Size regulation of portally deprived liver lobe and future liver remnant following simultaneous portal vein ligation and partial hepatectomy

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by Chuanfeng Hua
born on 12.04.1990 in Anhui

Reviewers:

1. **Frau Prof. Dr. med. Uta Dahmen**

   Experimentelle Transplantationschirurgie,

   Klinik für Allgemein-, Viszeral- und Gefäßchirurgie, Universitätsklinikum Jena

2. **Frau PD Dr. med. Yuan Chen**

   Experimentelle Transplantationschirurgie,

   Klinik für Allgemein-, Viszeral- und Gefäßchirurgie, Universitätsklinikum Jena

3. **Herr doc. MU Dr. Václav Liška, Ph.D.**

   Laboratory of Cancer Treatment and Tissue Regeneration

   Faculty of Medicine in Pilsen Biomedical Center , Charles University

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Abbreviations

PVL: Portal vein ligation

PHx: Partial hepatectomy

PVE: Portal vein embolization

FLR: Future liver remnant

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

BrdU: 5-bromo-2-deoxyuridine

TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

PI: Proliferation index

POD: Postoperative day

PCNA: Proliferating cell nuclear antigen

Zusammenfassung

Einleitung: Die partielle Hepatektomie (PHx) stellt nach wie vor die einzige kurative Behandlung für Pateinten mit malignen Lebertumoren dar. Aufgrund der Schädigung der Leber sowie der unzureichenden Größe des verbleibenden, gesunden Restlebergewebes (engl. future liver remnant = FLR) nach der Leberresektion ist eine erweiterte Leberresektion mit einem hohen Risiko für ein postoperatives Leberversagen verbunden.
Um die Indikationen für eine chirurgische Behandlung bei Patienten mit primär nicht resezierbaren Lebertumoren zu erweitern, wird zunehmend eine Pfortaderligatur vor der erweiterten Leberresektion angewendet. Die Durchführbarkeit der nachfolgenden erweiterten Leberresektion hängt einerseits von Ausmaß der Hypertrophie der FLR ab, und ist andererseits durch das Fortschreiten des Tumors limitiert. Bei einem Drittel der Patienten mit multiplen bilateralen Lebertumoren ist die geplante erweiterte Resektion aus diesen Gründen (unzureichende Regeneration bzw Tumor progress) nicht möglich.
Deswegen wird mittlerweile im ersten Eingriff nicht nur die Pfortaderligatur durchgeführt, sondern die Tumoren im FLR chirurgisch entfernt. Bisher wurde die intrahepatische Größenregulation nach diesem kombinierten Verfahren noch nicht untersucht. Es gibt kein gut charakterisiertes experimentelles Modell, um den zugrunde liegenden Mechanismus adäquat zu beschreiben. Aus diesem Grund haben wir ein chirurgisches Modell entwickelt, indem zeitgleich zur Pfortaderligatur eine Leberresektion durchgeführt wird um die intrahepatische Größenregulation zu untersuchen.

Ziel: Ziel der vorliegenden Studie war es das experimentelle Modell von simultaner PVL und PHx zu charakterisieren. Dazu wurden PVL und PHx von unterschiedlichem Ausmaß durchgeführt und Schlüsselprozeße der intrahepatischen Größenregulation untersucht: Hepatozytenproliferation, Apoptose und Autophagie. Wir haben ferner ein prospektives Experiment entworfen, um den zugrunde liegenden Regulierungsmechanismus zu erforschen.

Methoden und Ergebnisse: **Wie im Manuskript I (Scientific Reports 2020, IF 3.998)** beschrieben, haben wir zunächst den Einfluß einer kombinierten PVL und PHx unterschiedlichen Ausmaßes auf die Atrophie des ligierten Leberlappens im Rattenmodell untersucht. Dabei haben wir zwei Größenkombinationen verwendet: sowohl die Kombination von 20% PVL mit 70% PHx, als auch die Kombination von 70% PVL und 20% PHx. Die alleinige 20% PVL und die alleinige 70% PVL wurden als Kontrolle verwendet.

Nach der alleinigen Pfortaderligatur kam es, wie zu erwarten, zu einer Größenreduktion (Atrophie) bis auf der 30% der ursprünglichen Größe. Bei der zeitgleichen Durchführung einer Resektion wurde der Atrophie entgegengewirkt. Bei der gleichzeitigen kleinen Resektion wurde das Ausmaß der Atrophie reduziert, wohingegen es bei gleichzeitiger großer Resektion sogar zur Hypertrophie der ligierten Leberlappen kam. In beiden Fällen konnten wir proliferierende Hepatozyten im ligierten Leberlappen nachweisen, nach größerer Resektion mehr als bei kleiner Resektion, jedoch nach alleiniger PVL gar nicht. Gleichzeitig war die Apoptoserate im ligierten Leberlappen nach zusätzlicher PHx im Vergleich zur alleinigen PVL signifikant verringert, nach großer Resektion deutlicher als nach kleiner Resektion Gleichermaßen wurde die Autophagie in den ligierten Lappen durch die zeitgleiche PHx aktiviert, ebenfalls bei großer Resektion mehr als bei kleiner Resektion.

Wie in **Manuskript II (eingereicht in Scientific Reports 2020, IF 3,998)** beschrieben, wurde auch der Einfluss einer zusätzlichen PHx auf den nicht ligierten FLR untersucht. Wir verglichen das Ausmaß der Hepatozytenproliferation in den nicht ligierten Leberlappen wieder nach gleichzeitiger 20% PVL und 70% PHx bzw. gleichzeitiger 70% PVL und 20% PHx. Zu Kontrollzwecken wurde eine 90%PVL durchgeführt. Die zusätzliche PHx-verstärkte die Hypertrophie der FLR im Vergleich zu 90% PVL und führte zu einer verstärkten Induktion der Autophagie. Es wurde jedoch kein statistisch signifikanter Unterschied zwischen einer zusätzlichen großen Resektion und einer kleinen Resektion beobachtet.

Basierend auf den vorgestellten Experimenten haben wir die relevante Rolle der Autophagie in unserem experimentellen Modell erkannt. Wir planen eine prospektive Studie, um den zugrunde liegenden Mechanismus mithilfe eines Pfortader-Ligaturmodells in der Ratte zu untersuchen. Um die Rolle der Autophagie bei der Größenregulierung von ligiertem und nicht ligiertem Leberlappen besser zu verstehen, wollen wir verschiedene Autophagie-Modulatoren anwenden und das Ausmaß der postoperativen Leberschädigung, das Regenerationsverhalten beider Leberlappen sowie Apoptose und Autophagie untersuchen.

Zusammenfassung: Unser experimentelles Modell eignet sich zur Untersuchung der intrahepatischen Größenregulation nach gleichzeitiger PVL und PHx. Basierend auf unseren Beobachtungen wurde die Autophagie sowohl im ligierten, als auch im nicht ligierten Leberlappen aktiviert, begleitet von einer Hemmung der Apoptose und einer Förderung der Proliferation. Die Ergebnisse der geplanten Studie könnten zu einer neuartigen Strategie beitragen, die die Hypertrophie der FLR durch Modulation der Autophagie beschleunigt und daher die Indikationen auf eine erweiterte Hepatektomie nach PVL erweitert.

Summary

Background: Partial hepatectomy (PHx) remains the only curative treatment for patients with malignant liver tumors. Due to the hepatic injury and the inadequate size of future liver remnant (FLR) after liver resection, major hepatectomy is associated with a high risk of post-hepatectomy liver failure.

To expand the indications for surgical treatment for patients with primarily irresectable liver tumors, portal vein occlusion prior to major hepatectomy is adopted in liver surgery. The feasibility of the subsequent major hepatectomy is dependent on the hypertrophy of FLR but limited by tumor progression. Thus, about one third of patients with multiple bilateral liver tumors are not subjected to major hepatectomy.

Combined two-sequential hepatectomy and portal vein occlusion is an effective surgical strategy to clear tumor of the non-occluded FLR in the first step. However, intrahepatic liver size regulation after this combined procedure is not investigated. There is no well-characterized experimental model to study the underlying regulatory mechanism. Therefore, we designed a surgical model consisting of simultaneous portal vein ligation (PVL) and PHx to study intrahepatic size regulation.

Aim: The aim of the study was to characterize the experimental model of simultaneous PVL and PHx. Different extents of PVL respectively PHx were performed to study intrahepatic size regulation and investigate the underlying key processes: hepatocyte proliferation, apoptosis and autophagy. We further designed a prospective experiment to explore the underlying regulatory mechanism.

Methods and results: As reported in **manuscript I (Scientific Reports 2020, IF 3,998)**, we focused on the effect of different extents of combined PVL and PHx on atrophy of the ligated liver lobes. Rats were subjected to two experimental procedures: either simultaneous 20%PVL and 70%PHx or simultaneous 70%PVL and 20%PHx. For control, 20% PVL alone respectively 70%PVL alone were performed.

The ligated liver lobes underwent atrophy after PVL. However, atrophy of the ligated liver lobes was counteracted by additional PHx. Even more, the ligated liver lobes developed substantial hypertrophy after additional large resection due to the induction of hepatocyte proliferation. At the same time, apoptosis in the ligated lobes was significantly decreased after additional PHx compared to PVL alone. Furthermore, autophagy was substantially activated in the ligated lobes after additional PHx compared to PVL alone. In comparison with

additional small PHx, additional large PHx induced more hepatocyte proliferation and more autophagy in the ligated lobes, accompanied by decreased apoptosis.

As described in **manuscript II (Submitted to Scientific Reports 2020, IF 3,998),** the influence of additional PHx on the non-ligated FLR was also investigated. We compared the amount of hepatocyte proliferation in the non-ligated liver lobes after simultaneous 20%PVL and 70%PHx respectively simultaneous 70%PVL and 20%PHx. For control purposes, 90%PVL alone was performed.
Additional PHx augmented hypertrophy of FLR compared to 90%PVL alone, accompanied by substantially higher induction of autophagy. However, no statistically significant difference in autophagy was observed between additional large resection and additional small resection.

Based on both presented studies, we realized the important role of autophagy in our experimental model. We are planning a subsequent prospective study to explore the underlying mechanism using first the PVL model in rats and later the combined model. To explore the role of autophagy in size regulation of both ligated and non-ligated liver lobes, autophagy modulators will be applied to animals. The postoperative hepatic injury, hepatocyte proliferation, apoptosis and autophagy will be investigated.

Conclusion: Our experimental model is suitable to investigate intrahepatic size regulation following simultaneous PVL and PHx. Based on our observations autophagy was activated in both ligated and non-ligated liver lobes, accompanied by suppression of apoptosis and promotion of proliferation. Therefore we concluded that autophagy may play an essential role in intrahepatic size regulation. The expected findings of the planned study may contribute to a novel strategy which accelerates the hypertrophy of FLR by modulating autophagy and therefore expand the indications for extended hepatectomy following PVL.

Introduction

Due to the advancement of surgical techniques and the development of postoperative care, surgical treatment improves the long term survival (Orcutt et al. 2018). Liver resection remains the first choice for patients diagnosed with malignant liver tumors, since it is the only potentially curative treatment. Patients with multiple bilateral tumors require extended hepatectomy to remove all lesions. Extensive removal of liver mass is associated with a high risk of postoperative liver failure due to massive hepatic injury and the small size of FLR (Lee et al. 2020, Uemura et al. 2020).

Concept of portal vein occlusion

Extended hepatectomy is not possible in patients with an inadequate size of expected FLR. In order to expand the indications for surgical treatment, portal vein occlusion prior to major hepatectomy was established (Isfordink et al. 2017, van Gulik et al. 2008). This concept was first identified early in 1897, by Sir James Cantlie. He reported his observations of an autopsy on a patient's liver, in which the left part was significantly hypertrophied whereas the right side of the liver was atrophied. He also found that the hepatic vessels to the right side of the liver were devastated. Therefore, he speculated that ligation of hepatic vessels to one side of liver lobe may drive the contralateral liver lobe to compensatory hypertrophy (van Gulik et al. 2010). About two decades later, this striking theory was proven in a rabbit model reported by Rous and Larimore (Rous et al. 1920). After performing PVL, the authors observed that the portally deprived liver lobes underwent atrophy whereas the non-ligated liver lobes underwent compensatory hypertrophy (Rous et al. 1920). Similar findings were demonstrated by Rozga et al. using a rat model with different extents of PVL (Rozga et al. 1986). They further reported that the intensity of compensatory hypertrophy induced by PVL in the non-ligated lobes was positively correlated to the extent of atrophy in the ligated lobes (Rous et al. 1920)

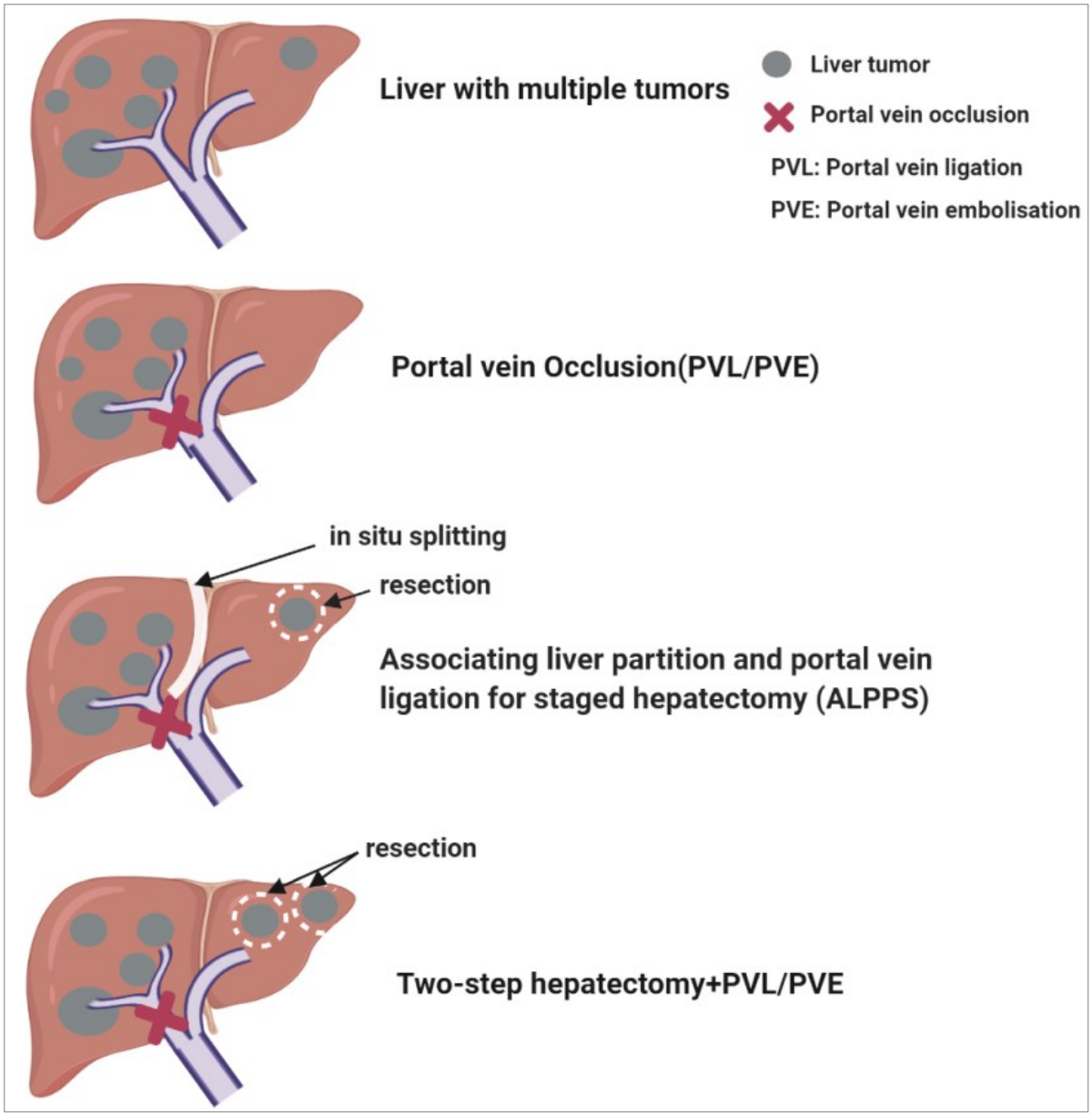

Figure S1: Surgical variations of of portal vein occlusion

Further development of different portal vein occlusion techniques (Figure S1)

Following portal vein occlusion, the occluded liver lobe develops atrophy whereas the non-occluded liver lobe undergoes hypertrophy. This phenomenon was termed as atrophy/hypertrophy complex (Ren et al. 2015). In the late 1980s, Makuuchi and coworkers first performed portal vein embolization (PVE) prior to major hepatectomy in patients with initially irresectable hilar bile duct carcinoma (Makuuchi et al. 1990). PVE successfully induced atrophy of the portal vein occluded lobe and hypertrophy of the non-occluded lobe. By increasing the volume of FLR following this strategy the indication for extended PHx was expanded, thereby increasing the chance for curative surgical treatment for more patients

(Makuuchi et al. 1990). Since then, this strategy was widely accepted in liver surgery. However, the second step major hepatectomy cannot be performed in around 1/3 of the patients subjected to portal vein occlusion due to insufficient hypertrophy and unpredicted tumor progression (Abdalla 2010, Garcia-Perez et al. 2015). The waiting time between portal vein occlusion and major hepatectomy is usually above 1 month and tumor growth often continues in this waiting period (Fischer et al. 2013, Hoekstra et al. 2012).

To shorten the waiting period, PVL combined with in situ parenchymal partition was firstly applied by Schnitzbauer and colleagues (Schnitzbauer et al. 2012). This novel procedure induced rapid hypertrophy of the non-ligated tumor-free left lobe. The procedure was later termed as associating liver partition and portal vein ligation for staged hepatectomy (ALPPS). The ALPPS procedure is considered as an effective technique to trigger faster hypertrophy of the FLR compared with selective portal vein occlusion alone (mean of waiting period: 9 days vs 4 weeks) (Baili et al. 2019, Garcia-Perez et al. 2015). The ALPPS strategy attracts increasing interest and has been adapted in many specialized liver surgery centers. The postoperative morbidity and mortality varies substantially between the centers due to the highly variable case loads and the resulting highly different levels of expertise (Wanis et al. 2020). Besides, the parenchymal transection results in enormous surgical trauma and high morbidity rate (Baili et al. 2019). Nevertheless, it was confirmed repeatedly that the ALPPS procedure induces fast hypertrophy of the FLR mass. However, the functional capacity of the regenerated liver and the process of intrahepatic size regulation are less well explored.

In patients with bilateral lesions, evidence is accumulating that the growth rate of the metastases in FLR is more rapid compared to that of normal parenchyma (Elias et al. 1999). Adam and colleagues performed two-sequential hepatectomy in patients with initially irresectable multiple bilateral liver tumors (Adam et al. 2000). The purpose of first step hepatectomy is to remove the highest possible amount of tumor mass and the second hepatectomy is performed when the hypertrophy of the FLR is deemed sufficient to sustain the metabolic needs of the patient (Adam et al. 2000).

Two-sequential hepatectomy was then combined with PVE by Jaeck D and others (Jaeck et al. 2003). They reported their successful sequence of surgical procedures, which consists of PVE after first-step hepatectomy, followed by a second step major hepatectomy. The first step hepatectomy clears all metastases of the FLR (usually left liver lobe). The major resection (right hepatectomy) is performed when the FLR reaches the desired extent of hypertrophy considered to cover the metabolic needs of the patient. The reported surgical outcome suggests that the procedure is a safe and effective treatment for patients with primarily

irresectable multiple bilateral liver tumors (Jaeck et al. 2003). By reducing the tumor burden, the first-stage clearance of the left lobe leads to favorable treatment outcomes. Surgical stress in the first step could be further decreased by a laparoscopic approach (Di Fabio et al. 2012, Levi Sandri et al. 2015).

Intrahepatic size regulation and underlying cellular processes

It is well known that hepatic atrophy and hypertrophy complex is governing intrahepatic size regulation. However, the relative contributions of the underlying key processes are not well explored. Furthermore, it is widely accepted that hepatocyte proliferation is leading to hypertrophy and cell death like apoptosis is leading to atrophy (Picard et al. 2004, Ren et al. 2015, Starkel et al. 1999). The balance of these two key processes taking place in the liver determines intrahepatic size regulation. Current evidence suggests that autophagy as energy producing process might play a role as well (Jia et al. 2019).

Regeneration: hepatocytes proliferation

One of the unique characteristics of the liver is its remarkable capability of regeneration to compensate the loss of liver cells (Tao et al. 2017). Hepatocytes are the main components of liver parenchymal cells (Alevra Sarika et al. 2020). In normal adult rat liver, the cellular mitosis rate is lower than 0.01%. Almost all hepatocytes and non-parenchymal cells remain in G0 phase (F. Xu et al. 2020). When facing various injuries including surgical trauma and chemical damage, the residual hepatocytes enter into the cell cycle rapidly from the quiescent state (Clemens et al. 2019, Zhou et al. 2015). Liver regeneration via hepatocyte proliferation compensates for the loss of liver cells and restores hepatic function (Abu Rmilah et al. 2019).
Liver regeneration after loss of liver mass is a highly complex and well-orchestrated process. Liver regeneration following partial hepatectomy is divided into 3 stages (Figure **S2**). The first and critical priming stage is initiated by pro-inflammatory cytokines like tumor necrosis factor (TNF) and interleukin-6 (IL-6). Quiescent hepatocytes (G0) enter into G1 phase during this step. The second stage is the proliferation stage, which is mainly controlled by hepatocyte growth factor (HGF) and transforming growth factor (TGF-α). In this stage, hepatocytes enter into synthesis and subsequent mitosis phase. In the final stage, the so-called termination phase, hepatocyte proliferation is ceased upon sensing the sufficient liver size. The termination of hepatocyte proliferation is related to transforming growth factor (TGF-β) and interleukin-1 (IL-1) (Hayashi et al. 2005, Jia 2011, Tao et al. 2017, Zimmermann 2004).

Liver size: balance of cell proliferation and cell death

The regulation of cell proliferation and apoptosis is essential to control the organ size (Patel et al. 2017). In response to the loss of liver mass caused by hepatic injury and PHx, the liver size can be maintained by promoting proliferation and inhibiting apoptosis (Kim et al. 2019).

Apoptosis, the programmed cell death, plays an essential role in the maintenance of the homeostatic process (Guicciardi et al. 2005). The morphological alterations observed during apoptosis consist of chromatin condensation and marginalisation, plasma membrane blebbing and cell shrinkage. The programmed form of cell death is activated by initiator and excecutioner caspases and executed by cleaving proteins and DNA into small fragments (Schwabe et al. 2018). Recently, death receptors such as TNF-R and FASL-R were reported to regulate both hepatocyte proliferation and apoptosis, indicating an important role of apoptosis during the process of liver regeneration (Zhou et al. 2015).

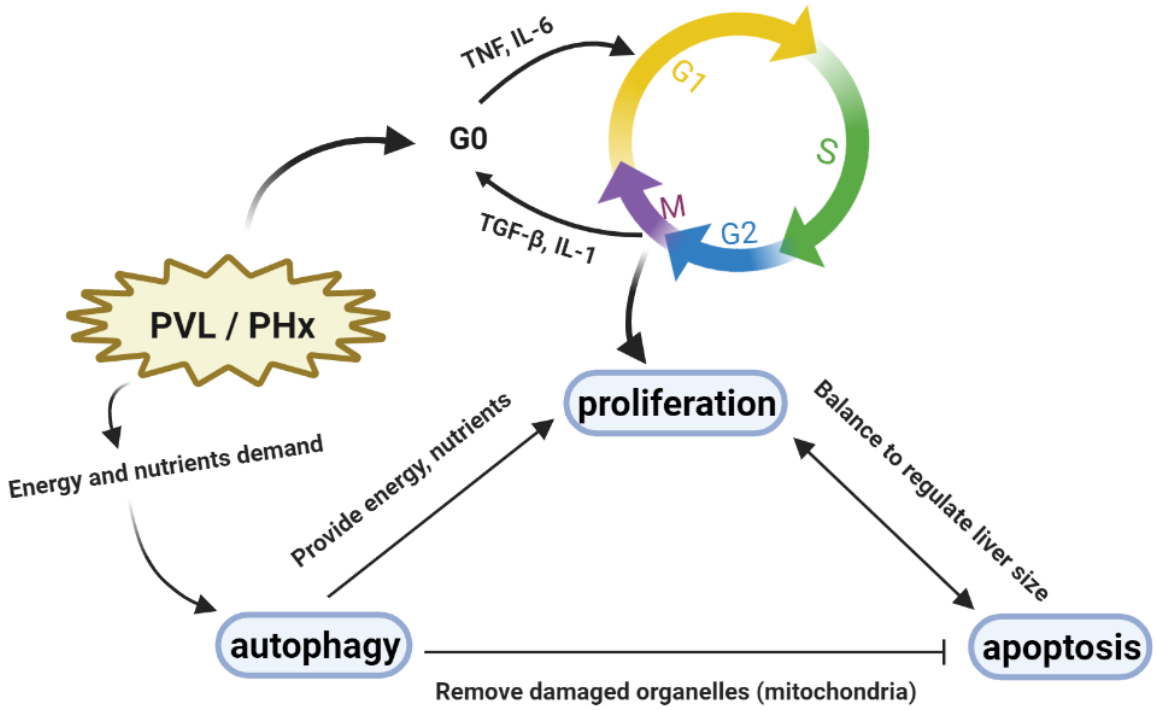

Figure S2. Interplay of proliferation, apoptosis and autophagy after simultaneous PVL and PHx

Liver regeneration and autophagy

Autophagy is a highly conserved catabolic process which is essential for maintaining intracellular homeostasis (Giampieri et al. 2019). Autophagy plays a protective role in cellular survival processes in response to various types of cellular stress (Giampieri et al. 2019, Kawabata et al. 2020). Liver resection as well as portal vein occlusion cause hepatic injury

and loss of hepatic parenchymal cells. The compensatory liver regeneration is highly dependent on energy and nutrients. Autophagy can break down misfolded and aggregated proteins and remove damaged organelles. Besides, autophagy recycles cellular components and provides amino acids, free fatty acids and glucose as energy and bricks for construction of new products (Czaja et al. 2013, Tan et al. 2020).

The role of autophagy following acute liver injury and liver surgery is gaining attention. Lin and coworkers reported that activation of autophagy promoted the regeneration of FLR and alleviated the hepatic injury after PHx (Lin et al. 2015). In a recent study, the activation of autophagy in the early stage after PVL was observed by Jia and others (Jia et al. 2019). They further reported that activation of autophagy contributed to the rapid liver regeneration in their PVL model (Jia et al. 2019).

Gap of knowledge regarding the interplay of cellular processes after simultaneous PVL and PHx

Portal vein occlusion prior to major hepatectomy for primarily irresectable liver malignant diseases is an effective strategy and has been widely adopted by surgeons in recent decades. However, the atrophy and hypertrophy complex governing intrahepatic size regulation is not fully explored. It is well known that hepatocyte proliferation and apoptosis are key processes in intrahepatic size regulation. Autophagy might play a role in interaction of hepatocyte proliferation and apoptosis.

A better understanding of the molecular mechanism underlying intrahepatic size regulation after PVL might help to improve surgical treatment. Several experimental studies investigated the intensity and dynamics of hypertrophy of FLR after portal vein occlusion (Bax et al. 1956, Rozga et al. 1986, Weinbren 1955). The relation of the extent of hypertrophy to the extent of atrophy was discussed in these studies. It remains controversial whether the extent of atrophy of portal vein occluded lobes determines the extent of hypertrophy of non-occluded lobes. On the one hand, different strategies were applied to promote hypertrophy of the non-occluded lobe, while inducing more atrophy in the occluded lobe (D. Kawaguchi et al. 2019, Schadde et al. 2019, Sugimoto et al. 2009). These studies elucidated the positive relation between the course of atrophy and the subsequent FLR hypertrophy after portal vein occlusion. However, on the other hand, it was observed in different animal models that the extent of atrophy did not always determine the extent of hypertrophy (Furrer et al. 2008, Picard et al. 2003, Picard et al. 2004, Wilms et al. 2008). The impact of atrophy and hypertrophy complex in surgical practice such as combined two-sequential hepatectomy and portal vein occlusion is not fully

understood. No suitable experimental model was introduced to study intrahepatic liver size regulation in the case of two concurrent stimuli such as PVL and PHx acting on the same liver lobe.

In a previous study of our group, Wei and colleagues established a rat model with simultaneous small 20%PVL and large 70%PHx to study intrahepatic size regulation in this situation (W. Wei et al. 2016). Compared to PVL alone, they observed attenuated apoptosis and moderate hepatocyte proliferation in the ligated liver lobes after combined PVL and PHx. It suggested that our experimental model could be a suitable model to study intrahepatic size regulation.

A better characterization of our model could improve the understanding of the balance between hepatocyte proliferation and apoptosis in the atrophied and hypertrophied liver lobes. Furthremore, it is of great intrest to better understand the role of autophagy in our experimental model with simultaneous PVL and PHx. A more profound knowledge of the molecular interplay of the intertwingled processes of autophagy, proliferation and apoptosis could be the basis for developing a promising strategy to improve liver regeneration in the FLR.

Aims and Hypotheses

Aims

In this study, we set three aims:

1. characterize size regulation of the ligated liver lobes after simultaneous PVL and PHx and evaluate the underlying key processes: hepatocyte proliferation, apoptosis and autophagy;
2. investigate liver volume recovery of the non-ligated liver lobes after simultaneous PVL and PHx by detecting hepatocyte proliferation and autophagy
3. design a future interventional experimental study using autophagy modulators to explore the interrelated mechanism

Hypotheses

(1) regarding size regulation of the ligated liver lobes, we hypothesized that (a) additional PHx counteracts atrophy through promoting proliferation, inducing autophagy and reducing apoptosis, and that (b) the effect is related to the extent of resection;

(2) regarding the extent of hypertrophy in the non-ligated liver lobes, we hypothesized that (a) liver growth is dependent on the course of atrophy in the ligated lobes and that (b) regeneration through hepatocyte proliferation is related to autophagy induction;

(3) regarding the role of autophagy, we hypothesized that application of autophagy inducer further promotes hypertrophy of the non-ligated liver lobes and reduces atrophy of the ligated liver lobes via accelerating proliferation and suppressing apoptosis.

Study design

To achieve these goals, we designed a study with three parts.

1. To explore the interaction of regeneration and atrophy in portally deprived liver lobe, the experimental model of simultaneous PVL and PHx with different extents was used. In this study, we calculated the liver volume and investigated hepatocyte proliferation, apoptosis and autophagy of the ligated liver lobes. We described the interplay between proliferation, apoptosis as well as autophagy in intrahepatic size regulation. An original research paper entitled *"Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis"* was published (Wei W, Hua C, Scientific Reports 2020).

2. To identify the effect of attenuated atrophy of the ligated liver lobes on the hypertrophy development of non-ligated FLR, we evaluated the liver volume, the proliferation index as well as mRNA expression level of proliferating cell nuclear antigen (PCNA). We further investigated the autophagy related protein and explored the interaction of autophagy and proliferation in our surgical model. A corresponding manuscript entitled *"Additional partial hepatectomy at the time of portal vein ligation accelerates the regeneration of future liver remnant"* was submitted to "Scientific Reports".

3. To further explore the underlying mechanism, we designed a subsequent study by using autophagy modulators in animal model with PVL alone, and later combined PVL and PHx. Autophagy promoter ezetimibe will be applied to induce autophagy via mTOR-independent pathway. Autophagy related proteins, proliferation makers and apoptosis indicators will be evaluated. The prospective project entitled *"Ezetimibe augments liver regeneration and suppresses apoptosis after portal vein ligation via autophagy activation"* is under preparation.

Manuscripts

Manuscript I

Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis

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Weiwei Wei, **Chuanfeng Hua**, Tianjiao Zhang, Olaf Dirsch, Felix Gremse, André Homeyer, Utz Settmacher, Uta Dahmen

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Authorship

First author

Authors' Contribution

W.Wei, C.Hua and U.Dahmen contributed to conception and design;

C.Hua, W.Wei and U.Dahmen for analysis and interpretation;

T.Zhang, F.Gremse and A.Homeyer helped in data acquisition

C.Hua, W.Wei and T.Zhang is responsible for performing the experiments and drafting of manuscript

O.Dirsch, U.Settmacher and U.Dahmen helped in manuscript revision

U.Dahmen and O.Dirsch for obtaining funding.

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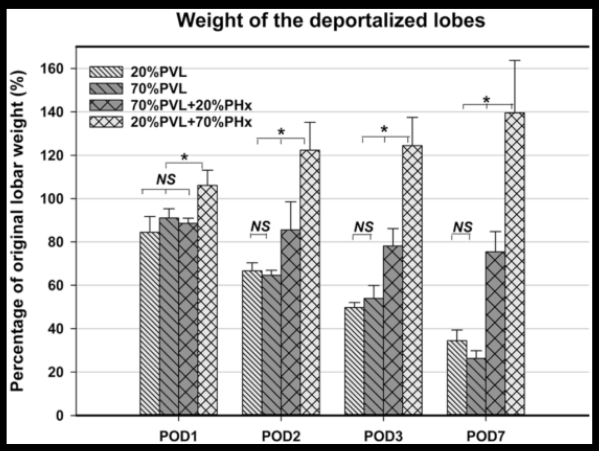
Uta.Dahmen@med.uni-jena.de


Weight of the deportalized lobes
Percentage of original lobar weight (%)
20%PVL
70%PVL
70%PVL+20%PHx
20%PVL+70%PHx
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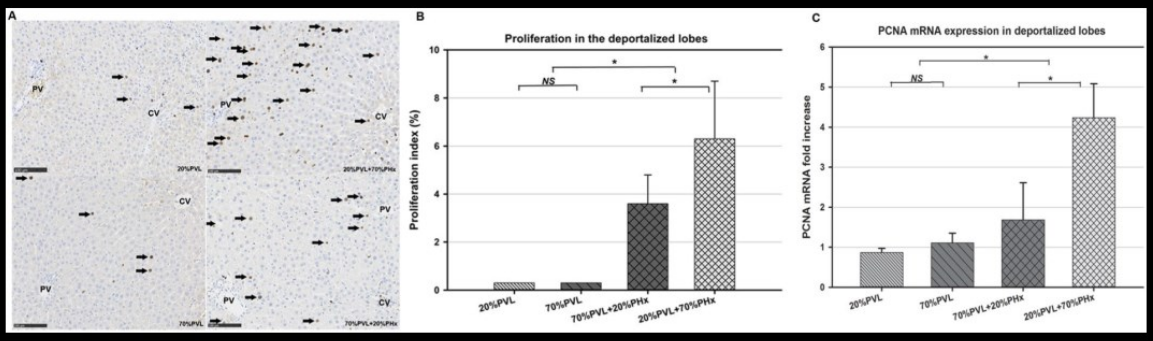

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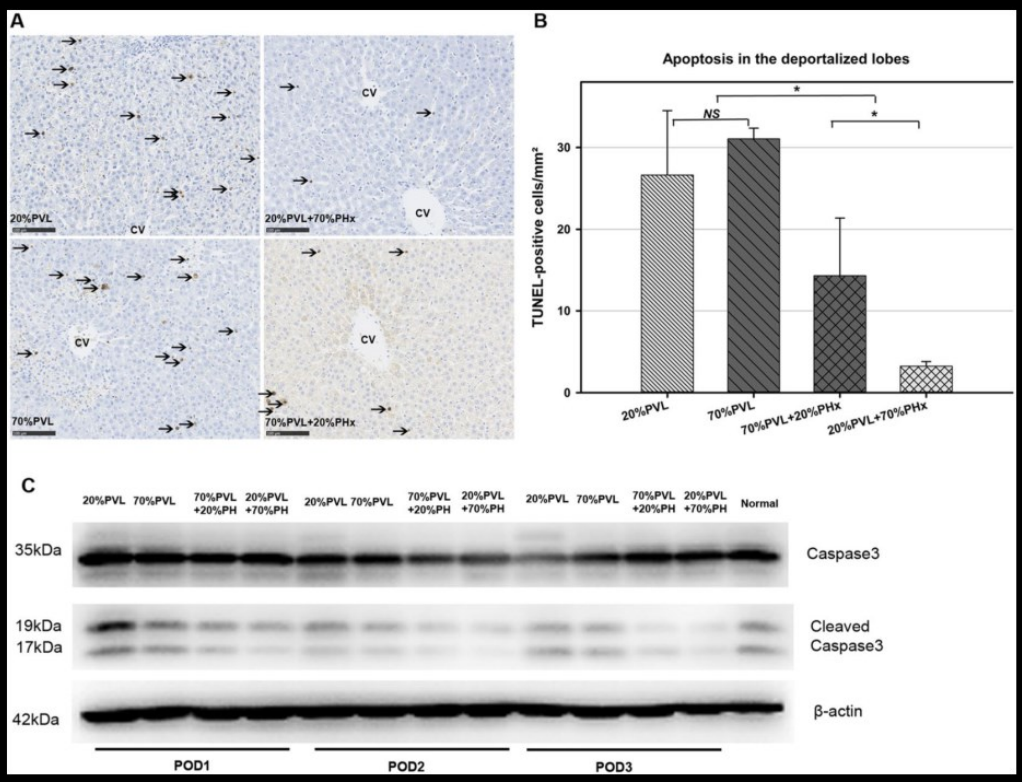

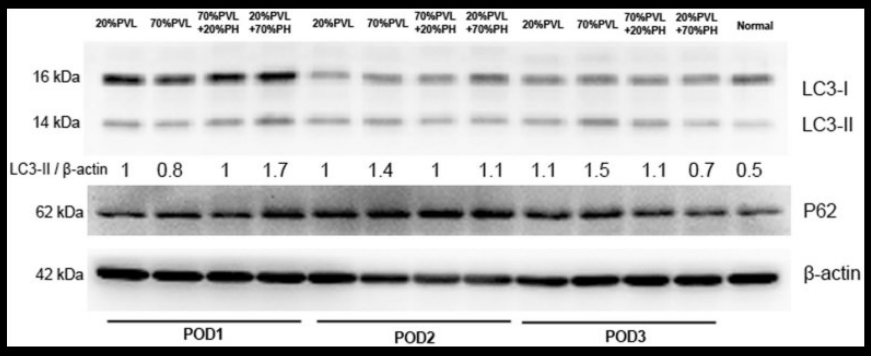

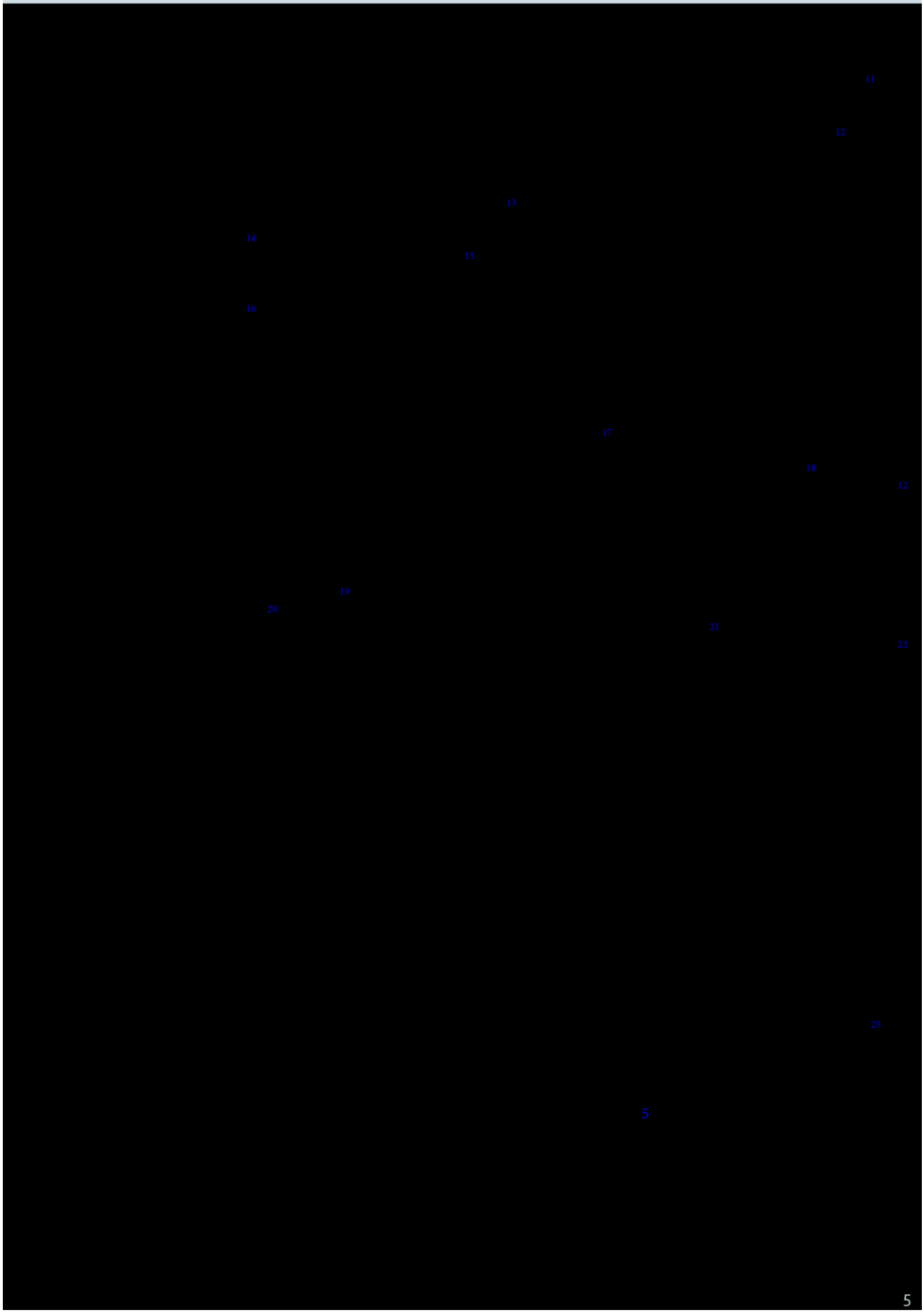
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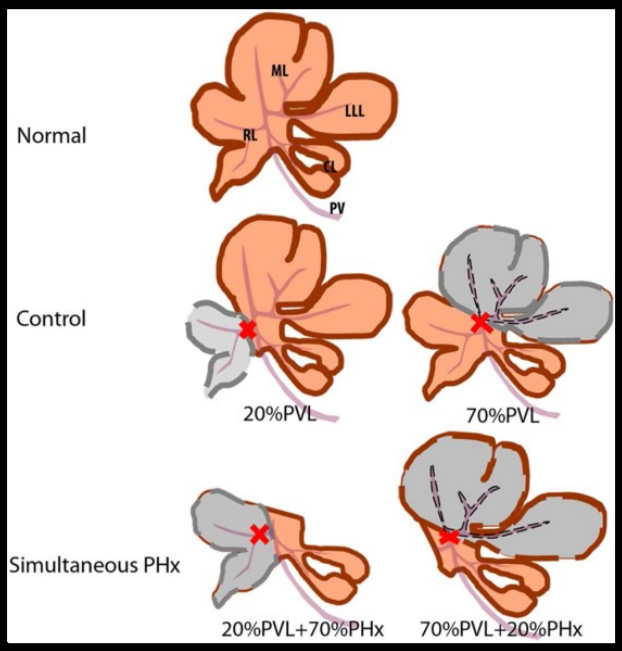
Normal
ML
LLL
RL
CL
PV
Control
20%PVL
70%PVL
Simultaneous PHx
20%PVL+70%PHx
70%PVL+20%PHx

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Manuscript II

Additional partial hepatectomy at the time of portal vein ligation accelerates the regeneration of future liver remnant

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Chuanfeng Hua, *MS* Weiwei Wei, *MD* Tianjiao Zhang, *MD* Fengming Xu, MS Olaf Dirsch, *PhD* André Homeyer, *PhD* Utz Settmacher, *MD* Uta Dahmen, *MD*

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Authors' Contribution

C.Hua, W.Wei and U.Dahmen contributed to conception and design;

C.Hua, W.Wei and T. Zhang for experiment performance, results analysis and interpretation and drafting manuscript;

A.Homeyer helped in providing analysis tool and data acquisition

O.Dirsch, U.Settmacher and U.Dahmen helped in manuscript revision

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Additional partial hepatectomy at the time of portal vein ligation accelerates the regeneration of the future liver remnant

Chuanfeng Hua, *MS* [a]* Weiwei Wei, *MD* [a]* Tianjiao Zhang, *MD* [b] Fengming Xu, MS[a] Olaf Dirsch, *PhD* [c] André Homeyer, *PhD* [d] Utz Settmacher, *MD* [a] Uta Dahmen, *MD*[a#]

[a] Department of General, Visceral and Vascular Surgery, Jena University Hospital, Jena, Germany

[b] Department of Radiotherapy and Radiation Oncology, Jena University Hospital, Jena, Germany

[c] Institute of Pathology, Klinikum Chemnitz gGmbH, Chemnitz, Germany

[d] Fraunhofer Institute for Digital Medicine MEVIS, Bremen, Germany

* These authors contributed equally to this work.

Corresponding author

E-mail: Uta.Dahmen@med.uni-jena.de Tel.: 03641-9325350 Fax: 03641-9325352

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Conflict of Interest

The authors disclose no conflicts of interest.

Abstract

Background Portal vein ligation (PVL) has been adopted to induce hypertrophy of the future liver remnant (FLR) in patients with primarily irresectable liver tumor. However, regeneration of the FLR is not always sufficient to allow curative resection of the portally-deprived tumor-bearing liver lobe. We hypothesize that simultaneous hepatectomy (PHx) and PVL augments regeneration of FLR and that the effect is related to the extent of the additional resection.

Method Seventy-two Lewis rats were enrolled into 3 groups: (1) 20%PVL + 70%PHx; (2) 70%PVL+20%PHx; (3) 90%PVL. Animals were observed for 1, 2, 3 and 7 days postoperatively (n=6/time point). Liver enzymes, caudate liver/body-weight-ratio, BrdU-proliferation-index (PI), proliferating-cell-nuclear-antigen (PCNA)-mRNA-expression level and autophagy-related-proteins were evaluated.

Result Compared with 90% PVL, additional PHx induced significantly more hypertrophy during the observation time, which was confirmed by significantly higher PI and higher level of PCNA-mRNA expression. Similarly, the additional PHx induced more autophagy in FLR compared with PVL alone. However, both effects were not clearly related to the extent of the additional resection.

Conclusion Additional resection augmented liver regeneration and autophagy substantially compared with PVL alone. Therefore, we concluded, that autophagy might play a critical role in regulating hepatocyte proliferation and the size of the FLR after simultaneous PVL+PHx.

Keywords: portal vein ligation, partial hepatectomy, hepatic hypertrophy, hepatocyte proliferation, autophagy.

Abbreviations

PHx,	partial hepatectomy
PVL,	portal vein ligation
PVE,	portal vein embolization
FLR,	future liver remnant
AST,	aspartate aminotransferase
ALT,	alanine aminotransferase
BrdU,	5-bromo-2-deoxyuridine
PI,	proliferation index
POD,	postoperative day
PCNA,	proliferating cell nuclear antigen

Introduction

The liver is the primary site of metastasis for many tumors, especially of colorectal cancer. Surgical resection is the only curative treatment for malignant liver tumors and offers the patients a chance for long-term survival[1]. At the time of diagnosis, the majority of patients have multiple metastases[2]. High metastatic burden requires extended liver resection. An important risk of extended liver resection is the inadequate size and function of the future liver remnant (FLR), which is associated with substantial postoperative morbidity and mortality[3,4]. Therefore, patients with a high tumor load are often excluded from this potentially life-saving therapeutic option[5].

In the case of initially irresectable liver tumor, different staged procedures were introduced in the clinic: portal vein occlusion followed by PHx, two sequential hepatectomies and the combination of portal vein occlusion and two sequential hepatectomies.

Portal vein occlusion prior to performing the major liver resection is used most frequently to induce hypertrophy of the FLR. Two main technologies were established: portal vein ligation (PVL) and portal vein embolization (PVE)[6,7]. Portal deprivation causes atrophy of the deportalized liver lobe and compensatory hypertrophy of the portally supplied contralateral lobe. The first successful clinical application of this concept was reported by Makuuchi and coworkers[8], who performed PVE of the tumor-bearing lobe prior to major hepatectomy. They demonstrated that the technique was feasible and decreased the post-hepatectomy liver failure.

Performing portal vein occlusion prior to the major resection broadens the indications of surgical treatment and is now adopted as one standard procedure for patients with initially irresectable tumors. However, around one-third of the patients undergoing portal vein occlusion in the first stage cannot be subjected to the second stage procedure[9,10]. The most

frequently reported reasons are insufficient hypertrophy of the FLR and progression of tumor growth[10]. Portal vein occlusion prior to major hepatectomy can not only induce hypertrophy in FLR, but also stimulates tumor growth in both, the occluded lobe and the non-occluded lobe[11,12]. It was reported that the growth rate of the tumor tissue in the non-ligated liver lobe may even be higher than the growth rate of the healthy non-tumor liver tissue, resulting in persistent irresectability[13,14]. Thus preoperative portal vein occlusion should not be performed in patients with initially irresectable multiple bilateral colorectal liver metastases.

Another procedure to increase the number of patients eligible for extended surgical resection was introduced by Adam and colleagues, who performed "two-stage (sequential) hepatectomy"[2]. The procedure consisted of two sequential liver resections to remove initially irresectable multiple bilateral liver tumors. Later, two-sequential hepatectomy was combined with portal vein occlusion[15,16]. Jaeke D et al. reported a small series of successful two-stage hepatectomy combined with PVE in patients with initially irresectable MBLMs. The first-stage hepatectomy cleared all the tumors located in the FLR (the left lobe) and followed by PVE of the right lobe to induce hypertrophy of FLR. The second-stage major hepatectomy was performed when the hypertrophy of FLR was sufficient[15]. Thus the tumor burden was reduced by "cleaning" the FLR completely in the first stage hepatectomy. Using this modified strategy allowed to expand the indications for resecting multiple bilateral colorectal liver metastases and provided selected patients with a chance of a curative treatment. Therefore it is of interest to better understand the effect of combined portal vein occlusion and partial hepatectomy on intrahepatic size regulation.

In our previous studies[17,18], we established a rat model combined PVL and PHx. We investigated the influence of the additional PHx on the ligated liver lobe. We found that additional PHx abrogated atrophy and induced mild hepatocyte proliferation in the ligated liver lobe[17]. We also observed that different extents of additional PHx abrogated the atrophy

of ligated lobe to different degrees. Performing a large additional resection (70%PHx) caused a slight increase in the size of the ligated liver lobe which normally undergoes substantial atrophy. However, performing a small additional resection (20%PHx) did not fully prevent atrophy of the ligated liver lobe, but reduced the extent of atrophy substantially compared to PVL alone. Furthermore, we observed an induction of proliferation, down-regulation of apoptosis and an up-regulation of autophagy in the ligated lobe[18]. In other words, the additional resection had a substantial impact on the size regulation of the ligated lobe, which was related to the extent of the additional resection.

The aim of the present study is to investigate the influence of the additional resection on the size regulation of the non-ligated lobe. We wanted to explore the amount of hypertrophy of the non-ligated FLR after simultaneous PVL and PHx of different extents compared with PVL alone. Furthermore, we wanted to figure out whether the extent of resection itself or the resulting different degrees of atrophy in the ligated lobe caused by the different extents of additional liver resection would also affect the extent of hypertrophy in FLR. We were also interested in exploring the role of autophagy in size regulation of the FLR.

We hypothesized that combining PVL and PHx augments the regenerative response in an "extent of resection-dependent" manner compared to PVL alone, possibly facilitated by the induction of autophagy in the FLR.

To investigate this hypothesis we investigated 3 experimental groups: (I) major PVL alone representing the conventional first step in two-stage hepatectomy without atypical resection; (II) major PVL with additional minor liver mass resection, similar to minor "cherry picking" resection; (III) minor PVL with additional major liver mass resection, mimicking a substantial liver resection at the time of portal vein occlusion.

The impact of additional liver resection on augmenting the regeneration of the FLR was compared with conventional simple PVL. Furthermore, the effect of performing different extents of additional resection on autophagy in the FLR was evaluated.

Results

As a first step, we assessed whether the additional resection aggravated the damage to the liver compared with extended PVL. We evaluated the postoperative liver enzyme release. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were selected as markers of acute liver injury following surgical treatment. As partly reported in our previous study[17,18], additional PHx did not induce significantly more injury to the liver compared with 90% extended PVL. The level of serum ALT and AST peaked on postoperative day (POD) 1 and decreased progressively to normal level on POD7. No significant differences were observed in the levels of AST and ALT after additional PHx and 90%PVL alone (Figure1).

Second, we assessed the hypertrophy of the FLR. Additional PHx induced more hypertrophy in the FLR compared with 90%PVL alone. To determine the extent of hypertrophy of the non-ligated liver lobe, the caudate lobe to body weight ratio was calculated. At all observation time points, the caudate liver lobe to body weight ratio in the non-ligated remnant liver was significantly higher after simultaneous PVL and PHx compared to PVL alone as shown in Figure 2 ($p < 0.05$). On POD2, the caudate liver lobe to body weight ratio after additional large resection was even about 2 fold higher than after PVL alone. However, no significant difference in the size of the caudated lobe (FLR) was observed when comparing large and small additional resection.

Third, we studied hepatocyte proliferation. We observed similar results as hypertrophy extent when quantifying hepatocyte proliferation in the FLR. Hepatocyte proliferation rate in the FLR was significantly higher on POD 2 and 3 after simultaneous PVL and PHx compared with 90%PVL without resection. As shown in Figure 3a, the proliferation in FLR on POD2 increased to 15% and 12% after 20%PVL+70%PHx respectively 70%PVL+20%PHx, which were significantly higher compared to 90%PVL alone (15% vs 6%, 12% vs 6%, $p<0.05$, Figure3b). However, there was no statistically significant difference in hepatocyte proliferation rate when comparing small and large additional liver resection.

For further confirmation we assessed proliferating cell nuclear antigen (PCNA) mRNA expression. Here, we observed a significantly higher expression level of PCNA mRNA in the FLR on POD1 after additional large resection compared with the additional small resection and with PVL alone (fold change 11 vs 8.4 vs 6.7, $p<0.05$, Figure3c). This effect was not seen on POD 2 and 3, where PCNA expression levels in all three groups were about 3-fold higher compared to normal liver tissue.

In the last step, we studied autophagy. To investigate the role of autophagy in promoting regeneration after simultaneous PVL and PHx, the expression levels of the autophagy related proteins LC3 and phosphorylated mTOR (p-mTOR) were determined (Figure4). In the early phase of liver regeneration, protein level of LC3II in the FLR was substantially higher on POD1 after simultaneous PVL and PHx compared with PVL alone, indicating a more pronounced induction of autophagy. Furthermore, the protein levels of p-mTOR in the FLR were higher after PVL alone compared with combined PVL and PHx. These results indicated that simultaneous PVL and PHx induced more autophagy compared to PVL alone.

Discussion

For patients with large or multiple malignant liver tumors, extended hepatectomy is the most successful strategy resulting in a rather favourable treatment outcome. However, substantial removal of liver mass also causes pronounced hepatic injury and a high risk of liver failure due to inadequate size of the FLR. Major hepatectomy is only considered to be feasible when the FLR is ≥40% for patients with cirrhotic liver and ≥30% in those with significant steatosis or fibrosis without cirrhosis. In patients with normal liver, a FLR of at least ≥20% is needed to sustain hepatic function after major hepatectomy[19].

Therefore staged liver surgery with or without portal vein occlusion was developed. It is well-known that upon various liver injuries and loss of liver mass, the remaining liver has the ability to compensate the loss of hepatic volume and to restore hepatic function[20,21]. In the past, different strategies were explored to induce regeneration of the FLR (first stage) prior to performing extended liver resection (second stage)[22]. These different strategies have in common that regeneration of the FLR is promoted by inflicting a controlled damage on the future resected liver lobes, resulting in regeneration of the FLR. The damage must be "strong enough" to induce regeneration but "weak enough" not to compromise hepatic function substantially. However, it is unknown which and how much damage should be inflicted on the liver to promote regeneration of the FLR while maintaining sufficient hepatic function during this critical period of time.

Theoretically various types of damage could serve the purpose as long as the damage is restricted to the future resected liver lobes and has little effect on the FLR. Portal vein occlusion including portal vein embolization and portal vein ligation was first established to improve resectability[23]. As outlined before, portal vein occlusion induces atrophy in the ligated liver and promotes hypertrophy in FLR, termed as atrophy/hypertrophy complex

(AHC)[24]. The non-ligated liver lobes undergo regeneration to compensate for the loss of hepatic function of the ligated lobes. Restoration of volume and function of the FLR improves the tolerance to the subsequent major hepatectomy performed as second stage operation[25]. The flaw of the procedure is that the waiting time between the two procedures may cause disease progression before proceeding to the second step.

In order to further enhance the regenerative process of the FLR under experimental conditions, several additional surgical strategies were developed. Besides PVL of given liver lobes, surgical modifications such as two-stage PVL, additional bile duct ligation and additional hepatic vein ligation were introduced in different experimental models (**Table1**).

Two-stage PVL, 70%PVL followed by the second 20%PVL seven days later, was established by Sugimoto et al. This new strategy was proven to induce more hypertrophy in FLR compared to the conventional one-stage PVL[26]. Ren and coworkers performed simultaneous bile duct and portal vein ligation in a rat model. They demonstrated that the simultaneous procedure accelerated the AHC and promoted hepatic proliferation in the FLR compared to PVL alone[27]. Additional hepatic vein ligation at the time of PVL was introduced by Kawaguchi et al. They found that additional hepatic vein occlusion resulted in more damage in the ligated lobe and induced more regeneration in the non-ligated liver compared with PVL alone[28].

In the present study, we suggest not to induce additional damage on the portally deprived liver but to remove part of the liver, as done clinically when “cleaning” the liver from bilobar metastasis. The additional resection serves two purposes: on the one hand to enhance regeneration of the FLR and on the other hand to mimic the clinical situation of “reducing the tumor load” of the liver. Furthermore using our novel model combining different extents of PVL with different extents of PHx, we can study the intrahepatic size regulation. In the previous study[18], we demonstrated that simultaneous PVL and PHx was a well-tolerated procedure allowing all rats to survive even when reducing the size of the healthy FLR to only

10% of the original liver mass. In the present study, we found that the combined procedure induced more hepatocyte proliferation and faster course of liver hypertrophy in the FLR than after PVL alone. However, we did not see a statistically significant additional effect on size regulation of the FLR when increasing the size of resection.

Using our model of simultaneous PVL and PHx, we observed previously in the ligated lobe that induction of the energy consuming process of hepatocyte proliferation was related to an increase in the energy providing process autophagy[18]. In the present study, we observed that increased regeneration of the non-ligated lobe after additional resection was also associated with an increased expression of autophagy related proteins.

The relationship between autophagy and liver regeneration is currently discussed in many experimental studies (see Table 2). Recently, experimental evidence is accumulating that autophagy activity plays an essential role in promoting regeneration and reducing organ damage[29-32]. It was reported that augmenter of liver regeneration promoted hepatocyte proliferation in mice through activation of autophagy in CCl_4-induced acute liver injury[31]. In a mouse model with 2/3 hepatectomy, Lu et al. found that miR-1907 accelerated hepatocyte proliferation via activating autophagy. They further observed a significant decrease in mIR-1907-induced liver regeneration after inhibiting autophagy[32]. Activation of autophagy in the non-ligated lobe following 70%PVL was also observed in a rat model. The study suggested that the increased autophagy activity was positively related to rapid hepatocyte proliferation[33]. Our present study further confirms these observations, since we also found that simultaneous PVL and PHx induced not only more regeneration but also more autophagy in FLR than PVL alone.

Based on these recent studies about the critical role of autophagy in liver regeneration, we suggest that the process of regeneration in the FLR after simultaneous PHx and PVL or PVL alone might also be further augmented by applying autophagy inducers. Application of an mTOR-independent autophagy promoter in an animal model of PVL or simultaneous PVL

and PHx seems to be an interesting approach to be investigated. This strategy might induce more proliferation and augment hypertrophy of FLR more than PVL alone, thereby shortening the interval time needed for restoring the original liver mass.

In summary, better understanding of the interaction between liver regeneration and autophagy after PVL and PHx is useful to refine therapeutic strategies for patients with primarily irresectable liver disease. Additional PHx will not only promote regeneration of the FLR but also reduce the tumor load in the first step, thereby preventing the increase of tumor burden during the waiting period. Additional application of mTOR-independent autophagy inducers could further promote liver regeneration of FLR. Modulation autophagy might shorten the period between the first and the second stage of this complex surgical strategy.

Conclusion

Simultaneous PVL and PHx procedures were performed on healthy rats without malignant disease resulting in augmented regeneration of the FLR and an enhancement of autophagy. These observations call for further exploration of promoting autophagy as novel strategy to augment liver regeneration in this complex surgical model.

Methods

Animals

Male Lewis rats weighing 250-300g (9-10weeks old), purchased from Charles River, Sulzfeld, Germany, were used in the present study. The rats were housed under constant room

temperature and humidity and a 12h-light-dark cycle in a conventional animal facility. Water and rat chow were provided ad libitum.

Ethics statement

The protocols were approved by the Thüringer Landesamt für Verbraucherschutz, Thuringia, Germany (Approval-Number: 02-024/13). All experiments and housing of animals were performed in compliance with the current German regulations and guidelines for animal welfare and the ARRIVE Guidelines for Reporting Animal Research[34].

Experimental design

Male Lewis rats were assigned into two experimental groups (Figure 5): **(1) 20%PVL+70%PHx**: ligation of right portal vein followed by resection of left lateral and median lobes (70% of the liver mass); **(2) 70%PVL+20%PHx**: ligation of right and left portal vein followed resection of right lobe (20% of the liver mass). The control consisted of additional twenty-four rats subjected to 90% PVL group which underwent ligation of left lateral, median and right lobes. Animals were observed for 24h, 48h, 72h and 7 days postoperatively (n=6/observation time point).

Operative procedures and postoperative management

The rats were acclimatized for 1 week before operation. Surgical procedures were conducted under inhalation anesthesia consisting of a mixture of 3% isoflurane and pure oxygen at a flow rate of 0.5L/min (isoflurane vaporizer, Sigma Delta, UK). Following skin disinfection, the abdominal cavity was opened via a transverse upper abdominal incision. The intestine was eventerated and covered by wet gauze. Portal vein branches were dissected from artery and bile duct and then ligated with 6-0 prolene suture using an operating microscope (Zeiss,

magnification 10-25×, Germany). Ligation of right portal vein represented 20%PVL, ligation of left portal vein represented 70%PVL, and ligation of both right and left portal vein represented 90%PVL. Additional PHx was then performed as described previously[17]. A Mosquito-clamp was placed on each liver lobe 2-3 mm distal from the inferior vena cava. The clamp was kept stable while removing the liver lobe and placing 2-4 piercing sutures. Next, the clamp was removed followed by replacing the abdominal viscera and closing abdomen. A dose of 0.05 mg/kg body weight of buprenorphine was applied as analgesic treatment to all animals postoperatively (Temgesic, Essex Pharma GmbH, Germany). Daily evaluation of general condition and activity was carried out after operation.

Harvesting and sampling

Rats were sacrificed postoperatively day (POD) 1, 2, 3 and 7. For detecting hepatocyte proliferation in FLR, rats were injected intravenously with a single dose of 50 mg/kg 5-bromo-2-deoxyuridine (BrdU, SIGMA-ALDRICH, St. Louis, USA) one hour before sacrifice. Blood collection was performed under anesthesia and liver tissue was harvested. The wet weight of remnant liver was measured and the liver weight/body weight ratio was calculated using the following formula: individual liver lobe weight (g)/ body weight (g)*100%.

Serum was isolated from the blood. The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by using the AEROSET System (Abbott Laboratories, Wiesbaden, Germany).

Histology and immunohistochemistry (IHC) analysis

Liver tissue was fixed by immersion in 4.5% buffered formalin for 48h and embedded in paraffin. Paraffin sections of 4μm thickness were prepared. BrdU-staining was performed for visualization of hepatocyte proliferation. A monoclonal anti-BrdU antibody (Dako, Hamburg, Germany) was use in the staining, following the protocol described previously[17].

Immunohistochemistry results were analyzed with the Histokat software (Fraunhofer MEVIS, Bremen, Germany) using a nuclei detection algorithm based on a previously published image analysis method[35]. The algorithm used machine learning techniques to recognize BrdU-positive and BrdU-negative nuclei, taking into account color, roundness and size features. The proliferation index was calculated as the fraction of the amount of BrdU-positive nuclei to the total number of hepatocyte nuclei, according to previously reported protocol.

Protein extraction and Western blotting

Liver tissues were homogenized in the RIPA buffer (sigma, R0278) containing the Protease and phosphatase inhibitor cocktail (Thermo Scientific, USA). The concentration of total proteins was measured by using BCA protein assay kit (Thermo Scientific, USA) and ELISA reader device. Equal amounts of protein were denatured with Laemmli sample buffer (Bio-Rad, USA). Proteins were separated in electrophoresis process and transferred to the polyvinylidene difluoride membranes. The membranes were washed and blocked as previously reported[36]. Primary antibodies rabbit anti-light chain 3 (LC3; 1:1000, Cell signaling Technology), rabbit anti-mammalian target of rapamycin (mTOR, 1:1000,Cell signaling Technology), rabbit anti-phospho-mTOR (Ser2448, 1:1000, Cell signaling Technology) and rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:10,000, Cell signaling Technology) were applied to the membranes and were incubated at 4℃ overnight. After the membranes being washed, the second antibody (Goat polyclonal antibody to rabbit IgG; 1:5000) was then used. After being probed by using enhanced chemiluminescence western blotting substrate (GE Healthcare), the signals were visualized by using Fusion FX7 (Labtech International Ltd, Heathfield, United Kingdom).

Real time PCR analysis

Total RNA was isolated from liver tissue sections using the RNeasy kit (Qiagen, Hilden, Germany) following the manufacture's instruction. RNA samples were reverse transcribed to cDNA by using the First-Strand cDNA synthesis KIT (Invitrogen, Carlsbad, USA). The mRNA expression of PCNA was investigated using the Brilliant probe-based QPCR Mater Mix kit (Agilent, Santa Clara, USA), performed by using M3000P QPCR System (Stratagene, La Jolla, USA). The mRNA level of HPRT was served as an endogenous control. The primers (eurofins Genomics, Germany) were listed as following: proliferating cell nuclear antigen (PCNA):forward5'-TGAACTTTTTCACAAAAGCCACT-3', reverse5'TGTCCCATGTCAGCAATTTTA-3'; hypoxanthine guanine phosphoribosyltransferase (HPRT): forward5'-GACCGGTTCTGTCATGTCG-3', reverse5'-ACCTGGTTCATCATCACTAATCAC-3'. Relative fold of gene expression of samples was calculated by the well-accepted $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

SigmaPlot 13.0 (Statcon, Witzenhausen, Germany) was adopted for data analysis. The differences between groups were compared using the one way independent ANOVA test. Statistical differences were considered significant when p values were less than 0.05.

Data availability

All data generated or analyzed during this study are included in this presented article. Parts of the data in this manuscript were presented in our previous paper[18] and Dr. Wei's thesis.

(https://www.dbthueringen.de/servlets/MCRFileNodeServlet/dbt_derivate_00039437/disswei.pdf).

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Author contributions:

C.H. and W.W. performed the experiments and drafted the manuscript. T.Z. helped in data acquisition and manuscript revision. F.X. took part in the manuscript revision. A.H. supported the image analysis. O.D. and U.D. supported the development of the experimental design and revised this manuscript. U.S. was involved in critical discussion of the manuscript.

References

1 Geng, L., Zong, R., Shi, Y. & Xu, K. Prognostic role of preoperative albumin-bilirubin grade on patients with hepatocellular carcinoma after surgical resection: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* **32**, 769-778, doi:10.1097/MEG.0000000000001618 (2020).

2 Adam, R., Laurent, A., Azoulay, D., Castaing, D. & Bismuth, H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg* **232**, 777-785, doi:10.1097/00000658-200012000-00006 (2000).

3 Isfordink, C. J. *et al.* Portal vein ligation versus portal vein embolization for induction of hypertrophy of the future liver remnant: A systematic review and meta-analysis. *Surg Oncol* **26**, 257-267, doi:10.1016/j.suronc.2017.05.001 (2017).

4 Garcea, G., Ong, S. L. & Maddern, G. J. Predicting liver failure following major hepatectomy. *Dig Liver Dis* **41**, 798-806, doi:10.1016/j.dld.2009.01.015 (2009).

5 Riddiough, G. E., Christophi, C., Jones, R. M., Muralidharan, V. & Perini, M. V. A systematic review of small for size syndrome after major hepatectomy and liver transplantation. *HPB (Oxford)* **22**, 487-496, doi:10.1016/j.hpb.2019.10.2445 (2020).

6 Rous, P. & Larimore, L. D. Relation of the Portal Blood to Liver Maintenance : A Demonstration of Liver Atrophy Conditional on Compensation. *J Exp Med* **31**, 609-632, doi:10.1084/jem.31.5.609 (1920).

7 van Gulik, T. M. & van den Esschert, J. W. James Cantlie's early messages for hepatic surgeons: how the concept of pre-operative portal vein occlusion was defined. *HPB (Oxford)* **12**, 81-83, doi:10.1111/j.1477-2574.2009.00124.x (2010).

8 Makuuchi, M. *et al.* Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* **107**, 521-527 (1990).

9 Garcia-Perez, R. *et al.* Associated Liver Partition and Portal Vein Ligation (ALPPS) vs Selective Portal Vein Ligation (PVL) for Staged Hepatectomy in a Rat Model. Similar Regenerative Response? *PLoS One* **10**, e0144096, doi:10.1371/journal.pone.0144096 (2015).

10 Abdalla, E. K. Portal vein embolization (prior to major hepatectomy) effects on regeneration, resectability, and outcome. *J Surg Oncol* **102**, 960-967, doi:10.1002/jso.21654 (2010).

11 Kokudo, N. *et al.* Proliferative activity of intrahepatic colorectal metastases after preoperative hemihepatic portal vein embolization. *Hepatology (Baltimore, Md.)* **34**, 267-272, doi:10.1053/jhep.2001.26513 (2001).

12 Hoekstra, L. T. *et al.* Tumor progression after preoperative portal vein embolization. *Ann Surg* **256**, 812-817; discussion 817-818, doi:10.1097/SLA.0b013e3182733f09 (2012).

13 Elias, D. *et al.* During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. *Br J Surg* **86**, 784-788, doi:10.1046/j.1365-2168.1999.01154.x (1999).

14 Bretagnol, F. *et al.* Selective portal vein embolization and colorectal liver metastases in rat: a new experimental model for tumor growth study. *J Surg Res* **171**, 669-674, doi:10.1016/j.jss.2010.03.047 (2011).

15 Jaeck, D. *et al.* One or two-stage hepatectomy combined with portal vein embolization for initially nonresectable colorectal liver metastases. *Am J Surg* **185**, 221-229, doi:10.1016/s0002-9610(02)01373-9 (2003).

16 Narita, M. *et al.* Two-stage hepatectomy for multiple bilobar colorectal liver metastases. *Br J Surg* **98**, 1463-1475, doi:10.1002/bjs.7580 (2011).

17 Wei, W. *et al.* Intrahepatic Size Regulation in a Surgical Model: Liver Resection-Induced Liver Regeneration Counteracts the Local Atrophy following Simultaneous Portal Vein Ligation. *Eur Surg Res* **57**, 125-137, doi:10.1159/000446875 (2016).

18 Wei, W. *et al.* Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis. *Sci Rep* **10**, 4893, doi:10.1038/s41598-020-60310-0 (2020).

19 Khan, A. S. *et al.* Assessment and optimization of liver volume before major hepatic resection: Current guidelines and a narrative review. *Int J Surg* **52**, 74-81, doi:10.1016/j.ijsu.2018.01.042 (2018).

20 Yamanaka, N. *et al.* Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology (Baltimore, Md.)* **18**, 79-85 (1993).

21 Ibis, C. *et al.* Factors Affecting Liver Regeneration in Living Donors After Hepatectomy. *Med Sci Monit* **23**, 5986-5993, doi:10.12659/msm.908136 (2017).

22 Szijarto, A. & Fulop, A. Triggered liver regeneration: from experimental model to clinical implications. *Eur Surg Res* **54**, 148-161, doi:10.1159/000368961 (2015).

23 Tustumi, F. *et al.* Preoperative strategies to improve resectability for hepatocellular carcinoma: a systematic review and meta-analysis. *HPB (Oxford)* **20**, 1109-1118, doi:10.1016/j.hpb.2018.06.1798 (2018).

24 Kovacs, T. *et al.* Functional shift with maintained regenerative potential following portal vein ligation. *Sci Rep* **7**, 18065, doi:10.1038/s41598-017-18309-7 (2017).

25 Nagano, Y. *et al.* Improved functional reserve of hypertrophied contra lateral liver after portal vein ligation in rats. *J Hepatol* **37**, 72-77, doi:10.1016/s0168-8278(02)00088-0 (2002).

26 Sugimoto, T. *et al.* Two-stage portal vein ligation facilitates liver regeneration in rats. *Eur Surg Res* **42**, 181-188, doi:10.1159/000203406 (2009).

27 Ren, W. *et al.* Simultaneous bile duct and portal vein ligation induces faster atrophy/hypertrophy complex than portal vein ligation: role of bile acids. *Sci Rep* **5**, 8455, doi:10.1038/srep08455 (2015).

28 Kawaguchi, D. *et al.* Effect of Portal Vein Ligation Plus Venous Congestion on Liver Regeneration in Rats. *Ann Hepatol* **18**, 89-100, doi:10.5604/01.3001.0012.7866 (2019).

29 Lin, C. W. *et al.* Amiodarone as an autophagy promoter reduces liver injury and enhances liver regeneration and survival in mice after partial hepatectomy. *Sci Rep* **5**, 15807, doi:10.1038/srep15807 (2015).

30 Toshima, T. *et al.* Suppression of autophagy during liver regeneration impairs energy charge and hepatocyte senescence in mice. *Hepatology (Baltimore, Md.)* **60**, 290-300, doi:10.1002/hep.27140 (2014).

31 Shi, H. *et al.* Augmenter of liver regeneration protects against carbon tetrachloride-induced liver injury by promoting autophagy in mice. *Oncotarget* **8**, 12637-12648, doi:10.18632/oncotarget.14478 (2017).

32 Lu, T. *et al.* Partial Hepatectomy-Induced Upregulation of miR-1907 Accelerates Liver Regeneration by Activation Autophagy. *Biomed Res Int* **2018**, 3817057, doi:10.1155/2018/3817057 (2018).

33 Jia, C. J., Sun, H. & Dai, C. L. Autophagy Contributes to Liver Regeneration After Portal Vein Ligation in Rats. *Med Sci Monit* **25**, 5674-5682, doi:10.12659/MSM.915404 (2019).

34 Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M. & Altman, D. G. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* **8**, e1000412, doi:10.1371/journal.pbio.1000412 (2010).

35 Homeyer, A. *et al.* Fast and accurate identification of fat droplets in histological images. *Comput Methods Programs Biomed* **121**, 59-65, doi:10.1016/j.cmpb.2015.05.009 (2015).

36 Liu, A., Fang, H., Dahmen, U. & Dirsch, O. Chronic lithium treatment protects against liver ischemia/reperfusion injury in rats. *Liver Transpl* **19**, 762-772, doi:10.1002/lt.23666 (2013).

37 Matsumoto, Y. *et al.* Ectopic localization of autophagosome in fatty liver is a key factor for liver regeneration. *Organogenesis* **15**, 24-34, doi:10.1080/15476278.2019.1633872 (2019).

Figure legends

Figure 1: Serum level of ALT and AST from serum sample after simultaneous PVL+ PHx and PVL alone. To evaluate the surgical stress and liver injury, the level of serum ALT and AST was measured. The serum ALT and AST were increased rapidly after simultaneous PVL+PHx and PVL alone and decreased to normal level within 7 days. No significant difference was observed between simultaneous PVL+PHx and PVL alone.

Figure 2: Liver weight to body weght ratio after simultaneous PVL+PHx and PVL alone. The liver/body weight ratio increased steadily after operation. The ratio was significantly higher after additional PHx compared with PVL alone (**p* value< 0.05, # *p* value< 0.01).

Figure 3: BrdU-staining, Hepatocyte proliferation index (PI), PCNA mRNA expression level of the non-ligated FLR. (A) Immunohistochemistcal analysis of BrdU incorporation of non-ligated FLR on POD2. More BrdU-positive cells were observed after simultaneous PVL+PHx compared with PVL alone (Scale marker:100 μm, magnification:200×). (B) Proliferation index (PI) in non-ligated FLR was significantly higher on POD2 after additional large PHx and additional small PHx compared with PVL alone (PI: 15% vs 6%,12% vs 6%, **p* value< 0.05). (C) PCNA mRNA expression level was substantially increased after additional PHx compared with PVL alone (fold change: 11 vs 8.4 vs 6.7, **p*＜0.05)

Figure 4: Autophagy related protein expression level in non-ligated FLR after simultaneous PHx+PVL and PVL alone. Expression level of LC3 and mTOR of total liver homogenates of FLR was investigated. Substantially higher level of LC3II was observed in additional large PHx and additional small PHx compared with PVL alone. The expression level of p-mTOR showed reverse results.

Figure 5: Sketches of experimental groups. (LLL: left lateral lobe, ML: median lobe, RL: right lobe, CL: caudate lobe, PV: portal vein; liver lobe with grey color: ligated lobe, liver lobe with orange color: non-ligated FLR, red cross: portal vein ligation)

Table1. Experimental studies investigating regeneration in non-ligated liver (FLR) after PVL in rat model

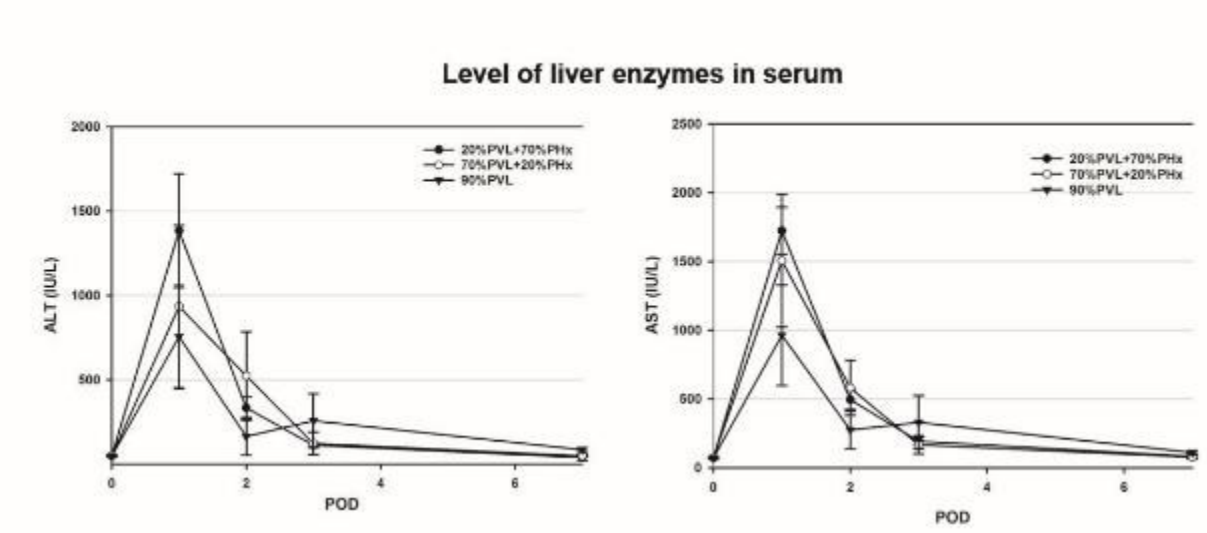
Year	Author	Animal	Surgical treatment	Hypertrophy of FLR		Regeneration of FLR		
				Extent[a] (%)	Observation time(POD)	Parameter	Enhanced regeneration	Observation time(POD9
2008	Sugimoto et al.(Sugimoto et al. 2009)	Wistar rats	90%PVL 2-stage 90%PVL[b]	315% 560%	POD7 POD14	PCNA Mitosis	+++ +++	POD2 POD8
2015	Ren et al.(Ren et al. 2015)	SD rats	90%PVL 90%PBL[c]	250% 310%	POD7 POD7	Ki67	++ +++	POD3 POD3
2019	Kawaguchi et al.(D. Kawaguchi et al. 2019)	Wistar rats	90%PVL 90%PVL+30%HVL	393% 777%	POD7 POD7	Ki67	++ +++	POD1 POD3
2019	Jia et al.(C. J. Jia et al. 2019)	SD rats	70%PVL	291%	POD7	Cyclin D1	+++	POD2

SD rats: Sprague-Dawley rats, HVL: hepatic vein ligation, + mild increase, ++ moderate increase, +++substantial increase
[a] Weight ratio of the non-ligated liver lobe to pre-operative value
[b] 2-stage PVL: First stage: 70%PVL, second stage: 20%PVL on POD7
[c] PBL: simultaneous portal vein ligation and bile duct ligation

Table2. Experimental studies investigating the protective role of autophagy during liver regeneration

Year	Author	Experimental model	Surgical strategy	Pharmacological intervention	Autophagy		Regeneration	
					Parameter	Observation time (POD)	Parameter	Observation time (POD)
2014	Toshima et al.(Toshima et al. 2014)	Wild-type Atg5 Mice	70%PHx	/	LC3-II ++, P62+	POD1	BrdU+++, Cyclin D+++	POD1
		L-Atg5KO mice[a]	70%PHx	/	LC3-II ---, P62+++	POD1	BrdU+, Cyclin D-	POD1
		Hepatocytes	/	HGF[b]	LC3-II +++	/	Cyclin D+++	/
2015	Lin et al.(Lin et al. 2015)	C57BL/6 Mice	70%PHx	/	LC3-II ++, P62+	POD1	Ki67++, PCNA++	POD2
				Amiodarone	LC3-II +++, P62-	POD1	Ki67+++, PCNA+++	POD2
				Chloroquine	LC3-II +++, P62+++	POD1	Ki67+, PCNA-	POD2
2016	Shi et al.(Shi et al. 2017)	BALB/c mice	/	CCl_4	LC3-II+++, P62+++	POD2	PCNA---, CyclinD---	POD2
				CCl_4+ALR	LC3-II+++, P62---	POD2	PCNA+++, Cyclin D+++	POD2
				CCl_4+3-MA[c]	/	/	PCNA---, CyclinD---	POD2
2018	Lu et al.(Lu et al. 2018)	C57BL/6 Mice	70%PHx	miR-1907	LC3-II+++, P62---	POD2	BrdU+++, PCNA+++	POD2
2019	Jia et al.(C. J. Jia et al. 2019)	SD rats	70%PVL	/	LC3-II +++	POD1	Cyclin D+++	POD1
2019	Matsumoto et al.(Matsumoto et al. 2019)	C57BL/KsJ m+/m+ mice(control)	70%PHx	/	LC3-II+++, P62-	POD1	PCNA+++, Cyclin D+++	POD2
		C57BL/KsJ db/db mice[d]	70%PHx	/	LC3-II+++, P62+++	POD1	PCNA+, Cyclin D-	POD2

[a] Atg5-deficient mice, [b] hepatocyte growth factor, [c] 3-MA: 3-Methyladenine, autophagy inhibitor
[d] model of liver steatosis and diabetes



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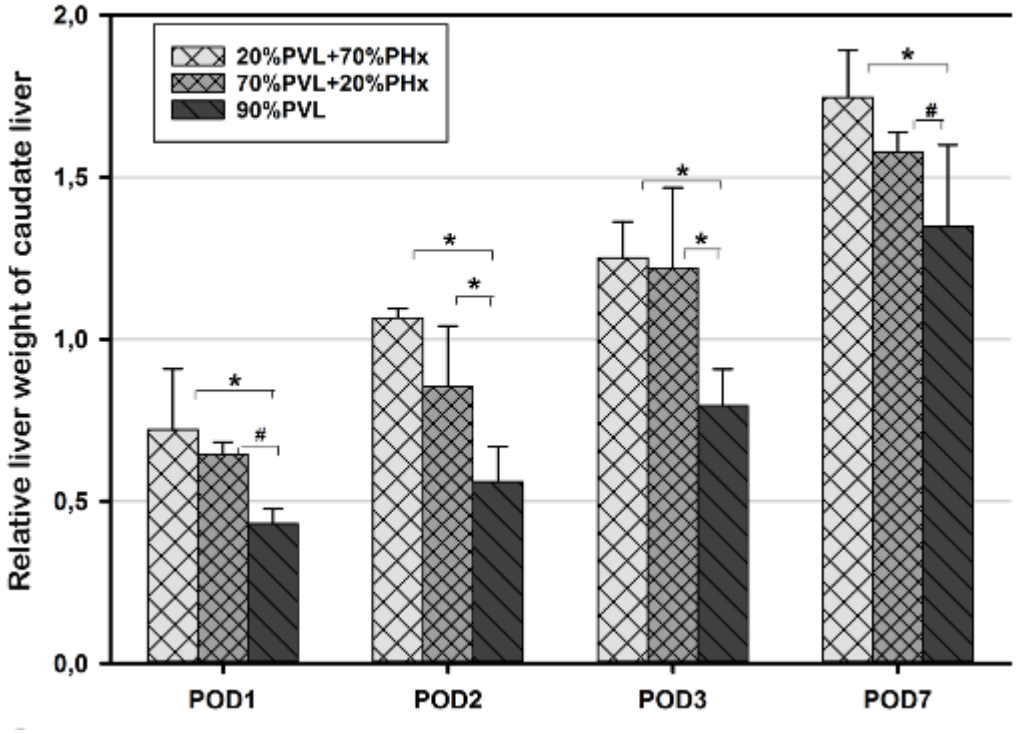

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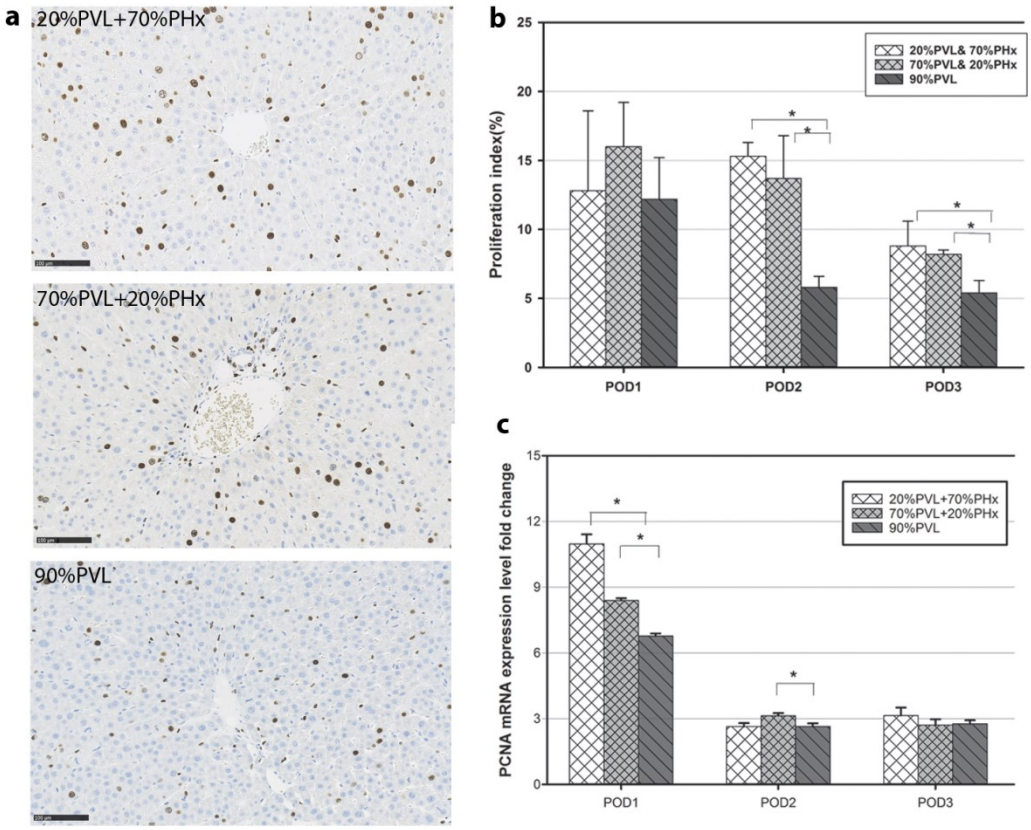

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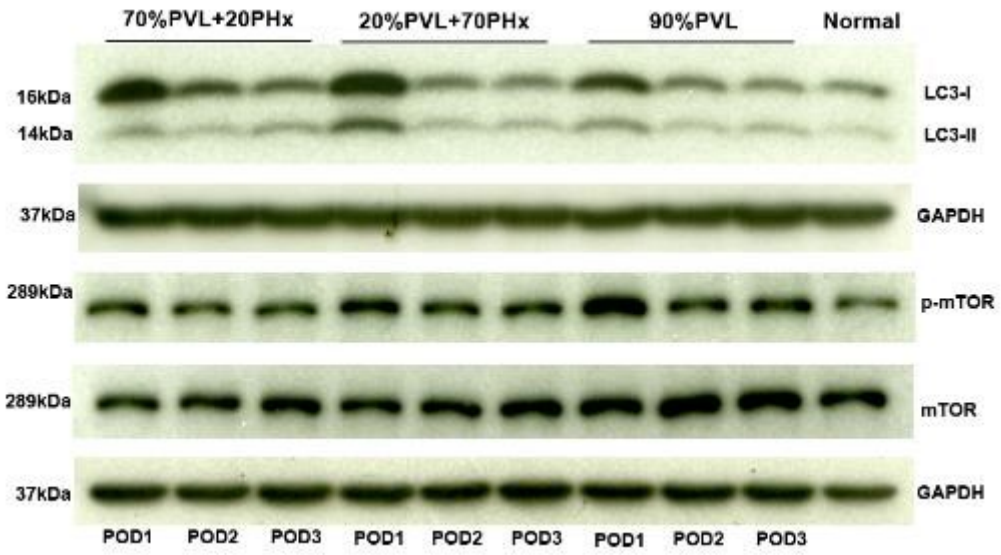

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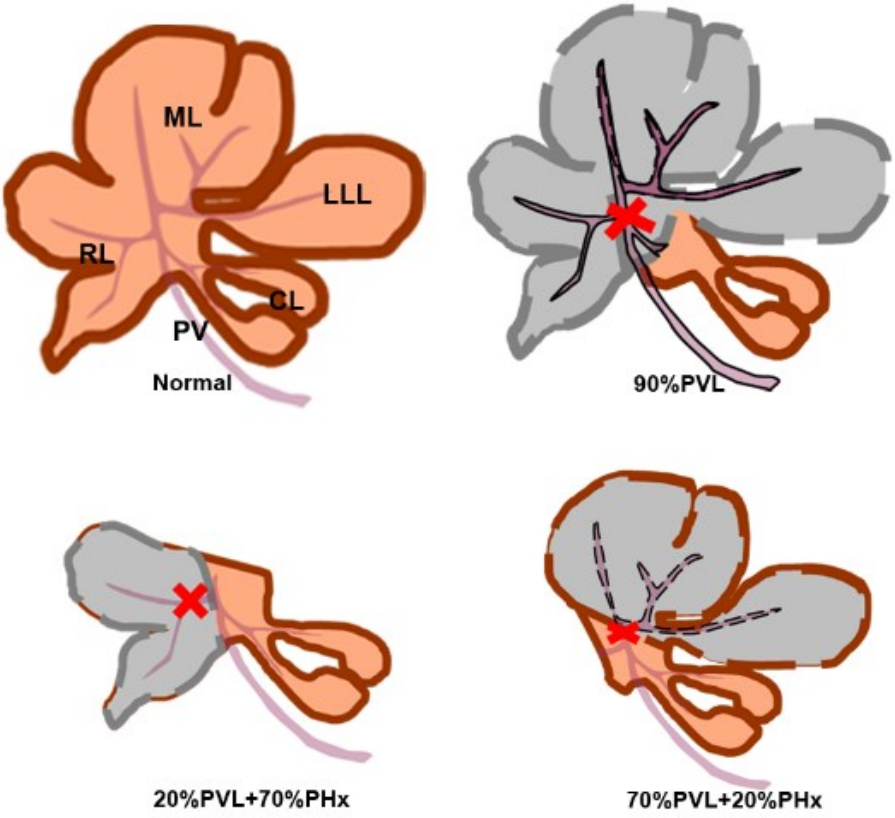

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Discussion

Hepatocyte proliferation in both occluded and non-occluded liver lobes

Regeneration of the portally deprived liver lobe was related to the extent of additional resection. Our novel finding was that the portally deprived lobes can proliferate even in the absence of portal vein supply after imposing a substantial regeneration stimulus via the additional liver resection. The extent of hepatocyte proliferation was related to the extent of additional resection. This finding was accompanied by autophagy activation and apoptosis suppression of different degrees. Additional large resection induced more hepatocyte proliferation and autophagy in the ligated liver lobes, compared with additional small resection. On the other hand, additional large resection reduced apoptosis substantially in the ligated lobes compared to additional small resection.

In contrast, regeneration of the portally supplied liver lobes was not related to the extent of additional resection. The portally supplied liver lobes did proliferate significantly more in the case of additional liver resection compared to PVL alone. The pronounced liver regeneration concurred with higher level of autophagy activity. However this effect was not related to the extent of additional resection, which is potentially due to the induction of proliferation in the ligated liver lobes.

Intrahepatic size regulation

Does atrophy determine hypertrophy?

As explained before, the liver has the ability to restore hepatic function when facing liver injury and loss of liver mass (Gock et al. 2011, Lambotte et al. 2000). Following PVL, the ligated liver lobes undergo atrophy and the non-ligated liver lobes develop compensatory hypertrophy, referred to as the aforementioned atrophy-hypertrophy complex (Heinrich et al. 2006, Riddiough et al. 2020) (**TableS1**).

Previous studies suggested that hypertrophy of the non-ligated liver lobes was proportional to atrophy of the ligated lobes (Ogasawara et al. 1996, Rozga et al. 1986). Rozga and coworkers conducted PVL of different extents in a rat model. They demonstrated that the extent of compensatory hypertrophy in the non-ligated lobes was related to the amount of atrophy in the ligated lobes. Therefore, the total liver maintained its original

volume as before operation throughout the postoperative observation time of 7 days (Rozga et al. 1986).

Other more complex experimental models also suggested a positive relation between atrophy of the ligated liver lobes and hypertrophy of the non-ligated lobes. Ren and colleagues performed additional bile duct ligation at the time of PVL in rats (Ren et al. 2015). Compared to PVL alone, simultaneous bile duct and portal vein ligation accelerated the course of atrophy in ligated liver lobes, accompanied with substantially promoted regeneration in the non-ligated liver lobes (Ren et al. 2015). Similar findings were also observed in an experimental model with simultaneous PVL and left lateral hepatic vein ligation (D. Kawaguchi et al. 2019). Combined with hepatic vein congestion, the procedure caused an increase in atrophy of the ligated lobes and significantly more hypertrophy in the non-ligated lobes (D. Kawaguchi et al. 2019).

In addition to the studies in rodents, the phenomenon was further confirmed in a pig model with simultaneous hepatic and portal vein ligation reported by Schadde and others (Schadde et al. 2019). Simultaneous ligation of both portal and hepatic vein induced rapid atrophy of the vascular deprived lobes and resulted in accelerated hypertrophy of the non-deprived lobes compared to PVL alone (Schadde et al. 2019). Taken together, there is accumulating evidence that the extent of atrophy and hypertrophy is tightly related.

However, the extent of hypertrophy of the non-ligated liver lobes is not always determined by the extent of atrophy in the ligated lobes. Furrer et al. compared the different hypertrophy responses in rats after PVL and PVE. According to their experiment, PVL induced less atrophy of the deprived lobes but more hypertrophy of the non-deprived lobes compared with PVE (Furrer et al. 2008). In contrast to the former rodent model, Wilms and coworkers demonstrated completely different results in a pig model (Wilms et al. 2008). In their porcine model, PVE was reported as a more effective strategy because it induced more hypertrophy of the non-deprived lobe compared to PVL. However, PVL induced more atrophy of the deprived lobe than PVE (Wilms et al. 2008). The controversial results from different experimental models can be explained by anatomical differences in the respective animal species. Compared to large animal or human liver, the rat liver has distinct lobes, either completely separated with small pedicles (left lateral and caudate lobes) or just connected at their wide bases (median and right lobes). Therefore, the formation of collaterals after portal vein occlusion is less likely than in large animal. These studies suggested that the extent of hypertrophy may not be dependent on the extent of atrophy.

Or the opposite: does hypertrophy determine atrophy?

In contrast, the opposite seems likely since the extent of hypertrophy of the non-deprived liver lobes could affect the amount of atrophy in the deprived lobes, as suggested by the study from Picard and coworkers (Picard et al. 2003, Picard et al. 2004). They blocked hepatocyte proliferation by using retrorsine which compromised hypertrophy of the non-ligated liver lobes. Their study indicated that when hypertrophy of the non-ligated liver lobes was impaired, the atrophy of the ligated lobes was also inhibited (Picard et al. 2003, Picard et al. 2004).

Our study could be taken as confirmation of Picard's work. In our study, we also observed that after simultaneous PVL and PHx, the atrophy of the deprived liver lobes was somehow counteracted. In addition, we found that following simultaneous small PVL and large PHx, the ligated liver lobes underwent a mild increase in size whereas the non-ligated liver lobes still developed hypertrophy. The compensatory hypertrophy was considered to occur in response to liver injury and loss of liver mass (Lambotte et al. 2000). After PVL in rats, the atrophy of the ligated liver lobes occurred progressively in the first week, but liver regeneration in the non-ligated lobes was initiated earlier before the reduction of the liver mass took place (Lambotte et al. 2000, Starkel et al. 1999). Therefore, atrophy of the ligated liver lobes may not be absolutely necessary for compensatory hypertrophy in the non-ligated liver lobes. It rather seems that the hypertrophy of the non-ligated lobes is independent from the atrophy of the ligated lobes.

These contradictory results from the experiments described above indicate that there might be an "unknown homoeostatic mechanism" controlling intrahepatic size regulation (Szijarto et al. 2015).

Processes underlying intrahepatic size regulation: apoptosis, proliferation and autophagy

Controversy regarding the type of cell death contributing to atrophy

The liver volume is dependent on the number of liver cells, with hepatocytes making up for the majority of cells. The size of the total cell population in the liver is determined by the balance of cell proliferation and cell death. It was discussed controversially which type of cell death mainly contributed to the atrophy of the deprived lobes. Early experiments suggested that atrophy was predominantly the result of necrosis (A. R. Kim et al. 2019, Steiner et al.

1961). Meanwhile, several other experiments reported that apoptosis, the programmed cell death, also contributed to the cell loss of the ligated liver lobes (Ikeda et al. 1995, Kerr et al. 1972). Recently, evidence showed that instead of necrosis, apoptosis is the main cause of atrophy of the ligated liver lobes. In previous study (W. Wei et al. 2016), the authors did not observe any necrotic cells upon histologic examination of the ligated liver lobes after PVL respectively simultaneous PVL and PHx. This observation was further confirmed in a study using a pig model (Schadde et al. 2019). The authors reported that no necrotic cells in the ligated lobes were observed. They claimed that the absence of necrosis could be due to the preservation of the hepatic artery reducing the damage to the liver (Schadde et al. 2019).

Interaction between proliferation stimulus and apoptosis stimulus

In the present study, apoptotic cells were observed in the ligated liver lobes after PVL alone and after additional PHx, as expected. However, the number of apoptotic cells and the intensity of the cleaved caspase 3 signals were significantly decreased after additional PHx compared to PVL alone. At the same time, we observed proliferating hepatocytes in the ligated lobes, up to now, an unprecedented finding. So far, no reports exist, describing hepatocyte proliferation in a portally deprived lobe. Even when concurrent regenerative stimuli such as IL-6 and mesenchymal stromal cells were added, as performed by Liska and others in the pig model (Liska et al. 2009, Liska et al. 2009), the impact on the ligated lobes was not assessed.

Apparently, the additional PHx represented a strong proliferative stimulus for the ligated lobes which counteracted the apoptotic stimulus. As a result, simultaneous large PVL and small PHx attenuated the atrophy of the deprived lobes to a mild extent, whereas simultaneous large PVL and small PHx induced moderate extent of hypertrophy of the deprived lobes. However, the underlying mechanism remains unclear.

Interplay between hepatocyte proliferation, apoptosis and autophagy

As briefly introduced before, autophagy is a self-digestion process that recycles materials and maintains homeostasis in cases of various cellular stresses. Autophagy removes damaged organelles and unwanted proteins and then provides essential materials for reconstruction. Normal liver has basal level of autophagy. Basal autophagy may participate in fundamental hepatic functions such as synthesis of proteins and carbohydrate metabolisms by providing the necessary energy (Abdalla 2010, Wang 2015). Several studies reported that growth factors such as hepatocyte growth factor (HGF) and augmenter of liver regeneration (ALR)

upregulated autophagic activity (Hu et al. 2019, Peng et al. 2011, Toshima et al. 2014). The induction of autophagy is an important pro-survival process to protect against hepatic injury and cell death. In addition, suppressing autophagy caused a significant increase of hepatocyte apoptosis (Ding et al. 2010). Shi et al. investigated the role of autophagy in a mouse model of acute liver injury induced by carbon tetrachloride (CCl_4) (Shi et al. 2017). After treatment with ALR, a growth factor and autophagy inducer, hepatocyte proliferation and autophagy were promoted whereas apoptosis was suppressed. These effects were reversed when treating the mice with autophagy inhibitor 3-methyladenine (3-MA) (Shi et al. 2017). These studies indicated that autophagy induction is an essential mechanism for promoting liver regeneration and for protecting against apoptosis.

Role of autophagy in intrahepatic size regulation

Autophagy was induced in both, the non-ligated and the ligated lobes after PVL. Following PVL, autophagy activation was observed in the non-ligated lobe (Jia et al. 2019). Autophagy activation may contribute to hepatocyte proliferation in the non-ligated lobe, as reported by Jia et al (Jia et al. 2019). In our study, we observed induction of autophagy in both, the non-ligated lobe and ligated lobe. Autophagy was more promoted in both non-ligated and ligated lobe after simultaneous PVL and PHx compared to PVL alone (Manuscript I. Figure 4 and Manuscript II. Figure 4). This finding indicated that the induction of autophagy was more pronounced in the case of an additional proliferative stimulus caused by the additional PHx. We further found that the additional large PHx induced substantially more autophagy compared with the additional small PHx. This observation showed the stronger proliferative stimulus induced more autophagy, possibly to cover the increased demand for energy needed in the cell division process.

In the atrophied liver lobes subjected to portal deprivation, hepatocyte apoptosis was considered to account for the atrophy. In contrast, in the non-ligated lobes, hepatocyte proliferation was detected and accounted for the hypertrophy (Huang et al. 2015, Picard et al. 2003, Picard et al. 2004). However, in our study, concurrence of regeneration, autophagy and apoptosis was observed in the ligated lobes after simultaneous PVL and PHx. Furthermore, we found that the more pronounced induction of autophagy was accompanied by a lower level of apoptosis. These results indicated that autophagy could also play a role in attenuating apoptosis in the ligated lobe.

Modulation of autophagy affects liver regeneration. Modulation of autophagy represents a new prospective strategy for hepatic surgery. MicroRNAs such as miR-1907 are reported to regulate hepatocyte proliferation via induction of autophagy after PHx. When inhibiting autophagy by 3-MA, the effect of miR-1907 in enhancing proliferation was impaired (Lu et al. 2018). Toshima et al. investigated the role of autophagy in liver regeneration after PHx. In autophagy-related gene 5 knockout (ATG5 KO) mice, liver regeneration after PHx was remarkably impaired, accompanied by an impaired degradation of damaged mitochondrion (Toshima et al. 2014). The protective role of autophagy in liver regeneration was further indicated by an impressive experiment conducted by Lin and others (Lin et al. 2015). By promoting autophagy with amiodarone, liver growth and hepatic proliferation increased in the early stage after PHx. The promotion of autophagy also reduced liver injury and increased the removal of damaged mitochondria (Lin et al. 2015). In our present study, autophagy was induced to different extents by combining different extents of PVL and PHx. We assume that the enhanced autophagy by additional PHx might in turn promote hepatocyte proliferation and suppress apoptosis.

Prospective study: Ezetimibe augments liver regeneration and suppresses apoptosis after portal vein ligation via autophagy activation

Therefore, in the next step we plan to obtain better evidence to verify the protective role of autophagy in intrahepatic size regulation. We select ezetimibe to modulate autophagy via the mTOR-independent signaling pathway in a rat model with 70% PVL.

We **hypothesize** that induction of autophagy by administering ezetimibe promotes hepatocyte proliferation in both non-ligated lobes and ligated lobes. In contrast, hepatocyte apoptosis in the ligated liver lobes is suppressed via autophagy activation leading to a reduction in atrophy of the ligated lobes.

Selection of experimental model

In the planned study we want to explore the role of autophagy in intrahepatic size regulation using established parameters. As a first step, we decided to use 70% PVL without resection in rats as surgical model for the following study. In a subsequent study we will further elucidate the role of autophagy for intrahepatic size regulation in case of two contradictory signals induced by simultaneous PVL and PHx.

Selection of autophagy modulators

Mammalian target of rapamycin (mTOR) is a central kinase regulating many critical cellular processes, such as cell growth, cell survival and metabolism (Kim et al. 2015). mTOR consists of two complexes: mTOR complex1 (mTORC1) and mTOR complex 2 (mTORC2) (Yoon 2017).

The activation of mTORC1 is mainly responsible to control cell growth and metabolism through promoting protein synthesis, and down-regulate catabolic processes. Autophagy is the utmost essential catabolic process related to mTOR signaling pathway. The signal transduction pathways of autophagy are divided according to the main molecules (Wei et al. 2019). The mTOR-dependent signaling pathways are already well described. Autophagy promoters affecting mTOR-dependent pathways inhibited cell proliferation by down-regulating the cell growth and protein synthesis (Fouraschen et al. 2013, Jiang et al. 2001). Thus, mTOR-dependent autophagy promoters might compromise liver regeneration and cannot be used in promoting hypertrophy after PVL.

Ezetimibe, a drug mainly used to treat hypercholesterolemia, was recently proven to ameliorate liver and brain diseases by inducing autophagy (see **Table S2**). Chang et al. demonstrated that orally administered ezetimibe in rat attenuated hepatic steatosis through activation of autophagy in a rat model of liver steatosis (Chang et al. 2015). Kim and colleagues extended this study and reported in their well-designed experiment that ezetimibe protected hepatocytes against steatosis and apoptosis by activating autophagy through mTOR-independent signaling pathway (Kim et al. 2017). They found that ezetimibe upregulated AMPK activation and increased the transcription and translocation of TFEB. They further verified that autophagy was induced by AMPK-TFEB signaling pathway (Kim et al. 2017). Yu and coworkers injected ezetimibe either intranasally or intraperitoneally in a rat model of middle cerebral artery occlusion (MCAO) (Yu et al. 2018). They also reported that ezetimibe attenuated cell apoptosis through AMPK-dependent autophagy induction (Yu et al. 2018).

Chloroquine and its analogues were primarily used to treat malaria. Nowadays, more pharmacological effects of chloroquine were discovered. The role as an autophagy inhibitor has been investigated intensively (Al-Bari 2015, Xu et al. 2018). It was confirmed repeatedly that chloroquine inhibits autophagy via blocking the fusion of autophagosomes with lysosomes. Modulation of autophagy by using chloroquine impairs degradation of autophagic cargo as well as the recruited LC3-II and P62.

Therefore we want to use these two drugs to modulate regeneration after PVL by inducing respectively inhibiting autophagy.

Experimental design

In the first step, we plan to perform a dose finding study (**Figure S3**) since the reported dosage and mode of administration for experimental animals is highly variable (Almeciga-Diaz et al. 2019, Yu et al. 2018) (table 2). We decide to administer different doses of ezetimibe (5mg/kg, 10mg/kg and 15mg/kg) intraperitoneally to rats 30min before performing PVL and once per day after operation. We will select the dose which results in ideally the highest proliferation index, highest level of autophagy and lowest level of apoptosis.

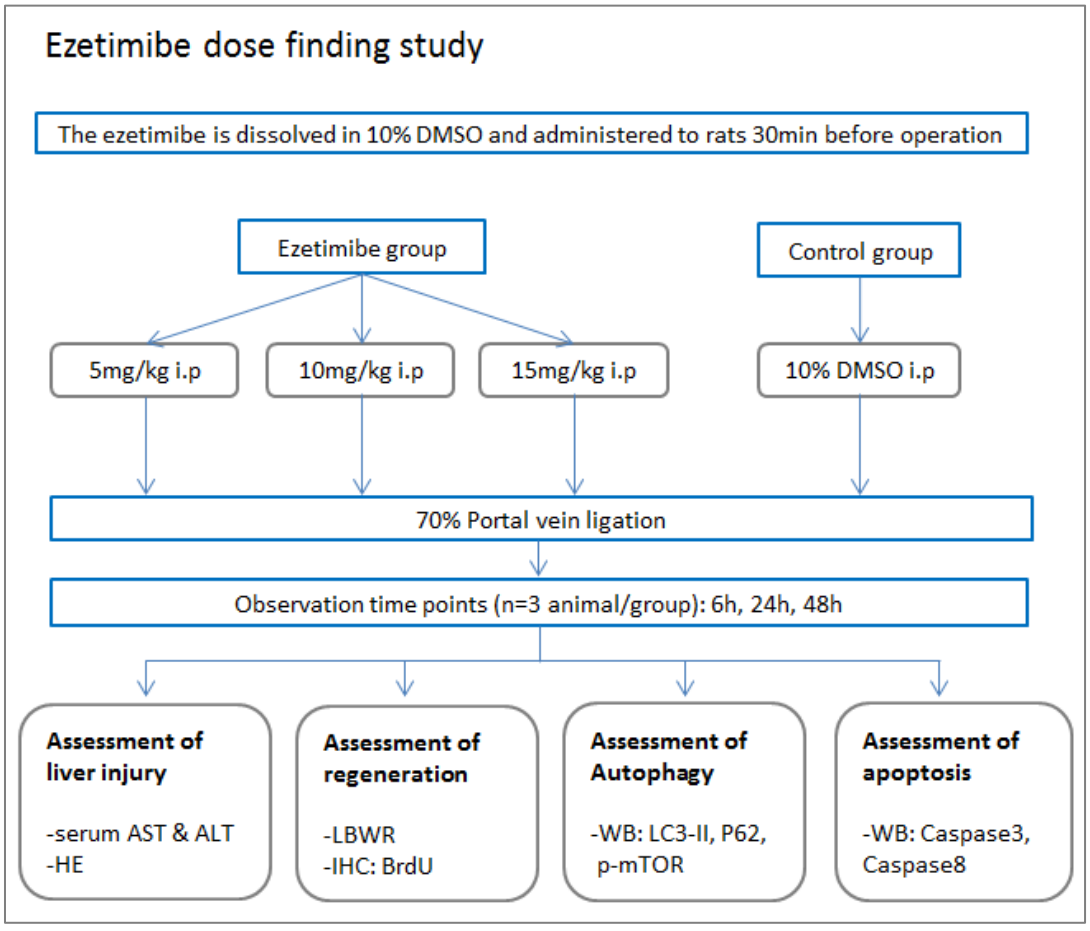

Figure S3.Experimental design for ezetimibe dose finding study

In the second step, animals will be distributed to 5 groups (n=6/group) (**Figure S4**): ezetimibe group, chloroquine group, combined ezetimibe and chloroquine group, vehicle-control group and sham-control group. We will choose the suitable dose of ezetimibe according to the result of dose finding study. The dose of chloroquine is selected according to a previous study from a mouse model (Lin et al. 2015). Ezetimibe, chloroquine or vehicle solution (10% DMSO)

will be applied intraperitoneally 30min before PVL to rats. After operation, the same doses of drugs will be applied to rats once per day in each group. Animals will be observed and evaluated after operation and will be sacrificed at different time points (6h, 12h, 24h, 48h, 72h and 168h, n=6/time point/group). Tissues and serum samples will be harvested upon sacrifice.

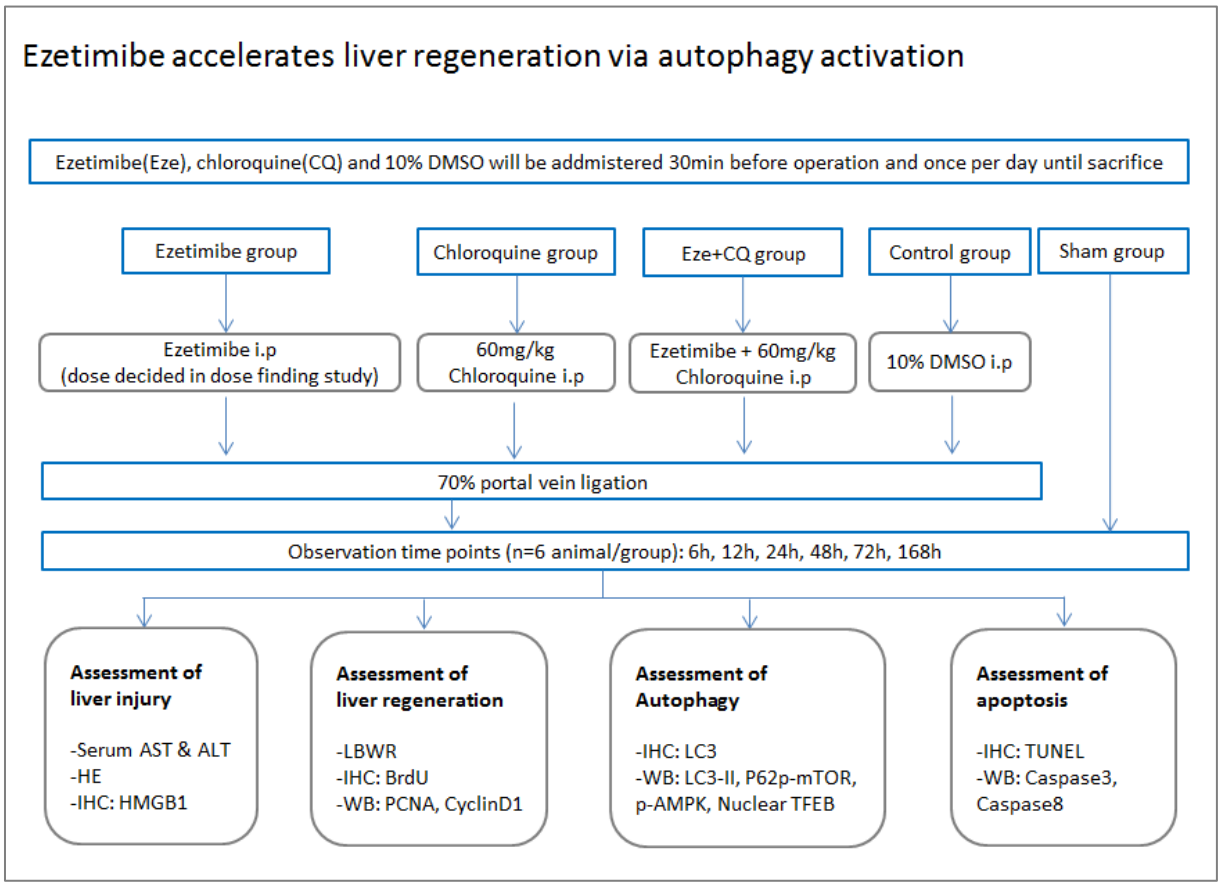

Figure S4. Experimental design to investigate impact of autophagy modulation on liver regeneration

Readout Parameters

To evaluate the liver damage after PVL, serum level of liver enzymes (ALT, AST) will be analyzed and histological examination will be conducted.

To determine autophagy activation, LC3 and p62 are selected as markers. Protein level of LC3-II and p62 can be determined by immunoblotting with anti-LC3 antibody and anti-p62 antibody. Autophagic flux can be evaluated by subtracting the LC3-II levels of animals not subjected to chloroquine treatment from the LC3-II levels of animals subjected to chloroquine treatment (Lin et al. 2015). Immunohistochemistry staining will be conducted to confirm the upregulated expression of LC3-II.

To investigate the signal transduction pathway of autophagy, the protein levels of p-mTOR, p-AMPK and Nuclear TFEB will be detected by western blots (**Figure S5**).

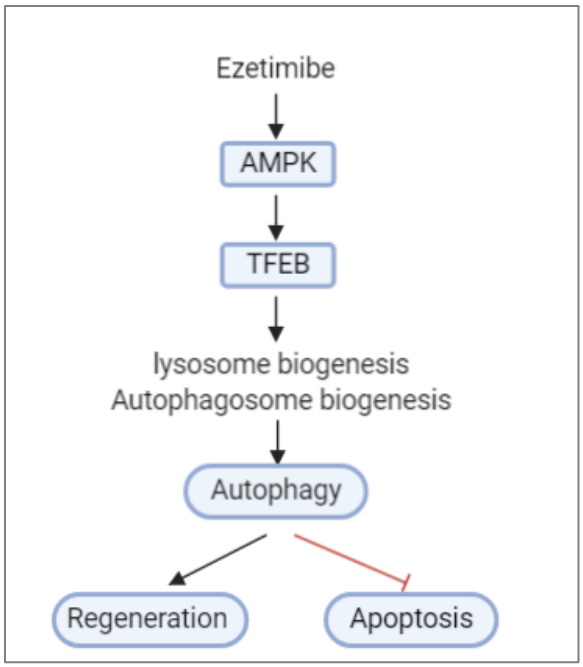

Figure S5. Schematic illustration of AMPK-TFEB autophagy pathway in PVL model

To quantify liver regeneration, the liver lobe to body weight ratio of the ligated and the non-ligated liver lobes will be calculated. Hepatocyte proliferation index will be determined based on the immunohistochemical analysis of proliferation marker BrdU followed by quantification using image analysis (Histokat, Fraunhofer MeVis). Immunoblotting analysis of PCNA and cyclin D1 and quantification will also be performed as proliferating markers.

To evaluate hepatocytes apoptosis, TUNEL-staining will be conducted and quantified. The levels of pro-apoptotic proteins such as caspase 3 and caspase 8 will be assessed by western blots.

Expected results

We expect that ezetimibe treatment will induce substantial autophagy in both the non-ligated lobe and the ligated lobes. Autophagy activation will accelerate liver regeneration in the non-ligated lobe. In both ligated and non-ligated lobes, autophagy activation will increase hepatocyte proliferation. The non-ligated liver lobes will undergo accelerated hypertrophy while the ligated lobes will undergo less atrophy. However, we expect that the effects will be compromised when administering the autophagy inhibitor.

Through autophagy activation, hepatic damage is expected to be reduced and apoptosis will be suppressed in the ligated liver lobes. In contrast, when suppressing autophagy, excessive hepatic damage and increased apoptotic hepatocytes will be observed.

Autophagy activation via an AMPK-TFEB mediated, mTOR-independent signaling pathway will be thoroughly investigated. Protein levels of p-AMPK and nuclear TFEB are supposed to increase in ezetimibe treated group. In contrast, the protein levels of p-mTOR are supposed to decrease in animals treated with ezetimibe.

If our expectations would come true, we may conclude that autophagy activation by using ezetimibe can reduce hepatic injury after PVL. Autophagy activation can accelerate hypertrophy of the non-occluded liver lobe without promoting atrophy of occluded liver lobe. It is expected that ezetimibe can augment hepatocyte proliferation and suppress apoptosis after PVL by enhancing autophagy via an mTOR-independent AMPK-TFEB mediated signaling pathway. In this case, application of ezetimibe might become an interesting therapeutic option to further expand indication for two stage hepatecomy.

Table S1. Experimental models of atrophy-hypertrophy complex

author	Animal species	Surgical model	Occluded lobe						Non-occluded lobe				
			Occluded liver lobe	extent	Atrophy		Necrosis	Apoptosis	Hypertrophy		Proliferation		
					Extent[a]	POD			Extent[b]	POD	Parameters	Level	POD
Rozga et al. 1986	Rats	PVL	RL	24%	18%	7	++ (POD2)	-	120%*	7	DNA synthesis Mitosis	+	1
			LLL	34%	16%	7	++ (POD2)		150%*	7		++	1
			ML and LLL	70%	20%	7	+++ (POD2)		300%*	7		++	1
Ren et al. 2015	Rats	PVL+BDL	ML, RL, LLL	90%	50%	7	-	Caspase3 ++	310%	7	Ki67	+++	3
		PVL			60%	7		Caspase3+	250%	7		++	3
Kawaguchi et al. 2019	Rats	PVL+LLHVL	RL, ML, LLL	90%	LLL:30% ML:55%	7	-	TUNEL+++	777%	7	Ki67	+++	1
		PVL			LLL:59% ML:55%	7		TUNEL++	393%	7		+++	3
Schadde et al. 2019	Pigs	PVL+HVL	RML, LML, LL	75%	82%	7	-	-	190%	7	Ki67	+	7
		PVL			Not significant	7			129%	7		+	7
Picard et al. 2004	Rats	Retrosine + PVL	ML and LLL	70%	38%	7	-	Caspase +	174%	7	Mitosis, BrdU	+	2
		PVL			26%	7		Caspase +++	236%	7		++	2
Furrer et al. 2008	Rats	PVL	ML and LLL	70%	67%*	7	++ (POD1)	-	185%	7	Ki67, PCNA	++	2
		PVE			55%*	7	+ (POD1)		151%	7		+	2
Wilms et al. 2008	Pigs	PVL	RML,LML, LLL	75%	LM: 67%, LL: 49%	28	not observed	-	149%	28	-	-	-
		PVE			LM: 96%, LL: 71%	28			206%	28			
Sugimoto et al. 2009	Rats	PVL	ML,LLL and RL	90%	61%	14	-	-	315%	7	PCNA, mitosis	+++	2
		2-stage PVL[c]			52%	14		-	560%	14		+++	8
Wei et al. 2016	Rats	PVL+70%PHx	RL	20%	-140%[d]	7	not observed	-	460%	7	BrdU	+++	2
		PVL			30%	7		-	120%*	7		+	2
Schadde et al. 2019	Pigs	PVL+HVL	RML, LML, LL	75%	82%	7	-	-	190%	7	Ki67	+	7
		PVL			Not significant	7			129%	7		+	7

*Data estimated from figures, not mentioned in published studies

RL: right lobe, LLL: left lateral lobe, ML: middle lobe, LL: left lobe, BDL: bile duct ligation, LLHVL: left lateral hepatic vein ligation

[a] Extent of atrophy: weight ratio of the ligated liver weight to pre-operative value

[b] Extent of hypertrophy: weight ratio of the non-ligated liver weight to pre-operative value

[c] 2-stage PVL: 70%PVL(ML+LLL) as first stage, after 7 days, 20%PVL(RL) as second stage

[d] The occluded RL underwent 140% hypertrophy

Table S2. Experimental studies using Ezetimibe as autophagy modulator

Author	Experimental model	Ezetimibe treatment		Autophagy	Treatment effect		
		Dose	Application route	Marker	Steatosis	Apoptosis	Other
Kim et al.	Atg7 wild-type mice, MCD [a]	10mg/kg	orally	LC3+++	↓↓↓	↓↓↓	
	Atg7 haploinsufficient mice, MCD			LC3---	No effect	No effect	
	Atg7 homozygous KO mice, MCD			LC3---	No effect	No effect	
Chang et al.	OLETF rats [b]	10mg/kg	Intragastrically	LC3+++	↓↓↓	-	
	Huh7 Hepatocytes [c], incubated with PA [d]	10µmol/L	1 h before PA	LC3+++	↓↓↓	-	
Yu et al.	SD Rats, MCAO [e]	10mg/kg	Intraperitoneally	LC3+++	-	↓↓↓	
Trocha et al	Wistar rats, 70% WIR[f]	5mg/kg	Intragastrically	-	-	-	Inflammation↓↓↓

[a] MCD: Methionine- and choline-deficient diet,

[b] Otsuka Long-Evans Tokushima Fatty rats: lack cholecystokinin-A receptor, closely resembles non-insulin-dependent diabetes mellitus in humans

[c] a well differentiated hepatocyte-derived carcinoma cell line

[d] PA: palmitic acid, the most cytotoxic fatty acid for the liver, leading to cell injury and death

[e] middle cerebral artery occlusion

[f] Warm ischemia reperfusion

Conclusion

In conclusion, our two manuscripts characterized a novel rat model suitable for studying the complex process of intrahepatic size regulation after simultaneous PVL and PHx. We observed that hypertrophy of the ligated lobes was due to induction of proliferation and suppression of apoptosis. Furthermore, we found that hypertrophy of the non-ligated lobes was independent from the extent of atrophy of the ligated lobes. We further observed a complex interaction between proliferation, apoptosis and autophagy of the ligated and the non-ligated liver lobes. The pronounced induction of autophagy after additional large PHx may play an essential role in regulating the balance between hepatocyte proliferation and apoptosis.

Our planned study is designed to offer convincing evidence of the role of autophagy in intrahepatic size regulation. The findings may provide the grounds for a promising novel strategy to accelerate the hypertrophy of FLR. In the long run, this might contribute to a further expansion of the indication for extended hepatectomy after portal vein occlusion.

References

Abdalla E K. 2010. Portal vein embolization (prior to major hepatectomy) effects on regeneration, resectability, and outcome. *J Surg Oncol, 102*(8), 960-967.

Abu Rmilah A, Zhou W, Nelson E, Lin L, Amiot B, Nyberg S L. 2019. Understanding the marvels behind liver regeneration. *Wiley Interdiscip Rev Dev Biol, 8*(3), e340.

Adam R, Laurent A, Azoulay D, Castaing D, Bismuth H. 2000. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg, 232*(6), 777-785.

Al-Bari M A. 2015. Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. *J Antimicrob Chemother, 70*(6), 1608-1621.

Alevra Sarika N, Payen V L, Fleron M, Ravau J, Brusa D, Najimi M, Taghdouini A E. 2020. Human Liver-Derived Extracellular Matrix for the Culture of Distinct Human Primary Liver Cells. *Cells, 9*(6).

Almeciga-Diaz C J, Hidalgo O A, Olarte-Avellaneda S, Rodriguez-Lopez A, Guzman E, Garzon R, … Zheng W. 2019. Identification of Ezetimibe and Pranlukast as Pharmacological Chaperones for the Treatment of the Rare Disease Mucopolysaccharidosis Type IVA. *J Med Chem, 62*(13), 6175-6189.

Baili E, Tsilimigras D I, Filippou D, Ioannidis A, Bakopoulos A, Machairas N, Moris D. 2019. Associating liver partition and portal vein ligation for staged hepatectomy in patients with primary liver malignancies: A systematic review of the literature. *J BUON, 24*(4), 1371-1381.

Bax H R, Mansens B J, Schalm L. 1956. Atrophy of the liver after occlusion of the bile ducts or portal vein and compensatory hypertrophy of the unoccluded portion and its clinical importance. *Gastroenterology, 31*(2), 131-155.

Bretagnol F, Maggiori L, Zappa M, Sibert A, Vilgrain V, Panis Y. 2011. Selective portal vein embolization and colorectal liver metastases in rat: a new experimental model for tumor growth study. *J Surg Res, 171*(2), 669-674.

Chang E, Kim L, Park S E, Rhee E J, Lee W Y, Oh K W, Park C Y. 2015. Ezetimibe improves hepatic steatosis in relation to autophagy in obese and diabetic rats. *World J Gastroenterol, 21*(25), 7754-7763.

Clemens M M, McGill M R, Apte U. 2019. Mechanisms and biomarkers of liver regeneration after drug-induced liver injury. *Adv Pharmacol, 85*, 241-262.

Czaja M J, Ding W X, Donohue T M, Jr., Friedman S L, Kim J S, Komatsu M, Yin X M. 2013. Functions of autophagy in normal and diseased liver. *Autophagy, 9*(8), 1131-1158.

Di Fabio F, Whistance R, Rahman S, Primrose J N, Pearce N W, Abu Hilal M. 2012. Exploring the role of laparoscopic surgery in two-stage hepatectomy for bilobar colorectal liver metastases. *J Laparoendosc Adv Surg Tech A, 22*(7), 647-650.

Ding W X, Li M, Chen X, Ni H M, Lin C W, Gao W, Yin X M. 2010. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology, 139*(5), 1740-1752.

Elias D, De Baere T, Roche A, Mducreux, Leclere J, Lasser P. 1999. During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. *Br J Surg, 86*(6), 784-788.

Fischer C, Melstrom L G, Arnaoutakis D, Jarnagin W, Brown K, D'Angelica M, Fong Y. 2013. Chemotherapy after portal vein embolization to protect against tumor growth during liver hypertrophy before hepatectomy. *JAMA Surg, 148*(12), 1103-1108.

Fouraschen S M, de Ruiter P E, Kwekkeboom J, de Bruin R W, Kazemier G, Metselaar H J, … de Jonge J. 2013. mTOR signaling in liver regeneration: Rapamycin combined with growth factor treatment. *World J Transplant, 3*(3), 36-47.

Furrer K, Tian Y, Pfammatter T, Jochum W, El-Badry A M, Graf R, Clavien P A. 2008. Selective portal vein embolization and ligation trigger different regenerative responses in the rat liver. *Hepatology, 47*(5), 1615-1623.

Garcea G, Ong S L, Maddern G J. 2009. Predicting liver failure following major hepatectomy. *Dig Liver Dis, 41*(11), 798-806.

Garcia-Perez R, Revilla-Nuin B, Martinez C M, Bernabe-Garcia A, Baroja Mazo A, Parrilla Paricio P. 2015. Associated Liver Partition and Portal Vein Ligation (ALPPS) vs Selective Portal Vein Ligation (PVL) for Staged Hepatectomy in a Rat Model. Similar Regenerative Response? *PLoS One, 10*(12), e0144096.

Geng L, Zong R, Shi Y, Xu K. 2020. Prognostic role of preoperative albumin-bilirubin grade on patients with hepatocellular carcinoma after surgical resection: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol, 32*(7), 769-778.

Giampieri F, Afrin S, Forbes-Hernandez T Y, Gasparrini M, Cianciosi D, Reboredo-Rodriguez P, … Battino M. 2019. Autophagy in Human Health and Disease: Novel Therapeutic Opportunities. *Antioxid Redox Signal, 30*(4), 577-634.

Gock M, Eipel C, Linnebacher M, Klar E, Vollmar B. 2011. Impact of portal branch ligation on tissue regeneration, microcirculatory response and microarchitecture in portal blood-deprived and undeprived liver tissue. *Microvasc Res, 81*(3), 274-280.

Guicciardi M E, Gores G J. 2005. Apoptosis: a mechanism of acute and chronic liver injury. *Gut, 54*(7), 1024-1033.

Hayashi H, Nagaki M, Imose M, Osawa Y, Kimura K, Takai S, … Moriwaki H. 2005. Normal liver regeneration and liver cell apoptosis after partial hepatectomy in tumor necrosis factor-alpha-deficient mice. *Liver Int, 25*(1), 162-170.

Heinrich S, Jochum W, Graf R, Clavien P A. 2006. Portal vein ligation and partial hepatectomy differentially influence growth of intrahepatic metastasis and liver regeneration in mice. *J Hepatol, 45*(1), 35-42.

Hoekstra L T, van Lienden K P, Doets A, Busch O R, Gouma D J, van Gulik T M. 2012. Tumor progression after preoperative portal vein embolization. *Ann Surg, 256*(5), 812-817; discussion 817-818.

Hu T, Sun H, Deng W Y, Huang W Q, Liu Q. 2019. Augmenter of Liver Regeneration Protects Against Acetaminophen-Induced Acute Liver Injury in Mice by Promoting Autophagy. *Shock, 52*(2), 274-283.

Huang B, Geng D, Zhan S, Li H, Xu X, Yi C. 2015. Magnetic resonance imaging characteristics of hepatocyte apoptosis (induced by right portal vein ligation) and necrosis (induced by combined right portal vein and right hepatic artery ligation) in rats. *J Int Med Res, 43*(1), 80-92.

Ibis C, Asenov Y, Akin M, Azamat I F, Sivrikoz N, Gurtekin B. 2017. Factors Affecting Liver Regeneration in Living Donors After Hepatectomy. *Med Sci Monit, 23*, 5986-5993.

Ikeda K, Kinoshita H, Hirohashi K, Kubo S, Kaneda K. 1995. The ultrastructure, kinetics and intralobular distribution of apoptotic hepatocytes after portal branch ligation with special reference to their relationship to necrotic hepatocytes. *Arch Histol Cytol, 58*(2), 171-184.

Isfordink C J, Samim M, Braat M, Almalki A M, Hagendoorn J, Borel Rinkes I H M, Molenaar I Q. 2017. Portal vein ligation versus portal vein embolization for induction of

hypertrophy of the future liver remnant: A systematic review and meta-analysis. *Surg Oncol, 26*(3), 257-267.

Jaeck D, Bachellier P, Nakano H, Oussoultzoglou E, Weber J C, Wolf P, Greget M. 2003. One or two-stage hepatectomy combined with portal vein embolization for initially nonresectable colorectal liver metastases. *Am J Surg, 185*(3), 221-229.

Jia C. 2011. Advances in the regulation of liver regeneration. *Expert Rev Gastroenterol Hepatol, 5*(1), 105-121.

Jia C J, Sun H, Dai C L. 2019. Autophagy Contributes to Liver Regeneration After Portal Vein Ligation in Rats. *Med Sci Monit, 25*, 5674-5682.

Jiang Y P, Ballou L M, Lin R Z. 2001. Rapamycin-insensitive regulation of 4e-BP1 in regenerating rat liver. *J Biol Chem, 276*(14), 10943-10951.

Kawabata T, Yoshimori T. 2020. Autophagosome biogenesis and human health. *Cell Discov, 6*, 33.

Kawaguchi D, Hiroshima Y, Kumamoto T, Mori R, Matsuyama R, Ichikawa Y, Endo I. 2019. Effect of Portal Vein Ligation Plus Venous Congestion on Liver Regeneration in Rats. *Ann Hepatol, 18*(1), 89-100.

Kawaguchi T, Kodama T, Hikita H, Tanaka S, Shigekawa M, Nawa T, Takehara T. 2013. Carbamazepine promotes liver regeneration and survival in mice. *J Hepatol, 59*(6), 1239-1245.

Kerr J F, Wyllie A H, Currie A R. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer, 26*(4), 239-257.

Khan A S, Garcia-Aroz S, Ansari M A, Atiq S M, Senter-Zapata M, Fowler K, Chapman W C. 2018. Assessment and optimization of liver volume before major hepatic resection: Current guidelines and a narrative review. *Int J Surg, 52*, 74-81.

Kilkenny C, Browne W J, Cuthill I C, Emerson M, Altman D G. 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol, 8*(6), e1000412.

Kim A R, Park J I, Oh H T, Kim K M, Hwang J H, Jeong M G, Hong J H. 2019. TAZ stimulates liver regeneration through interleukin-6-induced hepatocyte proliferation and inhibition of cell death after liver injury. *FASEB J, 33*(5), 5914-5923.

Kim S H, Kim G, Han D H, Lee M, Kim I, Kim B, Lee M S. 2017. Ezetimibe ameliorates steatohepatitis via AMP activated protein kinase-TFEB-mediated activation of autophagy and NLRP3 inflammasome inhibition. *Autophagy, 13*(10), 1767-1781.

Kim Y C, Guan K L. 2015. mTOR: a pharmacologic target for autophagy regulation. *J Clin Invest, 125*(1), 25-32.

Kokudo N, Tada K, Seki M, Ohta H, Azekura K, Ueno M, Kato Y. 2001. Proliferative activity of intrahepatic colorectal metastases after preoperative hemihepatic portal vein embolization. *Hepatology, 34*(2), 267-272.

Kovacs T, Mathe D, Fulop A, Jemnitz K, Batai-Konczos A, Veres Z, Szijarto A. 2017. Functional shift with maintained regenerative potential following portal vein ligation. *Sci Rep, 7*(1), 18065.

Lambotte L, Li B, Leclercq I, Sempoux C, Saliez A, Horsmans Y. 2000. The compensatory hyperplasia (liver regeneration) following ligation of a portal branch is initiated before the atrophy of the deprived lobes. *J Hepatol, 32*(6), 940-945.

Lee J W, Lee J H, Park Y, Lee W, Kwon J, Song K B, Kim S C. 2020. Risk factors of posthepatectomy liver failure for perihilar cholangiocarcinoma: Risk score and significance of future liver remnant volume-to-body weight ratio. *J Surg Oncol, 122*(3), 469-479.

Levi Sandri G B, Colace L, Vennarecci G, Santoro R, Lepiane P, Colasanti M, Ettorre G M. 2015. Laparoscopic first step approach in the two stage hepatectomy. *Hepatobiliary Surg Nutr, 4*(5), 345-347.

Lin C W, Chen Y S, Lin C C, Chen Y J, Lo G H, Lee P H, Yu M L. 2015. Amiodarone as an autophagy promoter reduces liver injury and enhances liver regeneration and survival in mice after partial hepatectomy. *Sci Rep, 5*, 15807.

Liska V, Slowik P, Eggenhofer E, Treska V, Renner P, Popp F C, Dahlke M H. 2009. Intraportal injection of porcine multipotent mesenchymal stromal cells augments liver regeneration after portal vein embolization. *In Vivo, 23*(2), 229-235.

Liska V, Treska V, Mirka H, Kobr J, Sykora R, Skalicky T, Matejovic M. 2009. Interleukin-6 augments activation of liver regeneration in porcine model of partial portal vein ligation. *Anticancer Res, 29*(6), 2371-2377.

Liu A, Fang H, Dahmen U, Dirsch O. 2013. Chronic lithium treatment protects against liver ischemia/reperfusion injury in rats. *Liver Transpl, 19*(7), 762-772.

Lu T, Hao J, Shen C, Gu G, Zhang J, Xu N. 2018. Partial Hepatectomy-Induced Upregulation of miR-1907 Accelerates Liver Regeneration by Activation Autophagy. *Biomed Res Int, 2018*, 3817057.

Makuuchi M, Thai B L, Takayasu K, Takayama T, Kosuge T, Gunven P, Ozaki H. 1990. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery, 107*(5), 521-527.

Matsumoto Y, Yoshizumi T, Toshima T, Takeishi K, Fukuhara T, Itoh S, Mori M. 2019. Ectopic localization of autophagosome in fatty liver is a key factor for liver regeneration. *Organogenesis, 15*(1), 24-34.

Nagano Y, Nagahori K, Kamiyama M, Fujii Y, Kubota T, Endo I, Shimada H. 2002. Improved functional reserve of hypertrophied contra lateral liver after portal vein ligation in rats. *J Hepatol, 37*(1), 72-77.

Narita M, Oussoultzoglou E, Jaeck D, Fuchschuber P, Rosso E, Pessaux P, Bachellier P. 2011. Two-stage hepatectomy for multiple bilobar colorectal liver metastases. *Br J Surg, 98*(10), 1463-1475.

Ogasawara K, Uchino J, Une Y, Fujioka Y. 1996. Selective portal vein embolization with absolute ethanol induces hepatic hypertrophy and makes more extensive hepatectomy possible. *Hepatology, 23*(2), 338-345.

Orcutt S T, Anaya D A. 2018. Liver Resection and Surgical Strategies for Management of Primary Liver Cancer. *Cancer Control, 25*(1), 1073274817744621.

Patel S H, Camargo F D, Yimlamai D. 2017. Hippo Signaling in the Liver Regulates Organ Size, Cell Fate, and Carcinogenesis. *Gastroenterology, 152*(3), 533-545.

Peng K Y, Horng L Y, Sung H C, Huang H C, Wu R T. 2011. Hepatocyte growth factor has a role in the amelioration of diabetic vascular complications via autophagic clearance of advanced glycation end products: Dispo85E, an HGF inducer, as a potential botanical drug. *Metabolism, 60*(6), 888-892.

Picard C, Lambotte L, Starkel P, Sempoux C, Saliez A, Van Den Berge V, Horsmans Y. 2003. Retrorsine: a kinetic study of its influence on rat liver regeneration in the portal branch ligation model. *J Hepatol, 39*(1), 99-105.

Picard C, Starkel P, Sempoux C, Saliez A, Lebrun V, Horsmans Y. 2004. Molecular mechanisms of apoptosis in the liver of rats after portal branch ligation with and without retrorsine. *Lab Invest, 84*(5), 618-628.

Ren W, Chen G, Wang X, Zhang A, Li C, Lv W, Dong J H. 2015. Simultaneous bile duct and portal vein ligation induces faster atrophy/hypertrophy complex than portal vein ligation: role of bile acids. *Sci Rep, 5*, 8455.

Riddiough G E, Christophi C, Jones R M, Muralidharan V, Perini M V. 2020. A systematic review of small for size syndrome after major hepatectomy and liver transplantation. *HPB (Oxford), 22*(4), 487-496.

Riddiough G E, Jalal Q, Perini M V, Majeed A W. 2020. Liver regeneration and liver metastasis. *Semin Cancer Biol*.

Rous P, Larimore L D. 1920. Relation of the Portal Blood to Liver Maintenance : A Demonstration of Liver Atrophy Conditional on Compensation. *J Exp Med, 31*(5), 609-632.

Rozga J, Jeppsson B, Bengmark S. 1986. Portal branch ligation in the rat. Reevaluation of a model. *Am J Pathol, 125*(2), 300-308.

Schadde E, Guiu B, Deal R, Kalil J, Arslan B, Tasse J, Hertl M. 2019. Simultaneous hepatic and portal vein ligation induces rapid liver hypertrophy: A study in pigs. *Surgery, 165*(3), 525-533.

Schnitzbauer A A, Lang S A, Goessmann H, Nadalin S, Baumgart J, Farkas S A, Schlitt H J. 2012. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg, 255*(3), 405-414.

Schwabe R F, Luedde T. 2018. Apoptosis and necroptosis in the liver: a matter of life and death. *Nat Rev Gastroenterol Hepatol, 15*(12), 738-752.

Shi H, Han W, Shi H, Ren F, Chen D, Chen Y, Duan Z. 2017. Augmenter of liver regeneration protects against carbon tetrachloride-induced liver injury by promoting autophagy in mice. *Oncotarget, 8*(8), 12637-12648.

Starkel P, Horsmans Y, Sempoux C, De Saeger C, Wary J, Lause P, Lambotte L. 1999. After portal branch ligation in rat, nuclear factor kappaB, interleukin-6, signal transducers and activators of transcription 3, c-fos, c-myc, and c-jun are similarly induced in the ligated and nonligated lobes. *Hepatology, 29*(5), 1463-1470.

Steiner P E, Martinez J B. 1961. Effects on the Rat Liver of Bile Duct, Portal Vein and Hepatic Artery Ligations. *Am J Pathol, 39*(3), 257-289.

Sugimoto T, Yamada T, Iwata H, Sekino T, Matsumoto S, Ishida N, Takemura H. 2009. Two-stage portal vein ligation facilitates liver regeneration in rats. *Eur Surg Res, 42*(3), 181-188.

Szijarto A, Fulop A. 2015. Triggered liver regeneration: from experimental model to clinical implications. *Eur Surg Res, 54*(3-4), 148-161.

Tan Q, Liu Y, Deng X, Chen J, Tsai P J, Chen P H, Su Z. 2020. Autophagy: a promising process for the treatment of acetaminophen-induced liver injury. *Arch Toxicol, 94*(9), 2925-2938.

Tao Y, Wang M, Chen E, Tang H. 2017. Liver Regeneration: Analysis of the Main Relevant Signaling Molecules. *Mediators Inflamm, 2017*, 4256352.

Toshima T, Shirabe K, Fukuhara T, Ikegami T, Yoshizumi T, Soejima Y, Maehara Y. 2014. Suppression of autophagy during liver regeneration impairs energy charge and hepatocyte senescence in mice. *Hepatology, 60*(1), 290-300.

Tustumi F, Ernani L, Coelho F F, Bernardo W M, Junior S S, Kruger J A P, Herman P. 2018. Preoperative strategies to improve resectability for hepatocellular carcinoma: a systematic review and meta-analysis. *HPB (Oxford), 20*(12), 1109-1118.

Uemura S, Higuchi R, Yazawa T, Izumo W, Otsubo T, Yamamoto M. 2020. Level of total bilirubin in the bile of the future remnant liver of patients with obstructive jaundice undergoing hepatectomy predicts postoperative liver failure. *J Hepatobiliary Pancreat Sci*.

van Gulik T M, van den Esschert J W. 2010. James Cantlie's early messages for hepatic surgeons: how the concept of pre-operative portal vein occlusion was defined. *HPB (Oxford), 12*(2), 81-83.

van Gulik T M, van den Esschert J W, de Graaf W, van Lienden K P, Busch O R, Heger M, Gouma D J. 2008. Controversies in the use of portal vein embolization. *Dig Surg, 25*(6), 436-444.

Wang K. 2015. Autophagy and apoptosis in liver injury. *Cell Cycle, 14*(11), 1631-1642.

Wanis K N, Linecker M, Madenci A L, Muller P C, Nussler N, Brusadin R, Hernandez-Alejandro R. 2020. Variation in complications and mortality following ALPPS at early-adopting centers. *HPB (Oxford)*.

Wei W, Hua C, Zhang T, Dirsch O, Gremse F, Homeyer A, … Dahmen U. 2020. Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis. *Sci Rep, 10*(1), 4893.

Wei W, Zhang T, Fang H, Dirsch O, Schenk A, Homeyer A, Dahmen U. 2016. Intrahepatic Size Regulation in a Surgical Model: Liver Resection-Induced Liver Regeneration Counteracts the Local Atrophy following Simultaneous Portal Vein Ligation. *Eur Surg Res, 57*(1-2), 125-137.

Wei X, Luo L, Chen J. 2019. Roles of mTOR Signaling in Tissue Regeneration. *Cells, 8*(9).

Weinbren K. 1955. The portal blood supply and regeneration of the rat liver. *Br J Exp Pathol, 36*(6), 583-591.

Wilms C, Mueller L, Lenk C, Wittkugel O, Helmke K, Krupski-Berdien G, Broering D C. 2008. Comparative study of portal vein embolization versus portal vein ligation for induction of hypertrophy of the future liver remnant using a mini-pig model. *Ann Surg, 247*(5), 825-834.

Xu F, Hua C, Tautenhahn H M, Dirsch O, Dahmen U. 2020. The Role of Autophagy for the Regeneration of the Aging Liver. *Int J Mol Sci, 21*(10).

Xu R, Ji Z, Xu C, Zhu J. 2018. The clinical value of using chloroquine or hydroxychloroquine as autophagy inhibitors in the treatment of cancers: A systematic review and meta-analysis. *Medicine (Baltimore), 97*(46), e12912.

Yamanaka N, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Tanaka W. 1993. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology, 18*(1), 79-85.

Yoon M S. 2017. The Role of Mammalian Target of Rapamycin (mTOR) in Insulin Signaling. *Nutrients, 9*(11).

Yu J, Li X, Matei N, McBride D, Tang J, Yan M, Zhang J H. 2018. Ezetimibe, a NPC1L1 inhibitor, attenuates neuronal apoptosis through AMPK dependent autophagy activation after MCAO in rats. *Exp Neurol, 307*, 12-23.

Zhou Y, Xu J C, Jia Y F, Xu C S. 2015. Role of death receptors in the regulation of hepatocyte proliferation and apoptosis during rat liver regeneration. *Genet Mol Res, 14*(4), 14066-14075.

Zimmermann A. 2004. Regulation of liver regeneration. *Nephrol Dial Transplant, 19 Suppl 4*, iv6-10.

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Ehrenwörtliche Erklärung

Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller-Universität bekannt ist,

ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben: Prof. Dr. Utz Settmacher, Prof. Dr. Uta Dahmen, Dr. Olaf Dirsch, Dr. Weiwei Wei, Prof. Dr. Haoshu Fang, Dr. André Homeyer, Dr. Felix Gremse, Isabel Jank, Dr. Janine Arlt, Dr. Claudia Schindler, Ana Lucia Paz, Juliana Neumann, Ulrike Vetterling, Dr. Tianjiao Zhang,

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dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Ort, Datum Unterschrift des Verfassers

Curriculum Vitae

Education:

Bachelor degree (Sep 2010 ~ Jun 2014)

Anhui Medical University, Anhui, China.

Master degree (Sep 2014 ~ Jun 2017)

The First Affiliated Hospital of Anhui Medical University, Anhui, China

Doctoral candidate (Jan 2018 ~ Feb. 2022)

Department of Experimental Transplantation Surgery, General Visceral Vascular Surgery, University hospital Jena, Jena, Germany

Language Skills:

Mother tongue: Chinese

Other languages: English B2, German B2

Experimental experience:

Molecular biological technique RNA-extraction, cDNA-synthesis, protein-extraction, qPCR

Immunological technique SDS-PAGE, Western blots, HE, Immunohistochemistry

Cell culture HepG2 cell culture, cell splitting, counting and preservation

Animal experiment

- Rat liver portal vein ligation (20%, 60%, 70%, 90%)
- Rat warm ischemia-reperfusion
- Rat and mouse partial liver resection (30%, 70%, 85%)
- Rat and mouse intraperitoneal injection, intravenous injection, blood sampling, organ and tissue harvesting
- Software “Histokat” for whole slides analysis

Scientific Achievement:

Publications:

1. Fang H, **Hua C**, Weiss S, Liu A., Cheng W, Claus R, Rödel J, Dirsch O, Dahmen U. Modulation of innate immunity by G-CSF and inflammatory response by LBPK95A improves the outcome of sepsis in a rat model. J Immunol Res, 2018 Nov 07; 6085095.
2. Fang H, Jin H, **Hua C**, Liu A, Song Z, Chen X, Dirsch O, Dahmen U. The LPS responsiveness in BN and LEW rats and its severity are modulated by the liver. J Immunol Res. 2018 Jul 30; 6328713.
3. Wei W, **Hua C**, Zhang T, Dirsch O, Gremse F, Homeyer A, Settmacher U, Dahmen U. Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis. 2020 Mar 17; 10:4893
4. Xu F, **Hua C**, Tautenhahn H, Dirsch O, Dahmen U. The Role of Autophagy for the Regeneration of the Aging Liver. Int J Mol Sci, 2020 May 20; 21(10).
5. **Hua C**, Wei W, Zhang T, Xu F, Dirsch O, Homeyer A, Settmacher U, Dahmen U. Additional partial hepatectomy at the time of portal vein ligation accelerates the regeneration of the future liver remnant. Scientific Reports under review.
6. Xu F, **Hua C**, Tautenhahn H, Dirsch O, Dahmen U. Modulation of Autophagy – a novel "rejuvenation" strategy for the aging liver. Oxidative Medicine and Cellular Longevity under review.

Meeting Presentation:

1. The LPS response in strain dependent and modulated by liver. 2018, 13th Jenaer Lebertag für Patientinnen und Patienten
2. Autophagy promotes liver regeneration by attenuating apoptosis in a rat model of simultaneous portal vein ligation and liver resection. 2018, 29th Biotest Wilsede-Workshop für experimentelle und klinische Lebertransplantation und Hepatologie
3. Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis. GBM/DGZ 2019 Fall conferece, Age-Related Human Diseases Special Focus: Autophagy