increased in mouse livers after 70%

partial hepatectomy [18]. Similarly, Yan et al. reported that the expression of both beclin 1 and LC3-II were increased when autophagy was induced in chronically ischemic myocardium of pigs [23]. Further research should be conducted to examine the interactions between these autophagy-related proteins.

Liver regeneration is tightly regulated by the cell cycle [24]. Cyclin D1 (a protein encoded by the CCND1 gene) is a major player in cell cycling [14,15]. In this study, increased cyclin D1 expression, along with a rapid liver regeneration, was observed in the non-ligated liver lobes as early as 12 h after PVL. This result is as expected, since increased cyclin D1 expression, which is positively correlated with p38 kinase expression, was repeatedly reported in partial hepatectomy-induced rapid liver regeneration models [3]. Of note, in the present study, the expression of cyclin D1 was linearly and positively correlated to LC3 II/I in the non-ligated liver lobes of PVL rats. Interestingly, while it is well accepted that both LC3-II and cyclin D1 are up-regulated in rapid liver regeneration models, autophagic activity is inversely correlated with cyclin D1 expression in some other autophagy models. Wu et al. reported that increased cyclin D1 was accompanied with decreased autophagic activity and was correlated with decreased overall survival rate in hepatocellular carcinoma patients [25]. Brown et al. reported that increased autophagy rate was observed in cyclin D1 deficient mice [26]. Due to these inconsistent results, we assume the autophagy in the rapid liver generation model is special to other autophagy models. The direct relationship between cyclin D1 and LC-II in the present PVL model remains unclear.

Conclusions

LC3 II/1 ratio and the expressions of beclin 1 and cyclin D1 were increased in rapid regeneration (non-ligated) liver lobes after PVL in rats.

Conflict of interests

None.

Supplementary Tables

Supplementary Table 1. The expression of LC3 II/I in the non-ligated liver lobe of sham and PVL rats[1].

Time after PVL[2]	Sham (%)	PVL (%)	P-value[3]
12 h	0.821±0.087	0.759±0.120[d]	0.334
24 h	0.718±0.100	5.176±0.213[a,*]	<0.001
48 h	0.654±0.107	3.463±0.235[b,*]	<0.001
72 h	0.532±0.138	2.397±0.138[c,*]	<0.001
168 h	0.750±0.056	0.890±0.084[d,*]	0.001
P-value[4]	0.087	<0.001	

* P<0.001 when compared to the Sham group within each time point. [a,b,c,d,e] Different superscripts in a row indicate differ significantly (P<0.05). [1] Data are shown as the mean±SD. PVL=portal vein ligation. The PVL model used in the current study is a 70% ligation model since the portal vein branches of the quadrate and caudate liver lobes were kept intact in all rats. All of the hepatic artery and bile duct were kept intact in all rats. [2] In PVL rats, the major portal vein branches of both left and right liver lobes were exposed and double ligated with 4-0 silk suture. In sham rats, the major portal vein branches of both left and right liver lobes were exposed but kept intact. [3] Two tailed independent Student's *t*-test (n=6) within each time point. [4] One-way ANOVA (n=6) within each group.

Supplementary Table 2. The expression of beclin 1 in the non-ligated liver lobe of sham and PVL rats[1].

Time after PVL[2]	Sham (%)	PVL (%)	P-value[3]
12 h	1.40±0.12[b]	3.30±0.17[c,*]	<0.001
24 h	1.63±0.74[a]	4.43±0.19[a,*]	<0.001
48 h	2.43±0.14[a]	3.80±0.50[b,*]	<0.001
72 h	2.20±0.10[a]	2.72±0.16[d,*]	<0.001
168 h	1.91±0.14[a]	1.99±0.09[e]	0.124
P-value[4]	<0.001	<0.001	

* P<0.001 when compared to the Sham group within each time point. [a, b, c, d, e] Different superscripts in a row indicate differ significantly (P<0.05). [1] Data are shown as the mean ± SD. PVL=portal vein ligation. The PVL model used in the current study is a 70% ligation model since the portal vein branches of the quadrate and caudate liver lobes were kept intact in all rats. All of the hepatic artery and bile duct were kept intact in all rats. [2] In PVL rats, the major portal vein branches of both left and right liver lobes were exposed and double ligated with 4-0 silk suture. In sham rats, the major portal vein branches of both left and right liver lobes were exposed but kept intact. [3] Two tailed independent Student's *t*-test (n=6) within each time point. [4] One-way ANOVA (n=6) within each group.

Supplementary Table 3. The expression of cyclin D1 in the non-ligated liver lobe of sham and PVL rats[1].

Time after PVL[2]	Sham (%)	PVL (%)	P-value[3]
12 h	0.081±0.007	0.322±0.014[c,*]	<0.001
24 h	0.076±0.008	0.446±0.022[a,*]	<0.001
48 h	0.082±0.008	0.366±0.015[b,*]	<0.001
72 h	0.087±0.005	0.387±0.012[b,*]	<0.001
168 h	0.087±0.012	0.311±0.017[c,*]	<0.001
P-value[4]	0.129	<0.001	

* P<0.001 when compared to the Sham group within each time point. [a, b, c, d, e] Different superscripts in a row indicate differ significantly (P<0.05). [1] Data are shown as the mean ±SD. PVL=portal vein ligation. The PVL model used in the current study is a 70% ligation model since the portal vein branches of the quadrate and caudate liver lobes were kept intact in all rats. All of the hepatic artery and bile duct were kept intact in all rats. [2] In PVL rats, the major portal vein branches of both left and right liver lobes were exposed and double ligated with 4-0 silk suture. In sham rats, the major portal vein branches of both left and right liver lobes were exposed but kept intact. [3] Two tailed independent Student's *t*-test (n=6) within each time point. [4] One-way ANOVA (n=6) within each group.

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