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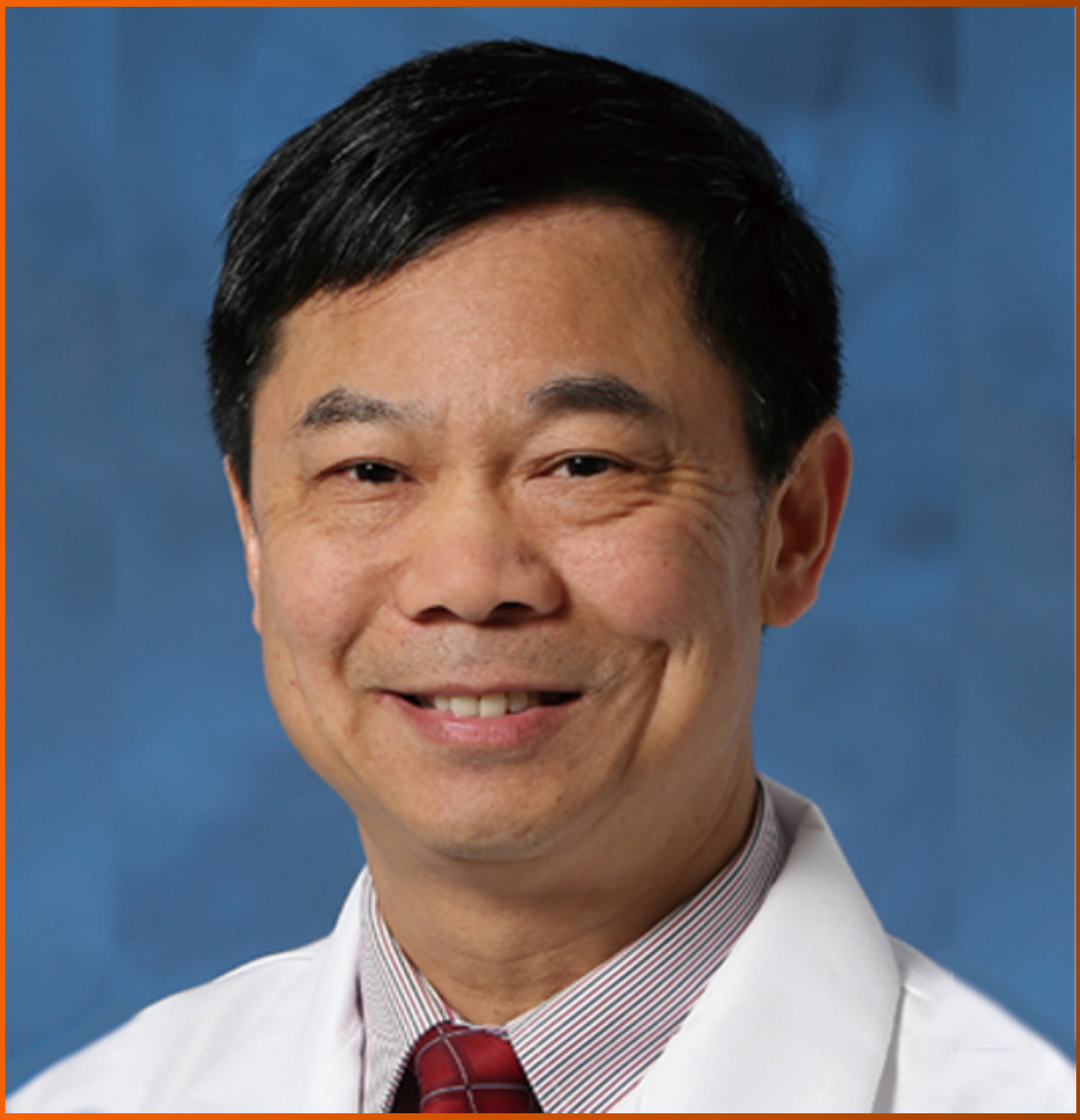

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World Journal of Hepatology

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ABOUT COVER

Editor-in-Chief of *World Journal of Hepatology*, Dr. Ke-Qin Hu is Director of Hepatology Services and Professor of Medicine in the Division of Gastroenterology and Hepatology, University of California, Irvine School of Medicine (United States). Dr. Hu's career efforts emphasize bridging research advances to bedside patient care. His clinical research has focused on the natural history and outcomes of various liver diseases and healthcare disparity. His basic science research has focused on molecular virology and diagnosis of hepatitis B and C virus infection, and chemoprevention of liver cancer. Dr. Hu has coauthored more than 150 research papers, book chapters, and review articles. He is Deputy Editor-in-Chief for Frontiers of Medicine. He is dedicated to community outreach, public health education, and reduction of healthcare disparity. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

INDEXING/ABSTRACTING

The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJH* as 0.52. The *WJH*'s CiteScore for 2021 is 3.6 and Scopus CiteScore rank 2021: Hepatology is 42/70.

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EDITORIAL

New Year's greeting and overview of *World Journal of Hepatology* in 2021

Ke-Qin Hu, Koo Jeong Kang, Nikolaos Pyrsopoulos, Xiang Li

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Abstract

The *World Journal of Hepatology* (*WJH*) was launched in October 2009. It mainly publishes articles reporting research findings in the field of hepatology, covering a wide range of topics, including viral hepatitis B and C, non-alcoholic fatty liver disease, alcoholic liver disease, autoimmune and chronic cholestatic liver disease, drug-induced liver injury, cirrhosis, liver failure, hepatocellular carcinoma, coronavirus disease 2019-related liver conditions, *etc*. As of December 31, 2020, the *WJH* has published 1349 articles, among which, the total cites is 18995 and the average cites per article is 14. In celebrating the New Year, we are pleased to share with you special a New Year's greeting from the *WJH* Editors-in-Chief, along with a detailed overview of the journal's submission, peer review and publishing metrics from 2020. In all, we are appreciative for the substantive support and submissions from authors worldwide, and the dedicated efforts and expertise provided by our invited reviewers and editorial board members.

Key Words: *World Journal of Hepatology*; New Year's greeting message; Editors-in-Chief; Editorial Board; Highly influential scientists; Baishideng Publishing Group Inc

Core Tip: The *World Journal of Hepatology* (*WJH*) mainly publishes articles reporting research results obtained in the field of hepatology and covering a wide range of topics, including a variety of different liver diseases, cirrhosis, hepatocellular carcinoma, and more recently coronavirus disease 2019-related liver conditions and management, and so on. Since its launch in October 2009, the *WJH* has published 1349 articles. As of December 31, 2020, the total cites among these articles is 18995 and the average cites per article is 14. The enthusiastic and excellent support and submissions from authors worldwide, complemented by the dedicated efforts and expertise of our invited reviewers, Editorial Board members, and Editorial Office staff, have been invaluable.

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INTRODUCTION

The *World Journal of Hepatology* (*WJH*, ISSN 1948-5182, https://www.wjgnet.com/1948-5182/index.htm) is a high-quality, monthly, online, open-access, single-blind peer-reviewed journal published by the Baishideng Publishing Group Inc (BPG). The primary aim of *WJH* is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. The *WJH* is abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), and Scopus.

Since its launch in October 2009, the *WJH* has published 1349 articles[1]. As of December 31, 2020, the total cites among these articles is 18995 and the average cites per article is 14.

A NEW YEAR'S GREETING FROM THE *WJH* EDITORS-IN-CHIEF

For all of us, 2020 was a very tough year due to coronavirus disease 2019 (COVID-19). As Editors-in-Chief of *WJH*, it is now our great pleasure to take this opportunity to wish all our authors, readers, Editorial Board members, independent expert referees, and staff of the Editorial Office a very Happy New Year. On behalf of the Editorial team, we would like to express our gratitude to all authors who contributed their valuable manuscripts, as well as all independent referees and readers for their continuous support, dedication, and encouragement. Together with an excellent team effort by our Editorial Board members and staff of the Editorial Office and BPG, *WJH* was able to advance in 2020 despite the ongoing COVID-19 pandemic.

As the chief editors, we strive to work with the journal's Editorial Office and BPG staff to make the manuscript submission process as simple as possible and ensure an efficient communication with the authors to provide our support and answer their questions. We are also open to any suggestions that could improve *WJH*'s operation and publication. Please feel free to contact us at (editorialoffice@wjgnet.com) with any question on your submission or suggestions for the journal in general.

OVERVIEW OF THE *WJH* IN 2020

In celebrating *WJH*'s 12-year anniversary and the 2021 New Year, we are very proud to share with you that we completed the following endeavors in submission, peer review and publishing in 2020.

Submission and acceptance

From 2013 to 2020, the *WJH* has received 2302 manuscripts, including invited manuscripts and unsolicited manuscripts, and the average submissions per year is 288. The submissions of unsolicited manuscripts are stable in recent years (Figure 1).

In 2020, we received 204 submissions from authors around the world and published 112 articles in 12 issues. Among those 112 articles, 57 (50.9%) were original articles, 31 (27.7%) were review articles, 1 was an editorial (0.9%), 15 (13.4%) were case reports and 8 (7.1%) were articles of 'other' types (Figure 2). The authors hailed from 32 countries, including 32 (28.6%) from the United States, 10 (8.9%) from Brazil, 6 (5.4%) each from Italy, Japan and Spain, and 5 (4.5%) each from the United Kingdom and France; the remaining 26 (23.2%) were from various individual countries (Figure 3).

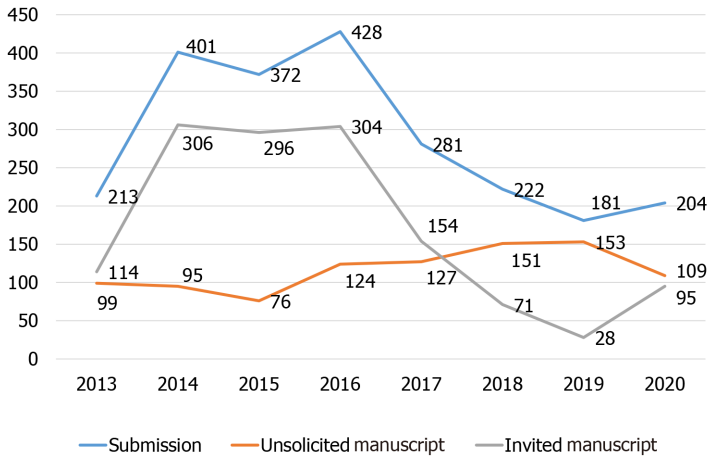

Figure 1 Annual submissions of *World Journal of Hepatology* from 2013 to 2020.

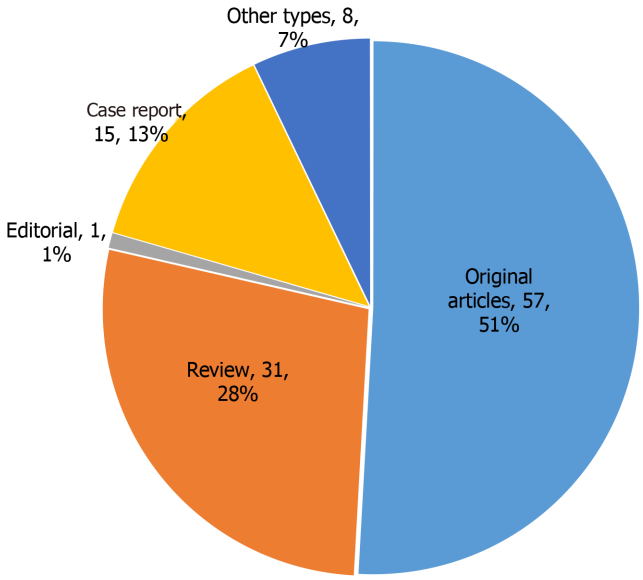

Figure 2 Article types among the 112 manuscripts published by *World Journal of Hepatology* in 2020.

Invitation for 2021

In November and December, invitations to contribute high-quality articles to *WJH* were sent out to distinguished scientists in the field of hepatology. As of December 31, 2020, *WJH* has accepted a total of 327 proposed titles for those invited manuscripts; these articles, to be submitted for publication in 2021, include 85 (26.0%) original articles, 215 (65.7%) review articles, 15 (4.6%) editorials, and 12 (3.7%) 'other' types (Figure 4). We are currently inviting highly influential scientists to submit Topic Highlight articles, commenting on and discussing hot topics in the field of hepatology. As of December 31, 2020, we have already received 14 submissions online.

Conducting peer review statistics

As of December 31, 2020, *WJH* had sent out 6120 invitations to peer reviewers and Editorial Board members to conduct peer review of manuscripts. Among the peer reviewers and Editorial Board members who accepted the invitation, 428 (35.0%) submitted the peer review report on time, 425 (34.7%) failed to submit the peer review report on time, and 370 (30.3%) have not submitted the peer review report yet.

Editorial Board members of WJH

The 2020 Editorial Board of *WJH* was composed of 195 members[2]. Among them, 3 were Editors-in-Chief (Professor Ke-Qin Hu, Professor Koo Jeong Kang, and Professor Nikolaos Pyrsopoulos), 5 were Associate Editors, and 187 were Editorial Board Members. The members were based in 45 countries and areas, including 23 (11.8%) in China, 22 (11.3%) in Italy, 19 (9.7%) in the United States, 18 (9.2%) in Turkey, 11 (5.6%) in Egypt, and 102 (52.3%) in various other countries (Figure 5). A total of 86 (44.1%) of

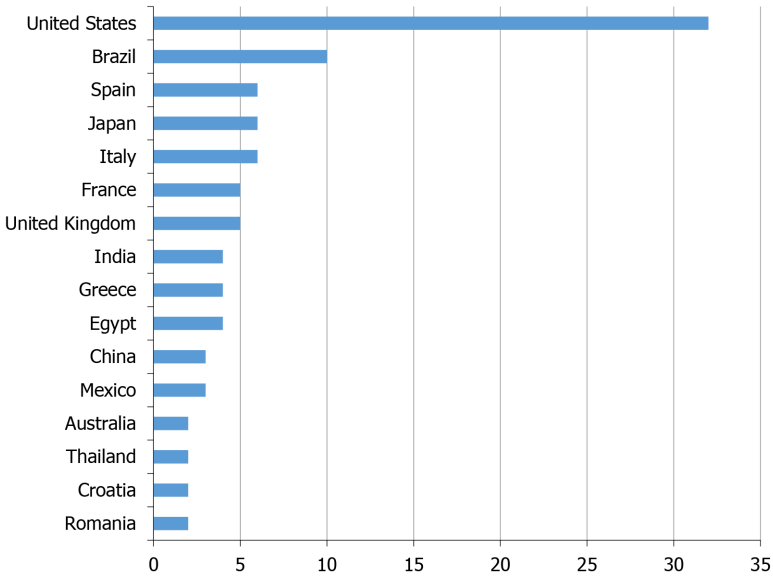

Figure 3 Top 16 countries by number of *World Journal of Hepatology* published manuscripts in 2020.

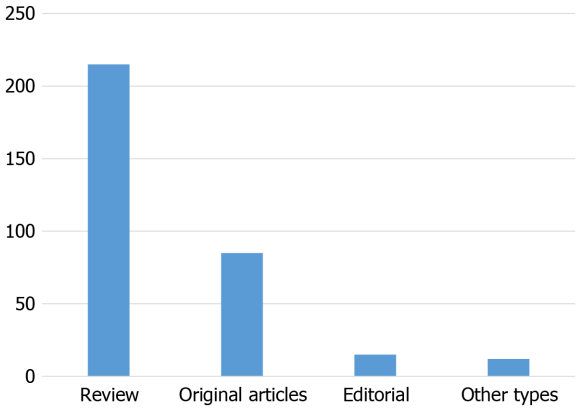

Figure 4 Article types of *World Journal of Hepatology* invited manuscripts for 2021.

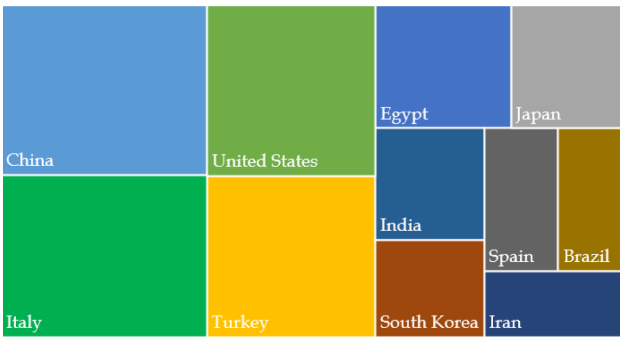

Figure 5 Countries of *World Journal of Hepatology* Editorial Board Members in 2020. Top 11 countries by the number of editorial members, where no less than five members are located in each country.

the Editorial Board Members served as peer reviewers in 2020.

We are pleased to have received 71 applications for Editorial Board membership (up to December 2020), which are currently under evaluation.

Journal metrics

According to data from the Web of Science (up to January 4, 2021), *WJH* published 258 articles between 2017 and 2018. These articles were cited 830 times in 2019, with a mean citation of 3.217 for each. On

behalf of *WJH*, BPG will submit an application to Clarivate Analytics for abstracting and indexing in Science Citation Index Expanded (SCIE), in the near future. *WJH*'s Scopus CiteScore for 2019 is 5.8, ranking 22/61 in the category of Hepatology.

Accurately pushing WJH articles and authors ahead

To enable more peers to read, share, and cite *WJH* authors' published research results and to help enhance their global academic influence and reputations, thereby also promoting the overall development of the field of hepatology, BPG sends *WJH*'s published articles to 1000-10000 highly influential experts in a topically-accurate manner. After completing this outreach activity, BPG formally notifies the paper's authors of the number of experts to whom their manuscript was sent *via* email. As of December 31, 2020, *WJH* articles included in the push email campaign were sent to 19905 in October, 5308 in November, and 11023 in December.

Challenges facing WJH in 2021

The development and growth of *WJH* rely on a large amount of high-quality manuscripts. We appreciate and encourage all authors to submit their topically-relevant manuscripts to *WJH*, to enjoy the benefits of this great platform and sharing resource in disseminating their medical research results. Our Editorial Board members are encouraged to continue their support by actively serving as peer reviewers, authors contributing articles, and journal representatives inviting high-quality articles from others. *WJH* Editorial Board members are also encouraged to communicate with the Editors-in-Chief actively, provide suggestions and analyze discipline hotspots to promote their academic influence through the *WJH*.

CONCLUSION

In 2021, *WJH* will publish more high-quality original and review articles, consistently improving its academic influence and moving closer towards its next goal of inclusion in the SCIE as soon as possible, which will ultimately promote the overall development of the field of hepatology. *WJH*'s Editors-in-Chief and Editorial Office staff expect to be more productive and have committed to working diligently with all of you to raise the academic rank of *WJH* in 2021. In order to achieve these goals, we recognize the importance of substantive support and submissions from authors like you in tandem with the dedicated efforts and expertise of our invited reviewers, many of whom also serve on our Editorial Board. Please feel free to contact our Editorial Office (editorialoffice@wjgnet.com) if you have further questions, need support, or wish to share your suggestions.

FOOTNOTES

Author contributions: Hu KQ and Li X drafted the editorial; Kang KJ and Pyrsopoulos N reviewed and revised the editorial.

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REFERENCES

1 ***World Journal of Hepatology.*** All published articles of *World Journal of Hepatology* from its launch in October 2009 to present. Available from: https://www.wjgnet.com/1948-5182/archive.htm

2 **The 2020 Editorial Board of *World Journal of Hepatology***. Available from: www.wjgnet.com/1948-5182/editorialboard.htm

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REVIEW

Autophagy in liver diseases

Elias Kouroumalis, Argryro Voumvouraki, Aikaterini Augoustaki, Dimitrios N Samonakis

Specialty type: Gastroenterology and hepatology

Peer-review report's scientific quality classification

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Grade C (Good): 0
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Abstract

Autophagy is the liver cell energy recycling system regulating a variety of homeostatic mechanisms. Damaged organelles, lipids and proteins are degraded in the lysosomes and their elements are re-used by the cell. Investigations on autophagy have led to the award of two Nobel Prizes and a health of important reports. In this review we describe the fundamental functions of autophagy in the liver including new data on the regulation of autophagy. Moreover we emphasize the fact that autophagy acts like a two edge sword in many occasions with the most prominent paradigm being its involvement in the initiation and progress of hepatocellular carcinoma. We also focused to the implication of autophagy and its specialized forms of lipophagy and mitophagy in the pathogenesis of various liver diseases. We analyzed autophagy not only in well studied diseases, like alcoholic and nonalcoholic fatty liver and liver fibrosis but also in viral hepatitis, biliary diseases, autoimmune hepatitis and rare diseases including inherited metabolic diseases and also acetaminophene hepatotoxicity. We also stressed the different consequences that activation or impairment of autophagy may have in hepatocytes as opposed to Kupffer cells, sinusoidal endothelial cells or hepatic stellate cells. Finally, we analyzed the limited clinical data compared to the extensive experimental evidence and the possible future therapeutic interventions based on autophagy manipulation.

Key Words: Autophagy; Lipophagy; Mitophagy; Fatty liver disease; Fibrosis; Liver sinusoidal cells

Core Tip: Extensive investigation of autophagy is mostly based on experimental data. However there is now enough evidence to support the notion that autophagy is not only the waste recycling mechanism of the hepatocyte, but is strongly involved in the pathogenesis of almost all liver diseases. It can be either a defensive mechanism against various insults or a detrimental machinery aggravating the underlying disease. Modulation of autophagy has different consequences in the hepatocyte than in the liver macrophages, the sinusoidal endothelium or the hepatic stellate cells. There is also an opportunity for future treatment applications of autophagy manipulation.

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INTRODUCTION

Autophagy in the liver

Autophagy (from the Greek self-eating) is a process crucial for cell survival[1,2]. Autophagy is a lysosomal degradation pathway that controls the disposition of intracellular waste including damaged organelles or invading pathogens. It can be characterized as the recycling energy system of the cell.

Under basal conditions autophagy degrades 1.5% of total hepatic protein per hour but in starvation, protein degradation increases to 4.5% of liver protein per hour[3]. When rodents are starved for 48 h, autophagy degrades up to 40% of liver protein[4].

Although It is accepted that the term "autophagy" was introduced in 1963 by the Belgian researcher Christian René de Duve, in fact the term autophagy was used almost a century earlier by Anselmier in a French journal[5].

However the modern era of autophagy started with the pioneer work of de Duve and Novicoff in the 1950s when acid phosphatase positive lysosomes were described in the rat liver[6-9] and the term lysosome was used for the first time[10].Later de Duve introduced the term autophagosome and Arstila and Trump proved that the autophagosomes originate from the endoplasmic reticulum (ER)[11]. The next important progress came when Takeshige *et al*[12] identified approximately fifteen Autophagy related genes (Atgs) involved in Saccharomyces cerevisiae autophagy[12-14]. Today, more than 40 Atgs in various animal and human cells have been identified and unified[15-17]. The importance of autophagy was recognized by the award of two Nobel Prizes for Physiology or Medicine, the first to Cristian De Duve in 1974 and the second to Yoshinori Ohsumi in 2016[18,19]. Landmarks of autophagy were recently described[20]. During the period 2008-2018 more than 33000 papers related to autophagy were published[21,22].

Autophagy has certain discrete stages including induction, phagophore formation, autophagosome formation, autolysosome formation and degradation[23-25]. Atg molecules are involved in various complexes essential for autophagy induction and autophagosome formation[26]. Initiation starts with activation of the unc-51-like kinase 1 complex (ULK1, Atg1 in yeast) followed by beclin 1 (Atg6 in yeast) and a subsequent cascade of Atg proteins leading to autophagosome formation where LC3 (Atg8 in yeast) is implicated[27]. LC3 is further processed to form initially LC3-I and then LC3-II[28]. Once the autophagosome is formed, a blockage of autophagic flux at late steps will downregulate the clearance of autophagosomes. A blockage of autophagic flux finally results in autophagy dependent cell death[29]. Detailed descriptions of the complex molecular steps of each stage of autophagy were recently published[20,28,30].

A commonly used marker for estimating autophagosome formation is the fusion protein green fluorescent protein-LC3 (GFP-LC3)[31]. Of the three members LC3A, LC3B, and LC3C of the human LC3 gene family, LC3B and LC3-II are mostly used for autophagy assays[32-34]. Autophagic flux into the lysosomes is estimated by measuring p62/SQSTM1 degradation. p62/SQSTM1 is a protein complex that binds to LC3 and is efficiently degraded by autophagy[35]. The total cellular level of p62/SQSTM1 inversely correlates with autophagic activity. Thus in autophagy-deficient cells, p62/SQSTM1 levels are increased after starvation in contrast to cells with normal autophagy[36].

It should be stressed that he level of LC3 is related to the induction of autophagy but might not reflect the final stages of autophagy and should not be used as a general marker of autophagy[34-36]. Further progress of autophagy is detected by a low level of p62 since p62 degradation depends on the function of the autophagosome-lysosome fusion[37]. Therefore an increase of both LC3 and p62 indicates formation of autophagosomes without lysosomal degradation[38].

As mentioned before, a major breakthrough in autophagy was the identification of Atgs. Evidence for the importance of autophagy in liver homeostasis was provided by the generation of of Atgs-knockout mice models[39]. Livers of mice with deletion of the autophagy gene Atg7 were markedly enlarged, up

to 30% of the body weight of the animal and hepatocytes were characterized by structural alterations of mitochondria and peroxisomes and aggregation of ubiquitinated proteins. These aggregates disappeared when the ATg7- knockout mouse was bred to a mouse null for SQSTM1/p62indicating that SQSTM1 is important to direct damaged cytosolic proteins into the autophagic pathway[40,41].

To date, three major types of autophagy, namely, macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA), have been described[22,42,43].

Macroautophagy is the classical pathway that engulfs the cytosolic components targeted for lysosomal degradation. Initiation of autophagy is controlled by two metabolic sensors the mammalian target of rapamycin complex 1 (mTORC1) and the AMP-activated protein kinase (AMPK). mTORC1 negatively regulates autophagy by direct phosphorylation of ULK1 thus inhibiting ULK1. AMPK suppresses mTORC1 activity by phosphorylation of tuberous sclerosis 2 and raptor, two essential regulators of mTORC1[44,45]. Recently it was reported that the final step in this activation process of mTOR is dependent on Rheb, a small GTPase that binds to mTOR and allosterically activates its kinase activity[46]. The long-term regulation of autophagy is carried out by transcription factor EB (TFEB)[47], the main regulator of lysosomal biogenesis and autophagy. Under nutrient-rich conditions, mTORC1 phosphorylates TFEB and retains TFEB in the cytosol[48-50]. Nutrient deprivation on the other hand leads to mTORC1 inhibition, dephosphorylation of TFEB and its translocation to the nucleus to initiate the rapid transcription of autophagy genes[51,52]. All subsequent series of complex events leading to the final degradation in lysosomes have elegantly been described[2,24,53].

A simplified scheme of macroautophagy is presented in Figure 1.

Microautophagy is the least studied type of autophagy where compounds or membranous vesicles are directly taken up by lysosomes[54]. Microautophagy is important during amino acid starvation[55, 56] and possibly three different types can be recognized[57].

Chaperone Mediated Autophagy (CMA) is a selective engulfment process of substrates containing the pentapeptide "Lys-Phe-Glu-Arg-Gln" (KFERQ) motifs. They are recognized by, the cytosolic chaperone heat-shock cognate protein of 70 kDa (HSC70), and transported into the lysosomes through the lysosomal membrane protein 2A (LAMP2A)[58,59]. CMA is induced by DNA damage, hypoxia and oxidative stress, among others[60-65].

Today macroautophagy is also divided into non selective autophagy and selective macroautophagy targeting special organelles or specific compounds for degradation[43,66,67]. Thus new names have appeared according to the coumpounds involved: Ribophagy (ribosomes)[68], pexophagy (peroxisomes)[69], ferritinophagy (iron-based compounds)[70] and most importantly reticulophagy (ER) [71] lipophagy (lipids)[72] and mitophagy (mitochondria)[73]. The last two are practically involved in every form of fatty liver.

Reticulophagy: Multiple receptors directly interact with LC3 and form autophagosomesduring reticulophagy, a very important form of macroautophagy thatpreserves the size and function of the ER in different conditions like starvation, non-alcoholic fatty liver disease (NAFLD), viral infections and fibrosis[74-79].

Lipophagy: Lipophagy is implicated in lipid homeostasis and metabolism in liver diseases. It is usually down-regulated in steatosis of either alcoholic or non-alcoholic liver disease[80-84], but it is up-regulated when fibrosis, cirrhosis or hepatocellular carcinoma are evolving[85-87]. Comprehensive reviews of lipophagy in liver disease were recently presented[88-91].

Mitophagy: The first step of mitophagy in mammals requires the induction of canonic Atg-dependentautophagy with either mTOR suppression induced by mitochondrial generated reactive oxygen species (ROS), or AMPK activation induced by adenosine triphosphate (ATP) depletion. The second step is the priming of the mitochondria involving molecular modifications leading to their recognition by the autophagosomes[92,93]. Even in the healthy liver, worn out mitochondria with a half-life of 10 to 25 d are removed by mitophagy[94,95]. Elimination of aged or damaged mitochondria protect cells from release of pro-apoptotic proteins, generation of toxic ROS and non proper hydrolysis of ATP[96-99]. When oxidative stress appears, autophagy rapidly acts to remove oxidized proteins or damaged mitochondria that generate more ROS. Recent data show that in autophagy deficiency there is acummulation of ROS and p62 probably mediated by the loss ofFOXO1/3. It has been reported that the p62-FOXO1/3 axis is the molecular basis for the reduction of antioxidant defense in autophagy deficiency [100]. Three different types of mitophagy have been described based in the different molecular pathways involved[101,102]. An extensive review of molecular mechanisms of mitophagy in liver diseases has been recently published[103].

New players in liver autophagy: It is clear today that apart from the known pathways regulating liver autophagy, there are additional mechanisms involved. The most important are the long non-coding RNAs (lncRNAs), microRNAs (miRNAs) and exosomes. Many recent studies have presented strong evidence that ncRNAs influence autophagy by regulating various autophagy pathways[104-110]. Equally, miRNAs regulate autophagy influencing the core autophagy pathways[111].

Evidence from experimental animals with liver specific deletions of Atgs has demonstrated the role of High mobility group box 1 (HMGB1)[112] and Yes-associated protein (YAP)[113] in the pathological

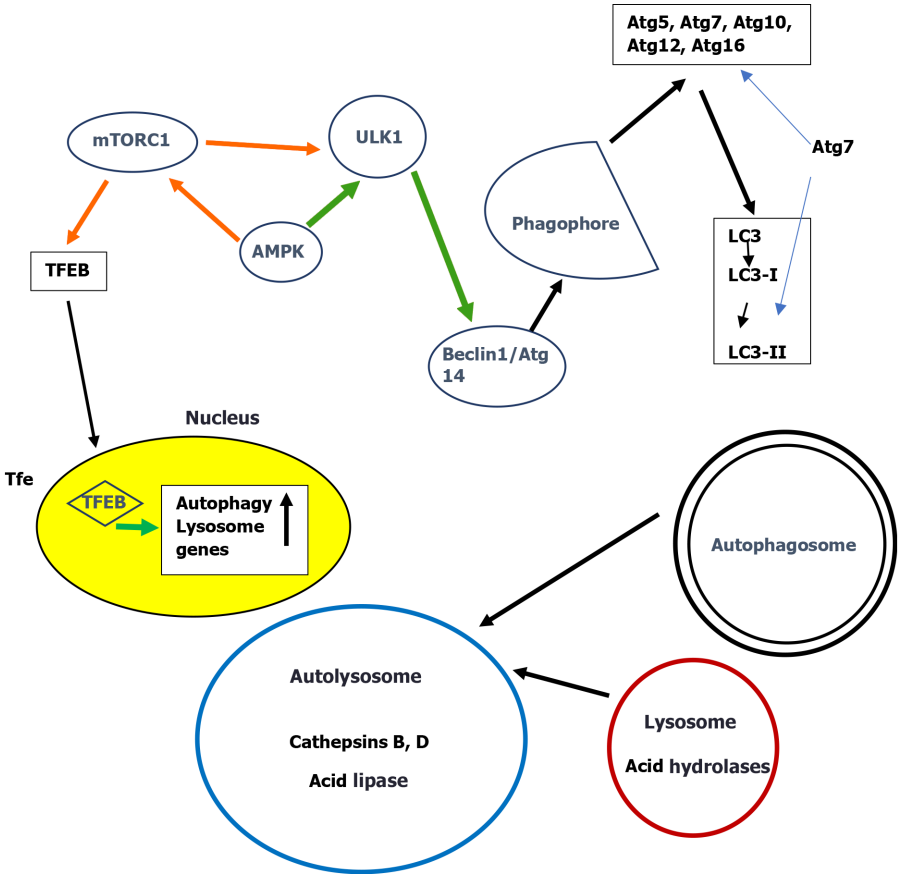

Figure 1 A simplified scheme of the macroautophagy pathways in the liver. Initiation starts with activation of the unc-51-like kinase 1 complex (ULK1, Atg1 in yeast) followed by beclin 1(Atg6 in yeast) and a subsequent cascade of Atg proteins leading to autophagosome formation where LC3 (Atg8 in yeast) is implicated. LC3 is further processed to form initially LC3-I and then LC3-II. Fusion of the autophagosomes with lysosomes form the autolysosome where acid proteases (among which cathepsins are important) and lipases degrade proteins and lipids. Initiation of autophagy is controlled by two metabolic sensors the mammalian target of rapamycin complex 1 (mTORC1) and the AMP-activated protein kinase (AMPK). mTORC1 negatively regulates autophagy inhibiting ULK1. AMPK suppresses mTORC1 activity. The long-term regulation of autophagy is carried out by transcription factor EB (TFEB), the main regulator of lysosomal biogenesis and autophagy. Under nutrient-rich conditions, mTORC1 phosphorylates TFEB and retains TFEB in the cytosol. Orange arrows: Inhibition. Green arrows: Positive regulation. For details see Ref.[21,29,31]. mTORC1: Mammalian target of rapamycin complex 1; TFEB: Transcription factor EB; ULK1: Unc-51-like kinase 1 complex.

changes induced by autophagy. Nuclear receptors were also reported to control autophagy. Activation of the farnesoid X receptor (FXR), occurs during feeding and suppresses Atgs expression. On the other hand during starvation, fasting-activated nuclear receptors, the peroxisome proliferator-activated receptor alpha (PPAR), and the cAMP response element-binding protein (CREB), induce expression of Atgs and therefore increase autophagy[114-116].

An association of autophagy with the formation and function of exosomes has also been described. Exosomes are extracellular vesicles originating from late endosomes, which do not fuse with lysosomes but are released extracellularly by exocytosis. Exosomes can either activate autophagy pathways or transfer extracellular vesicles to the lysosomes[117].The interplay between autophagy and exosome biogenesis has been recently described[118].

Most researchers have studied either the early or the late stages of autophagy. However equally important is the final stage, namely the lysosome reformation (ALR), leading to regeneration of functional lysosomes from autolysosomes. A series of proteins including clathrin, the motor protein KIF5B, and dynamin 2 are sequentially involved up to the maturation of functional lysosomes. Early lysosomes are pH-neutral but eventually they gain acidity and luminal proteins[119-122]. Accumulating evidence suggests that most, if not all, components of the molecular machinery for autophagy also mediate autophagy-independent functions. Autophagy is involved in various cell functions like endocytosis, phagocytosis, DNA repair, centrosome function, cell proliferation, cell death and immunological response including memory. Details were recently reported[123].

Autophagy and immunity: The implication of autophagy with the immune system has been investigated in the last few years[124-131]. Non-canonical forms of macroautophagy were described, resulting in the formation of autophagosomes that fuse with the lysosomes[132]. Only a subset of the Atgs machinery is used. Among these, LC3-associated phagocytosis (LAP) has been extensively studied because of its implication in immune regulation. LAP recruits LC3-II to the phagosomal membrane[133-

135] and is taken up by macrophages through innate immune receptors such as Toll-like receptors. In contrast to autophagy the LAPasome is a single membrane vacuole. In contrast to autophagy, ULK1 is not required for LAP[133]. Chaperone-mediated autophagy has also attracted attention because of its central role in antigen presentation and aging[136,137]. Autophagy is also implicated in the function of innate immunity interfering with macrophage autophagy. There is interplay between autophagy and innate immunity as interferon (IFN)-γpromotes autophagy in macrophages[138]. Mice fed with high fat diet had impaired autophagy in bone marrow-derived macrophages and peritoneal macrophages[139]. Mice with Atg5 deficient macrophages, developed hepatic inflammation when stimulated with lipopolysaccharide (LPS) after a high fat diet feeding. Acquired immunity is primarily a defense function against specific pathogens and is brought about by the different subsets of T cells and B cells. Interestingly there is evidence that high autophagic activity maintains the differentiation and function of important T-cell subsets such as regulatory T (Treg)-cells[140] and γ δ T-cells[141].

Autophagy and cell death: It has been proven that autophagy can be either a protective mechanism or a contributor to cellular death in certain instances[142-144]. Autophagy is involved in cellular death mostly by its effects on apoptosis. Autophagy is connected to apoptosis and these two cellular destructive phenomena are affecting each other[145-148]. This is particularly important in hepatic cell death[149].

Generally autophagy blocks the induction of caspase-dependent apoptosis, and apoptosis-associated caspase activation stops the autophagic process. Yet, in special cases, autophagy may induce apoptosis or necrosis, and autophagy has been shown to degrade the cytoplasm, leading to 'autophagic cell death' [150-152].

Autophagy is also implicated in caspase-independent cell death, leading to necrosis and necroptosis [153]. Induction of apoptosis eliminates cells damaged through the action of the tumor suppressor gene p53[154]. Apoptosis is counteracted, among others, by the mTOR/AKT pathway also involved in autophagy. The balance between p53 and AKT/mTOR is crucial for the fate of injured cells[155,156]. In addition, autophagy induces a particular mechanism of cell death named ferroptosis. It was initially reported as a specific iron-dependent form of malignant cell death. It soon became clear that ferroptosis is a more general form of cell death[157,158]. Many proteins implicated in autophagy (like Atgs and BECN1) were also involved in ferroptosis. Moreover activators of ferroptosis, like erastin, induced autophagosome accumulation and activation of autophagy led to ferroptotic cell death possibly by the turnover of ferritin through ferritinophagy[159-161].

A recent study has shown that ferroptosis is also interconnected with lipophagy. Lipids released during lipophagy and subsequent peroxidized increase ferroptosis. Therefore it might be that ferroptosis is a mechanism of cellular death in NAFLD[162].

Autophagy and inflammation: Autophagy is also closely associated with the inflammatory response in the liver. Inflammasome and autophagy regulate each other by the same inhibitory mechanisms which however are controlled by different input pathways. The NLRP3 inflammasome activation, usually through the stimulation by pathogen- and/or danger-associated molecular patterns[163,164], induces procaspase-1 activation which promotes interleukin interleukin (IL)-1β and IL-18 production leading to pyroptotic cell death. These events are counteracted by caspase-1-mediated activation of autophagy. In addition autophagy reduces inflammasome activation degrading the inflammasomes in the autophagosomes but also eliminating damaged cytoplasmic organelles that otherwise would produce DAMPS increasing activation of inflammasomes[165,166].

On the other hand, the negative correlation between inflammasomes and autophagy[167-169] leads to an increased production of the pro-inflammatory IL-1β[170] when autophagy is decreased[128]. However, the relationship between NLRP3 and autophagy has not been fully clarified, and recent studies have reported that nuclear factor-κappa beta (NF-κB) activation can modulate the NLRP3 and autophagy towards the same direction[171].

In view of the above is not surprising that many reviews on autophagy use the term "double-edged sword" stressing the fact that autophagy may have opposite effects on the same biological phenomenon [172]. Prominent general paradigms are cancer[173,174] and viral infections[175].

Another characterization pertinent to the liver is that autophagy behaves like Jekyll and Hyde depending on the cells involved. In hepatocytes, macroautophagy [in NAFLD and alcoholic liver disease (ALD)] and CMA (in NAFLD) is protective. It reduces fat accumulation and oxidative stress, it removes damaged mitochondria and favors regeneration. In macrophages, macroautophagy inhibits liver inflammation and fibrosis but it enhances fibrosis activated stellate cells. It is protective in early phases of hepatocellular carcinoma, but may be detrimental in late phases[176,177].

Autophagy in hepatocytes but also in the non-parenchymal sinusoidal cells of the liver is a key for liver physiology[178,179] and defects of autophagy are implicated in the pathophysiology of most liver diseases[180]. Both common diseases like alcoholic and non-alcoholic fatty liver or viral hepatitis and rare entities like Wilson's disease and a1 antitrypsin deficiency are related to autophagy defects[30,41, 57,181-184]. Defective autophagy also leads to accumulation of detrimental hepatocyte byproducts due to the fact that hepatocytes have a long half life of 6-12 mo [143]. Moreover, the liver is responsible for handling of a large number of xenobiotics and autophagy is a cytoprotective mechanism[99,185]

(Figure 2).

OBESITY, STEATOSIS AND NAFLD

NAFLD is the commonest liver disease worldwide. Recently it was suggested that it should be renamed as metabolic dysfunction-associated fatty liver disease (MAFLD)[186,187]. Pathological lesions in the liver vary from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. Current pathogenesis of NASH is mainly focused on the effects of insulin resistance and lipotoxicity in hepatocytes[188]. The abnormalities reported in Kupffer cells, stellate cells and endothelial cells are regarded as secondary events[189,190].

Obesity and insulin resistance are well documented risk factors for NAFLD development. Defects in liver autophagy have been established as fundamental abnormalities in both conditions.

Hepatic autophagy in obesity and insulin resistance

In the hepatocyte, lipids are catabolized by two major pathways. The first involves cytoplasmic neutral lipases and the second is lipophagy and acid lipases and hydrolases of the lysosomes. The end result is the production of free fatty acids that are further broken down by βI-oxidase in the mitochondria[191].

Lipid droplets have a core of lipids enwrapped in a phospholipid layer characterized by proteins called perilipins directing them to the autophagosome[72]. A crucial protein mediating lipolysis and autophagy is the adipose triglyceride lipase (ATGL). Cytoplasmic lipolysis and lipophagy are interconnected. The degradation of perilipins by autophagy facilitates actions of ATGL which in turn induces autophagy *via* sirtuin1 deacetylation of certain Atgs and activation of the transcription factors FoxO1 and FoxO3 thus promoting autophagy[192-194].

Lipophagy can prevent lipid accumulation in hepatocytes, while the inhibition of lipophagy promotes lipid droplets (LDs) accumulation, resulting in hepatocellular steatosis[195].

Characteristic changes of the metabolic syndrome like obesity, hyperglycemia, and dyslipidemia have been shown to exert a negative effect on autophagy because the regulatory control of forkhead box O1 (FoxO1) on the expression of *Atg* genes is lost leading to autophagy malfunction[196]. Macroautophagy and CMA are also down-regulated by increased intracellulal lipids due to either interference with the lysosomal stability of the CMA receptor or to the reduction of the ability of autophagosomes to fuse with lysosomes leading to the reduction of macroautophagic flux[196-198].

The severity of steatosis is related to the expression of three proteins, the damage regulated autophagy modulator (DRAM), BAX and p53. In mice livers, p53 expression increased in mild and severe steatosis. A DRAM expression increase was observed in mild hepatosteatosis, whereas high BAX expression was identified in severe hepatosteatosis[199].

A clinical study has confirmed the link between induction of autophagy and liver steatosis[200]. Autophagy-related genes (Atg5, LC3A, and LC3B) were overexpressed in obese patients compared with non obese patients.

Experimental evidence also suggests that defective autophagy is crucial in the development of obesity, oxidative stress, and the metabolic syndrome[201-203].

Insulin is intimately involved in autophagy regulation as the mTOR inhibitor of the FoxO and TFEB controllers of the transcription of autophagic genes is insulin-inducible[204]. Overactivation of mTOR in turn leads to insulin resistance[205,206]. Several mechanisms might explain this defect in obesity. Obesity increases calpain-2 by a still unknown signal pathway. Calpain is a protease that degrades Atg7 and modulates autophagy[201]. Autophagosome-lysosome fusion is also defective in livers of obese mice due to alterations of the lipids in cellular membranes induced by the high-fat diet[198]. A defective liver autophagy and the associated decrease of lysosomal degradation contribute to an additional increase in the ER stress which leads to insulin resistance and a vicious circle is completed[201,207,208]. Hyper-insulinemia decreases liver autophagy and reduced hepatic autophagy aggravates ER stress and insulin resistance.

An additional mechanism is a defect in acidification of lysosomes. Impaired substrate degradation in autolysosomes has also been reported for obese ob/ob mice. Activities of lysomal cathepsins were implicated in obesity. Cathepsin L was decreased in obese adipose tissue, while Cathepsin B was significantly elevated. Interestingly in obese adipose tissue inflammasomes were activated and further upregulation of cathepsin B resulted in additional activation of inflammasomes[209-212].

A study of the expression of 322 lysosomal/autophagic genes was recently reported in adipose tissue of lean and obese patients. Among 35 significantly expressed genes, 34 were upregulated. In isolated murine cells, tumor necrosis factor alpha (TNFα) stimulation resulted in upregulation of lysosomal/autophagic genes accompanied by upregulation of the autophagy associated SQSTM1/p62 receptor leading to increased degradation of perilipin 1. It seems that local inflammatory cytokines may impair lipid storage *via* autophagy induction[213].

An extensive review of lysosomal enzyme abnormalities in both adipose and liver tissue was recently published[214]. A recent report suggests an additional mechanism contributing to obesity-associated abnormalities. Obesity increases lysosomal iNOS and NO production leading to exacerbation of

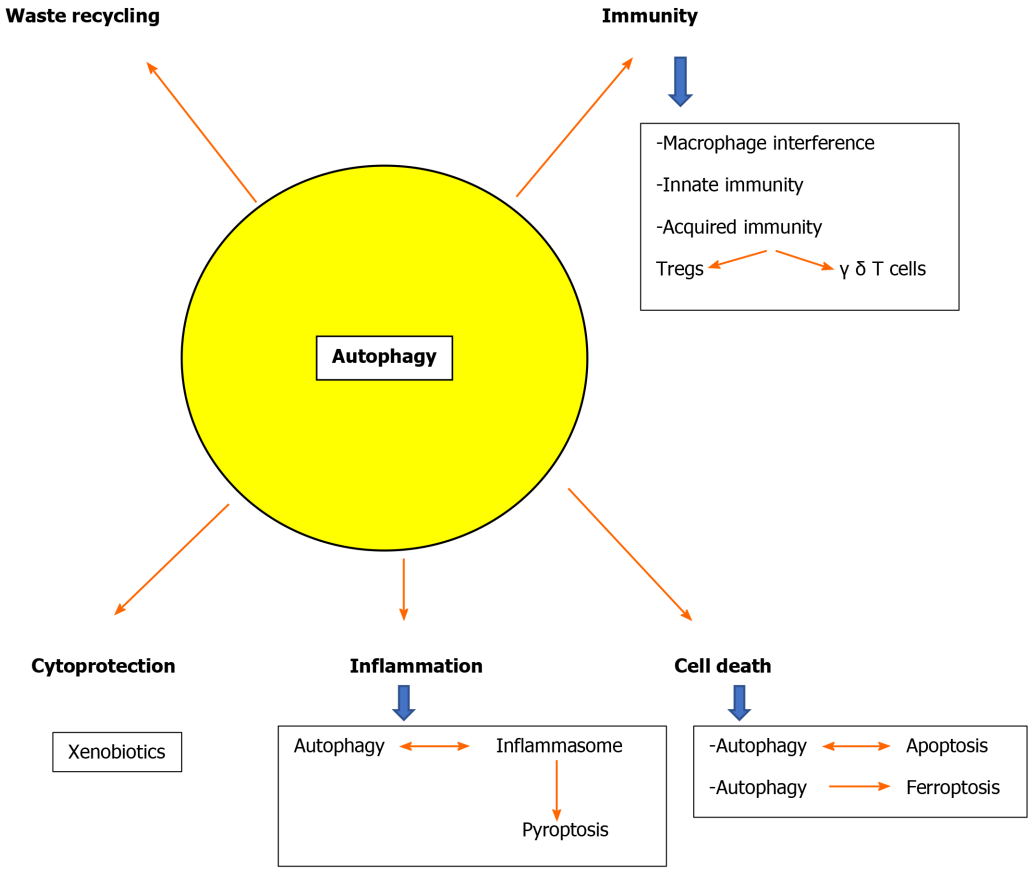

Figure 2 Implications of autophagy in critical cellular functions in the liver. For details see text.

lysosomal nitrosative stress, impairment of lysosomal function, defective autophagy and insulin resistance[215].

There is also evidence that mitophagy is negatively regulated by liver insulin resistance. Mitophagy can promote mitochondrial fatty acid oxidation to inhibit hepatic fatty acid accumulation and improve hepatic insulin resistance. Fundc1 is a recently characterized mitophagy receptor and mice lacking this receptor develop severe obesity and insulin resistance when maintained in a high-fat diet[216,217].

However, when autophagy is defective an alternative mechanism protects the liver from steatosis. An induction of fibroblast growth factor 21 (FGF21) was reported in mice with subsequent amelioration of insulin resistance and decreased diet-induced obesity[218,219]. This has been corroborated in a clinical study of overweight NAFLD patients, where increased FGF21 levels were correlated with steatosis grade, fibrosis and lobular inflammation. NASH patients had the highest levels[220]. An analogue of FGF21 has been tested in experimental animals and obese diabetic patients with promising results[221-223]. Nevertheless, the control of adipose tissue biology is very complex and is elegantly described in a recent publication[224].

NAFLD-NASH

Not surprisingly autophagy is strongly associated with NAFLD pathogenesis[179]. Diet-induced NAFLD in mice blocks hepatic autophagy and leads to oxidative stress and mitochondrial dysfunction [225], also reducing thyroid hormone-induced mitophagy[226]. The potential molecular pathways and possible therapeutic implications of thyroid hormones in NAFLD have been recently reviewed[227].

Mitophagy abnormalities are strongly implicated in NAFLD[228-230]. In particular an impairment of mitophagy seems to activate the NLRP3 inflammasome favoring the progression of NAFLD to NASH [38]. Accordingly, recent evidence indicates that restoration of mitophagy may improve NAFLD[231-234].

In addition to mitophagy, reticulophagy is also implicated in NAFLD. An extensive reticulophagic response is evident in hepatocytes after induction of NAFLD by oleic acid[228,235]. It is suggested that reticulophagy and mitophagy are independent, events involved in NAFLD progression[228].

Impaired lipophagy and lipotoxicity are also strongly involved in NAFLD[72,192,236,237]. Lipid accumulation in hepatocytes blocks autophagic flux and impaired autophagic flux favors the progress of

NAFLD[30].

This impaired flux and the subsequent ER stress can be improved by inhibition of the sterol regulatory element-binding protein 2 (SREBP-2) whose activation promotes accumulation of cholesterol in NAFLD. This improvement is associated with upregulation of autophagy genes[238].

Intracellular lipid trafficking is also regulated by store operated calcium entry and enhanced lipophagy is observed in cells defective in this system[239]. Moreover, the detrimental effects of diets rich in saturated FFA were increased bysirtuin-3, which enhanced lipotoxicity, reducing the autophagic flux[240]. The effect of lipophagy in liver steatosis is further supported by experimental evidence that various chemicals are involved in steatosis by interfering with autophagy. Caffeine reduces lipid content and stimulates beta-oxidation in hepatocytes through autophagy in mammalian liver cells in NAFLD[17]. In essence caffeine protects against fatty liver through the co-ordination of the induced lipophagy and mitochondrial β-oxidation[241,242]. Epidemiologic studies demonstrated that coffee consumption reduced the development of fatty liver, fibrosis, and hepatocellular carcinoma in NAFLD patients[243,244] supporting thus the experimental evidence.

Methionine is a well known inactivator of autophagy and lipophagy. The correlation between lipophagy and methionine in the liver from patients with liver steatosis has been studied. Increased levels of methionine inhibit autophagic catabolism of lipids and contribute to liver steatosis in NAFLD [83]. Mice fed with a methionine/choline deficient diet developed steatosis, inflammation, fibrosis and ER stress associated with mitochondrial dysfunction. The administration of the autophagy enhancer rapamycin ameliorated these lesions while chloroquine, a well established autophagy inhibitor, aggravated the liver injury[245]. Resveratrol, another autophagy activator, also attenuated liver lesions induced by a similar diet[246,247]. Consistent with these findings is a recent report that a traditional Chinese herb increased autophagy and considerably improved steatohepatitis induced by methionine/choline deficient diet in rats[248].

Other diet-supplied molecules affect autophagy and are possibly beneficial in NAFLD including the purple sweet potato color[249]. Likewise, the caffeic acid of vegetables has been reported to ameliorate hepatic steatosis[250] while curcumin, an antioxidant polyphenol of Curcuma longa, has been shown to inhibit apoptosis and induce autophagy with a potential protective effect on hepatocellular carcinoma [251].

A finding that might be useful in future treatment of NAFLD was recently reported. Celecoxib, a COX-2 inhibitor, attenuated steatosis and restored autophagic flux in cells treated with palmitate and rats fed a high fat diet[252].

Other lipids like the sphingolipid ceramide may be implicated in NAFLD as it is increased in Atg7 knockout mouse liver in parallel with the impaired autophagy[253]. Autophagy increased when sphingolipid de novo synthesis was upregulated, indicating that lipid degradation was activated to prevent excessive sphingolipid accumulation.

Interestingly, autophagic activity seems to be upregulated when the renin angiotensin system is overexpressed. The underlying mechanisms and its role in NAFLD have yet to be clarified as there are many controversial issues to be solved[254]. Overall there is extensive evidence that inhibition of lipophagy is detrimental for the liver in NAFLD[198,222,238,255].

Summarizing the above studies, a therapeutic approach against NAFLD would be the activation of lipophagy[90]. However, it is noteworthy that there is one study indicating the opposite, as suppression of autophagy through inhibition of c-Jun N-terminal Kinase (JNK) ameliorates insulin resistance in a rat NAFLD model[256].

Extensive reviews on the mechanisms of autophagy deregulation in NAFLD were recently published [183,257,258]. Not only impaired macroautophagy but also reduced liver chaperon mediated autophagy (CMA) favors steatosis due to failure in the timely removal of perilipins[259,260] and therefore an increase in lipogenic enzymes. When oxidative stress is increased in the liver, an upregulation of CMA occurs to selectively remove damaged proteins[62]. Loss of CMA leads to impairment of proteostasis and accumulation of oxidized protein aggregates perpetuating thus chronic oxidative stress[261].

Autophagy and NASH

Involvement of autophagy in the progression of NAFLD to NASH has not yet been clarified and molecular mechanisms are not fully understood.

One of the histological characteristics of NASH used in diagnosis and scoring systems is the formation of Mallory-Denk bodies (MDB)[262-264]. There is experimental evidence that inhibition of autophagy and accumulation of p62 is related to their formation while autophagy activation with rapamycin leads to their resolution[265]. Further support of the involvement of autophagy in NAFLD evolution to NASH was reported in a clinical and experimental study where a decrease of autophagic flux in parallel with an increase in ER stress was demonstrated both in the livers from NAFLD patients and mice models of NAFLD, and in lipid-overloaded human hepatocytes[266]. However tests for measurements of autophagic flux used in this paper are not full-proof as they can be influenced by autophagy independent factors. Therefore these findings should be corroborated in a different set up.

Patients with NASH and murine models of steatotic inflammation had reduced expression of Atg7 and TFEB while the autophagy inhibitor rubicon was increased[139,177,255].

In contrast, steatosis and liver injury were improved in parallel with restoration of autophagy and reduction of ER stress in mice with a deletion of the Rubicon or adenoviral delivery of Atg7[202,251]. Recent evidence also indicates that impaired mitophagy may contribute to liver injury during progression of NAFLD and formation of megamitochondria[229].

Transition of NAFLD to NASH also implicates Kupffer cells. These cells, constitute 80%-90% of tissue macrophages in the body and are critical cells in liver inflammation[20]. They are the main site of NLRP3 inflammasome activation and production of the pro-inflammatory cytokines compared to hepatocytes and stellate cells[267,268]. Activation of the NLRP3 inflammasome plays an important role in the transition from NAFLD to NASH[269].

An earlier report demonstrated that cathepsin B, a lysosomalcysteine protease, is released in the cytosol in response to FFAs and that this redistribution of cathepsin B is present in the liver of patients with NAFLD related to disease severity. Importantly in a dietary mouse model of NAFLD, inhibition of Cath B significantly decreased steatosis, liver inflammation and insulin resistance[270].

These findings were recently elaborated in more detail as it was reported that cathepsin B and activation of the NLRP3 inflammasome are interconnected in a murine model of NASH but also in isolated Kupffer cells stimulated with palmitate. Expression of cathepsin B and activation of NLRP3 inflammasome were increase in NASH animals. Moreover, an inhibition of Cathepsin B decreased liver inflammation, ballooning, and the pro-inflammatory cytokines IL-1β and IL-18. *In vitro* stimulation of Kupffer cells showed identical results in inflammasome activation, expression of Cath.B and cytokine production before and after Cath.B inhibition. These results indicate that NASH pathogenesis probably depends in part to inflammasome activation which in turn is regulated by the activity of aprotease tightly connected to autophagy[271].

Additional supporting evidence for the role of autophagy in NASH pathogenesis is the fact that impaired autophagy in obese mice is critical for macrophage polarization. M2 macrophage polarization relies on energy provided by FFA oxidation, suggesting a potential implication of autophagy in this process. Macrophages change to a pro-inflammatory phenotype due to both increased M1 and decreased M2 polarization[132] with a resultant upregulation of liver inflammation, a prominent feature of NASH.

The situation is controversial when adipose tissue macrophages from obese mice are concerned. Increased rather than decreased autophagy of macrophages has been demonstrated in adipose tissue [272,273]. Another cathepsin mostly found in Kupffer cells seems to be implicated in NASH. Lysosomal cholesterol accumulation inside murine Kupffer cells leads to increased liver Cathepsin D activity which is related to liver inflammation[274]. Kupffer cell cathepsin D may therefore be an additional key player in hepatic inflammation of NASH[275]. The impairment of macrophage autophagy with aging may explain in part the increased prevalence of the metabolic syndrome and steatohepatitis of older age in humans[276,277].

The oxidative stress is also involved in the progression to NASH. Hepatocytes exposed to palmitate concentrations similar to those found in patients with the metabolic syndrome and NAFLD showed mitochondrial membrane permeabilization and production of ROS. Similarly, an inhibition of Cathepsin B ameliorated mitochondrial dysfunction and oxidative stress, indicating an additional mechanism of NASH progression[229,278].

Under normal conditions, damaged mitochondria are removed through mitophagy. In certain cases of NAFLD however mitophagy is defective and the oxidation of biomolecules by mitochondrial ROS starts a vicious cycle of increasing mitochondrial dysfunction and aggravation of hepatocellular oxidative damage. This ultimately leads to hepatic inflammation and liver failure[279,280], since impaired mitophagy triggers liver NLRP3 inflammasome activation *in vivo* and *in vitro* in isolated murine hepatocytes[38].

Impairment of autophagy in other liver sinusoidal cells may also participate in the progression of NAFLD to NASH. Decreased autophagy has been observed in the liver endothelial cells of patients with NASH or in mice with endothelial deletion of Atg5 and features of inflammation[180,190,281]. A very recent study has convincingly shown that impaired autophagy of liver endothelial cells (LSECs) occurs in NASH patients but not in simple steatosis. Deficiency in autophagy in LSECs induces endothelial inflammation ultimately leading to liver inflammation and fibrosis. This defective autophagy, in part due to inflammatory mediators of the portal blood, might well be one of the missing links of the progression of simple steatosis to NASH and cirrhosis[282].

A further mechanisms leading to NASH involves multivesicular bodies (MVBs), a form of endosomes, whose contents are transported into lysosomes[283]. The MVB-lysosomal pathway was shown to participate in the development of steatohepatitis through lysosomal degradation of Toll-like receptor 4 reported to be critical for the progression of NASH[284].

Finally a role of the chemokine CXCL10 in the development of steatohepatitis has been proposed. Upregulation of CXCL10 impairs autophagic flux decreasing thus autolysosome formation. Autophagic protein degradation is inhibited followed by the accumulation of ubiquitinated proteins with ultimate development of steatohepatitis[285].

ALD

The liver is the organ mostly responsible for ethanol metabolism. Oxidation of ethanol happens through three pathways namely alcohol dehydrogenase in the cytosol, cytochrome P450 (CYP2E1) in the ER and microsomes and the enzyme catalase in peroxisomes[286]. Ethanol oxidation also produces ROS, including superoxide anion, and hydroxyl radicals that may damage hepatocytes[287].

Ethanol induces autophagosome formation in the liver. Reduction of autophagy results in the accumulation of lipid droplets and apoptosis of hepatocytes[288]. On the other hand activation of autophagy by rapamycin attenuates steatosis and injury induced by a combination of ethanol and lipopolysaccharide[289].

Induction of autophagy by acute ethanol exposure is mediated through many mechanisms Ethanol-induced autophagy requires ethanol oxidation to acetaldehyde and ROS generation[290,291]. ROS activates autophagy by suppressing mTOR and proteasome activity[292,293] and inactivation of Atg4 [294].

Oxidants differentially influence the activities of the proteasome (the other major pathway of protein degradation.) Proteasomes are reduced when autophagosomes are increased[295]. Proteasome inhibition further triggers ER stress activates autophagy through JNK activation. Ethanol may also suppress Akt and mTOR through the upregulation of PTEN[296,297]. Metals, like zinc, are also implicated in autophagy alterations after ethanol treatment[298].

A caution should be exercised on CYP2E1 ethanol oxidation as oxidative products resulting from the expression of CYP2E1 may in fact impair autophagy leading to lipid accumulation in the liver. In cells expressing CYP2E1, hepatocyte lipids and generation of ROS were increased by an inhibitor of autophagy and decreased when a stimulator of autophagy was used[299]. Similar results were found after acute alcohol in CYP2E1 knockout mice[291]. These findings also support the idea that autophagy protects against ethanol/CYP2E1-dependent hepatic injury.

It has also been shown that hepatic autophagy depends on the level of acetaldehyde produced during ethanol metabolism. Mice expressing the ALDH2 isoenzyme, clear acetaldehyde more rapidly and have increased autophagy and lower levels of hepatic triglycerides[300]. Cannabinoid receptor 2 can also induce macrophage autophagy to protect from alcoholic liver damage[301].

It should be stresses however that acute and chronic ethanol exposure may have different effects in liver autophagy[302]. Increased autophagosome formation and autophagy flux were shown in cultured hepatocytes after short term incubation with ethanol or in livers of mice after acute alcohol administration[288,302]. Enhanced autophagy parallel a higher hepatocyte nuclear content of TFEB, the main transcriptional regulator of genes involved in lysosome biogenesis[49,50].

Alcohol also has an effect on the transcription factor forkhead box O3a (FoxO3a) that modulates liver autophagy[303]. The activity of FoxO3a is largely controlled by multiple post-transcriptional modifications, including phosphorylation and acetylation[304]. Acute ethanol exposure increases nuclear translocation of FoxO3a inducing its dephosphorylation and acetylation.

However, results are not uniform for the chronic ethanol effect. Chronic ethanol administration (Lieber-DeCarli model) for 4 wk or 10 wk increased autophagosome numbers in murine livers, suggesting the induction of autophagy[305]. In another similar murine model, mice were given gradually increasing ethanol ethanol concentrations for 10 d and autophagic flux was reduced[302].

The discrepancy seems to be solved by the report that autophagy response was dependent on the alcohol concentration used. In a murine model on Lieber-DeCarli diet with different levels of alcohol for 4 wk, autophagy is increased by a lower dose of alcohol (29% of the caloric need), but decreased by a higher dose (36% of the caloric need). Liver injury was aggravated by further reduction of autophagy and attenuated by autophagy activation[306].

Earlier studies have also demonstrated that chronic alcohol exposure disrupts lysosome function [307]. Overall results have demonstrated that autophagy is suppressed in chronic alcohol consumption due to either the defect of lysosomal function and biogenesis from TFEB suppression[302,308] or to a reduction in AMPK activity and inhibition of autophagosome formation[309,310].

After ethanol-induced reduction of autophagy, there is accumulation of aggregated proteins and SQSTM1/p62, leading to activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and damage to the mitochondria and cell death[309,311].

How the other autophagy-related transcriptional factors, such as TFEB and farnesoid X receptor (FXR) are interconnected with FoxO3a in the expression of autophagy genes is unknown. Moreover, how ROS generation in acute or chronic alcoholic condition systematically affects the mTORC1 activation or TFEB translocation is unclear.

Autophagy is also protective against CYP2E1-dependent liver lesions in a chronically ethanol-fed murine model[312]. Autophagy in ALD can be further affected by additional factors identified in various experimental models. Augmenter of liver regeneration (ALR) is a factor that can promote liver growth. It was reported to protect mice from ethanol-induced liver injury through inhibition of mTOR and therefore activation of autophagy[313]. Moreover an interesting recent study used many genetic models of autophagy impairment, with different functional levels and different alcohol regimens. Deficiencies of either Atg7 or Atg5 demonstrated variable responses to ethanol feeding according to the timing of autophagy dysfunction, the gene being affected, and the alcohol scheme used[314].

It should be stressed that in acute alcohol administration, ethanol-induced autophagy may protect the liver by three basic mechanisms namely mitophagy[80,102,315,316], lipophagy[72,293,317] and clearance of Mallory-Denk bodies by proteophagy[265,318,319].

However, chronic alcohol exposure impairs autophagy and lipophagy[308,320] most likely due to the activation of mTOR signaling and a decrease in lysosomal biogenesis. Administration of the mTOR inhibitor Torin- 1 restores lysosomal biogenesis and attenuates liver lesions[308]. An additional pathway through which chronic alcohol exposure could reduce liver autophagy is the inactivation of the guanosine triphosphateRab7 and reduction of dynamin 2 activity leading to depletion of lysosomes and inhibition of hepatocyte lipophagy[320,321].

Ethanol Induced steatosis activates mitophagy by elevating PINK1 expression on mitochondria[305]. PINK1-dependent mitophagy was correlated with the mitochondrial expression of Parkin and the level of an indicator of oxidative mtDNA damage[322-325]. Mitophagy has a dominant role in protection of the hepatocyte from alcohol-induced hepatic injury as evidenced by a report that enhancement of mitophagy by quercetin, a natural flavonoid, attenuated ethanol-induced mitochondrial damage[326].

Regulation of mitophagy is related to three receptors namelyFUN14 domain containing 1 (FUNDC1), BCL2 interacting protein 3 (Bnip3), and Parkin[327].

DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is a newly described housekeeper of liver mitochondrial fission. DNA-PKcs is overexpressed in murine livers after exposure to ethanol and was positively correlated with steatosis, mitochondrial damage andfibrosis. On the other hand this over expression repressed FUNDC1-required mitophagy[328].

An additional significant point is the effect that ethanol might have on the different sinusoidal cell subpopulations. There is strong evidence that autophagy in macrophages is crucial to protect the liver from ethanol-induced damage. Investigations were mostly performed in macrophage specific deletions of either Atg7 or Atg5. The cannabinoid CB2 receptors of macrophages were found to have a protective rolein ALD, which was abrogated by Atg5-deletion in macrophages[301]. Increased mortality in Atg5 deleted mice was also demonstrated after chronic ethanol feeding plus LPS challenge[329]. Similar findings were reported after Atg7 deletion[330]. Both studies demonstrated an activation of the inflammasome and an augmented IL-1 production.

In contrast to hepatocytes and macrophages the effect of autophagy in hepatic stellate cells after ethanol exposure has not been clarified. A recent study in immortalized rat stellate cells demonstrated that autophagy could contribute to ethanol-induced stellate cell activation[331]. Induction of fibrosis by alcohol in current murine models is not feasible unless accompanied by steatosis induced by a high-fat diet[332].

Most autophagy studies in ALD are focused on the involvement of macroautophagy. Recent evidence however indicates that CMA is also important in alcoholic liver disease through the CMA negative regulator sorting nexin 10 (snx10). Snx10 knockout mice fed with Lieber-DeCarli diet were resistant to alcohol-induced liver injury associated with an increase of lysosome-associated membrane protein 2A (LAMP2A) and CMA activation through inhibition of the enzyme Cathepsin A which is responsible for LAMP2A degradation[333]. Deficiency therefore of a CMA negative regulator, protects animals from ALD. Deficiency of another CMA negative regulator, Lipocaline-2 (LCN2), also maintains hepatic CMA activity in murine livers after chronic alcohol administration[334] verifying the idea that impaired CMA may be responsible at least in part in alcohol-induced liver injury.

Involvement of miRNAs is an additional factor in the regulation of autophagy in ALD that has emerged from recent evidence. Several miRNAs were reported to alter autophagy and alcoholic steatosis[335]. miR-26a ameliorates alcohol-induced acute liver injury by two MAPKs inhibitors thus inducing Beclin-1 expression and autophagy[335]. Another report provided evidence that miR-155 is a mediator of alcohol-related exosome production and autophagy impairment in both hepatocytes and macrophages[336]. Deletion of miR-155 protected mice from alcoholic steatosis and inflammation. Interestingly in this study serum levels of exosomes were increased in ALD patients and alcohol exposed mice, whereas miR-155 deficient mice had significantly reduced exosome release from both hepatocytes and Kupffer cells. It was suggested therefore that autophagy is an atypical promoter of exosome release in ALD.

Clinically important observations indicate that withdrawal of ethanol from ethanol-fed rats resolves steatosis[337] suggesting that removal of ethanol oxidation and restoration of lipophagy may be the mechanism of steatosis resolution observed in humans after ethanol abstinence[338,339]. Informative reviews of autophagy in ALD were recently published[90,181,182,340-342].

In view of the fundamental role of lipophagy in the pathogenesis of ALD, it is not surprising that pharmacological inducers of lipophagy like carvamazepine, rapamycin, resveratrol and simvastatin were tested in alcohol-fed animals with a resultant attenuation of liver lesions. By contrast chloroquine exacerbated hepatic steatosis[312,343,344]. Recently plant-derived agents were also used to activate lipophagy. Thus, corosolic acid[345], quercetin[346] and Salvianolic acid A[347] all had a favorable result on alcohol-induced liver lesions activating lipophagy through different pathways.

Summarizing, it is evident that whether ethanol causes an increase or decrease of autophagy depends on the duration of ethanol consumption/exposure, the amount of alcohol given, and the manner in which it is administered[290,302]. Moreover, lipophagy and mitophagy cannot act as defensive mechanisms in the long term as they do in acute ethanol consumption as they are inhibited by chronic

alcohol exposure[102,348].

VIRAL HEPATITIS

In the past decade, hepatic autophagy has been implicated in viral infection with either hepatitis B (HBV) or hepatitis C (HCV).

HBV

Recent studies have shown that autophagy is involved in the life cycle of Hepatitis B. Inhibition of autophagosome formation could reduce HBV production, while stimulation of autophagy could significantly contribute to HBV production[349,350].

However, the mechanism by which HBV activates autophagy is not clear. Previous reports have implicated either the HBx[351,352] the large HBsAg protein[353] or a mutant with a deletion in the preS2 region[354,355] as inducers of ER stress which in turn increases autophagy.

In contrast it was shown that HBx does not play a significant role in the induction of autophagy compared to the small HBsAg protein also increasing autophagy *via* the induction of ER stress. An HBV genome unable to express small HBsAg does not activate autophagy[356]. To reconcile the discrepancy, it has been suggested that autophagy can be stimulated both by HBx and the small surface HBsAg protein through upregulation of beclin-1 expression[357,358]. In addition HBx induces autophagy through its effect on the cytoplasmic high-mobility group box 1 (HMGB1), identified as a a positive regulator of autophagy. HBx binds to HMGB1 and triggers autophagy in hepatocytes[359]. This observation may be clinically relevant. Spontaneous and induced autophagy of peripheral Treg cells from 98 patients with chronic hepatitis B were assessed[360]. No difference of spontaneous autophagy was found between patients and normal controls but induced autophagy was significantly higher in patients. It was also related to HMGB1 as it was significantly decreased when HMGB1 was blocked with a neutralizing antibody.

HBx further impairs lysosomal acidification with a final result the accumulation of immature lysosomes. Moreover immature lysosomal hydrolase cathepsin D was shown in human liver tissues with chronic HBV infection suggesting that a repressive effect of HBx on lysosomes may be responsible for the inhibition of autophagic degradation[350]. Interestingly, although HBV could impair lysosomal acidification it was unable to induce autophagic protein degradation, due to the inability of HBV to increase the sequestration of proteins destined for degradation by autophagy[350]. Therefore, it is usually stated that HBV induces incomplete autophagy. In addition, it was clearly shown that HBV specifically targets damaged mitochondria and mitophagy. Either the whole HBV genome or HBx alone were able to induce Parkin-mediated mitophagy[361,362]. In addition, HBx-induced autophagy inhibited mitochondrial apoptosis increasing the survival of HBV DNA-transfected cells[349]. Another clinically important observation is that different HBV genotypes have a variant effect on autophagy. HBV genotype C was a more potent inducer of autophagy than HBV genotype B. HBV-C is associated with more severe disease than HBV-B but however attractive such an association between autophagy and severity of liver disease may be, it has to be verified[363,364].

It is important to realize that many viruses, including HBV, have developed strategies to hijack autophagy to benefit their replication and dissemination[356,365,366]. So far, HBV is the only DNA virus known to exploit autophagy for its own replication as it is RNA, but not DNA viruses, that commonly use autophagic function to promote replication[367].

HBV infection induced the early-stage formation of autophagic vacuoles increasing the PI(3)K enzyme activity to promote HBV DNA replication. HBx can directly bind and activate the PI3KC3 complex[368,369]. Ablation of Atg5 has been shown to inhibit autophagy and impair nuclear localization of the HBV core protein. HBV DNA level in sera was decreased by more than 90% accompanied by practically undetectable levels of the HBV DNA replicative intermediate in the liver [370].

Autophagy was responsible for the degradation of an oncogenic microRNA-224 in the liver of HBV patients with hepatocellular carcinoma (HCC) and HBx-transgenic mice. In HCC patients, the combination of low-Atg5 expression and high miR-224, was significantly correlated with a poor overall survival rate[371]. The list of the mechanisms used by HBV to subvert autophagy and the detrimental consequences in the liver is by no means complete as new factors are constantly reported including release of pro-inflammatory cytokines and chemokines and inhibition of neutrophil extracellular trap [372-375].

Further evidence of autophagy subversion by HBV was recently reported. In HBV-replicating hepatocyte cultures, the silencing of Atg5, Atg12, and Atg16L1, interfered with viral core/nucleocapsid (NC) formation/stability and significantly reduced virus yields. It was further demonstrated that a covalent conjugation of Atg12 to Atg5 was essential for HBV replication. In addition the virus required Atg10 and Atg3 which are necessary for Atg5-12 conjugation. Deletion of Atg10 and Atg3 decreased HBV yields, while Atg3 overexpression increased virus production. HBV was associated with the Atg5-12/16L1 *via* interaction of HBV core protein with the Atg12 unit of the complex. Subsequent autopha-

gosome maturation events were not necessary for HBV replication. These data indicate that HBV subverts early, non degradative autophagy components avoiding thus autophagosomal destruction[178,376,377].

Death receptors of TNFSF10 (tumor necrosis factor superfamily member 10) participate in the immune defense against several viruses by promoting apoptosis. HBx impairs TNFSF10 receptor signaling through autophagy mediated lysosomal and not proteasomal degradation. Importantly a significant reduction of the protein TNFRSF10B was demonstrated not only in cell lines but also in the liver of chronic HBV patients[378].

It was very recently reported that the hepatitis D virus also utilizes autophagy to assist its life cycle as it increases autophagosome accumulation and impairs autophagic flux. Both the small HDAg and large HDAg proteins are capable to disturb the autophagy machinery, in particular the proteins Atg7, Atg5, and LC3 involved in the early elongation stage of autophagy. Unexpectedly, deletion of Atg5 and Atg7 reduced the intracellular HDV RNA level in hepatocyte cell lines without an effect on HDV secretion [379]. Reviews of autophagy in HBV have recently been published[366,380].

HCV

Reported data have shown that HCV could induce autophagy to support its own replication[381,382]. Several mechanisms for HCV induction of autophagy have been investigated using hepatocyte cell lines [383,384]. HCV infection initiates the formation of phagophores after induction of the localization of Atg5 to the ER. Phagophores fuse to form autophagosomes. HCV-induced autophagosomes were further reported to be required for viral RNA replication as the autophagosomal membrane provided a platform containing HCV NS5A, NS5B, and viral RNA for replication[385-387] but subsequently HCV blocks the fusion of autophagosomes and lysosomes through Rubicon overexpression. As a result autophagosomes accumulate and HCV RNA replication and assembly of infectious virions[385,388,389,390,391] are supported.

However, several studies have contradicted the need for co-localization of viral proteins in the autophagosomal membrane suggesting that this is not a necessity for viral replication[392-395].

Autophagy favors HCV replication with an additional mechanism. The entire autophagic process may be manipulated leading to the suppression of the HCV associated innate antiviral response[393,396]. After silencing different Atgs, HCV viral infectivity was suppressed in parallel with an upregulation of interferon-stimulated gene expression[390]. Moreover, HCV seems to activate autophagy to degrade the tumor necrosis factor receptor -associated factor 6 (TRAF6), thus subverting innate host immunity[389,397-399]. HCV induced unfolded protein response strongly activates autophagy to sustain viral replication through inhibition of cellular apoptosis[396]. Different HCV genotypes may have variable influence on autophagy[391,400].

HCV was also found to selectively activate lipophagy to counteract the HCV induced lipid abnormalities. This may be clinically important as the levels of autophagy in the liver of chronic HCV patients were inversely correlated to steatosis[401]. Inhibition of autophagic degradation of lipophagy may account for the characteristic occurrence of hepatic steatosis in chronic HCV infection. Mitophagy is also selectively activated *via* the PINK1–Parkin axis in infected cells, thereby promoting HCV viral RNA replication[361,402]. Virus-activated mitophagy further attenuates apoptosis and favors persistent viral infection[403]. In agreement with this finding, the viral non-structural protein 5A (NS5A) was shown to disrupt mitochondrial dynamics, thus increasing ROS production and mitophagy[404].

On the other hand, the viral core protein interacts with Parkin inhibiting its translocation to mitochondria. Mitophagy is suppressed and mitochondrial injury of infected hepatocytes is sustained and viral persistence is maintained[405].

Syntaxin 17 is an autophagosomal protein required for the fusion of autophagosomes with lysosomes and also the release of HCV. The amount of syntaxin 17 was reduced in HCV-replicating cells indicating that HCV impairs the late stages of autophagy affecting the equilibrium between the release and the lysososomal degradation of viral particles[406].

Recently CMA was also demonstrated to be activated by HCV leading to degradation of IFN-alpha receptor-1[407]. Moreover the HCV NS5A was found to interact with Hsc70, recruiting Hsc70 to hepatocyte nuclear factor 1 alpha thus targeting HNF-1α for CMA degradation[408]. Taken together these studies indicate that HCV induced CMA also facilitate HCV replication.

However, an opposite less permissive effect of the manipulation of autophagy by HCV has been suggested as a result of recent studies. Atg10 is critical for autophagy as it promotes the Atg5-Atg12 complex formation. Two isoforms of the Atg10 protein were described, namely Atg10 (a longer one) and Atg10S. They have a similar amino acid sequence except for an absence of a 36-amino acid fragment in Atg10S. Yet they differ in their effects on HCV genome replication. Atg10 with deleted or mutated two cysteins, (Cys^{44} and Cys^{135}) could trigger the expression of anti-HCV immunological genes combating the HCV replication[409,410].

Taken together these results indicate that autophagy is required for initiation of the HCV replicative phase but not for further replication[393]. However this might not be entirely true, as chloroquine an inhibitor of lysosomal acidification inhibits HCV replication offering an additional evidence for the permissive role of autophagy in HCV infectivity in the late phase[411].

Autophagy may additionaly be involved in HCV replication through the regulation of the exosomal pathway[390] and apolipoprotein transport[412], both critical steps in the egress of the HCV virion. The virion is associated to apolipoprotein E (ApoE) and its infectivity is enhanced. Autophagy has a central role in the trafficking of ApoE in HCV-infected cells leading to partial autophagic degradation of ApoE, but also to the interaction between ApoE and the viral protein E2 to increase the production of infectious viral particles[412].Molecular details of how HCV is using autophagy to its own advantage were recently published[380,413].

In summary, the life cycles of HBV and HCV in liver cells can be subdivided into 7 steps: Endocytosis, uncoating, genome replication, translation, envelopment, assembly and release. Both HBV and HCV drive autophagy largely by the ER stress response resulting from uncontrolled translation of viral proteins[414-416]. In addition HBx modulates autophagy for the benefit of HBV replication[357], while multiple HCV proteins including p7, NS3/4A and NS4B, modulate autophagy by direct or indirect association with moieties of the early autophagy machinery in favor of its replication[417-419]. Pharmacological or genetic manipulation of autophagy may limit the viral yield[183,369,420], making autophagy a feasible target for HBV and HCV treatment.

FIBROSIS-CIRRHOSIS

The liver responds to practically any insult with only a limited number of pathological lesions: Hepatitis (hepatocyte death), cholestasis, fibrosis-cirrhosis or a combination of the three. Autophagy participates in all liver pathological responses.

Liver fibrosis is a complex and dynamic cellular process implicated in the evolution of the majority of chronic liver disease towards cirrhosis. Most review articles have broadly concentrated on the role of autophagy in liver diseases, with restricted information on cell types implicated in liver fibrosis. Not unexpectedly, most research has focused on hepatic stellate cells (HSCs) and myofibroblasts, because they are the central elements in extracellular matrix production[421]. However, other liver cells, including hepatocytes, macrophages, sinusoidal endothelial cells (LSECs), infiltrating immune cells and the so-called ductular reaction (DR) are also important[422,423]. DR significantly correlates with the degree of fibrosis and involves cholangiocyte-like cells that dominate an interplay of extracellular matrix and inflammatory infiltrate[424-427].

HSC and autophagy

The fundamental event in fibrosis is the transformation of hepatic stellate cells into myofibroblasts and this is closely related to autophagy. Typical autophagosomes that contained LDs were found in cultured HSCs indicating a connection of liver fibrosis and lipid autophagy[428]. Increasing evidence supports the notion that inhibition of lipophagy in hepatocytes reduces HSC activation and fibrosis progression [429,430]. Inhibition of the activation of HSCs and the formation of autophagosomes have been reported and these seem to be connected with the downregulation of transforming growth factor beta 1/Smads pathway as an increase in TGFb/Smad3 Leads the transcription of Beclin-1, which is a critical player in the autophagy process[431-433].

In rat-derived HSCs, cytoplasmic LDs are degraded followed by fibrogenic genes expression. Moreover induced lipid accumulation by an alkaloid, was associated with quiescent HSCs due to autophagy blockade[434]. Inhibition of autophagy by chloroquine improved CCl4-induced liver fibrosis affecting the activation of hepatic stellate cells as expected[435]. On the other hand, dihydroceramide an inhibitor of autophagy promoted the progression of liver steatosis to fibrosis[436]. Similarly, inhibition of YAP degradation also led to liver fibrosis[113].

In addition, it has been suggested that the IL-17A/STAT3 signaling pathway is important in the evolution of liver fibrosis through suppression of hepatocellular autophagy since neutralization of IL-17A promotes the resolution of experimental fibrosis[437].

Based therefore on current evidence, it has been stated that autophagy at least in murine hepatocytes is a selective survival mechanism through clearance of excessive fat leading to attenuation of lipotoxicity [438]. This is certainly not the case for HSCs autophagy where lipid droplets are digested to supply energy for the activation of HSCs, promoting thus liver fibrosis. Non specific inhibition of stellate cell autophagy or specific inhibition of Atg5 or Atg7, blocked HSCs activation[439-441]. Lipophagy in HSCs is induced by ER stress[442] and is mediated through Rab25 in a ROS dependent manner as antioxidants were effective in stopping autophagy[87]. In agreement with experimental data, clinical research found that cirrhotic patients had significantly increased levels of several autophagy- related genes compared with non cirrhotics accompanied by increased maturation of lysosomal cathepsin D[85]. Furthermore, serum lipids were evaluated in patients with cirrhosis of viral etiology and compared to non cirrhotics. Low serum lipids were found in HCV and HBV cirrhosis which were negatively correlated with lipophagy[443].

Micro-RNAs interfere with the activation of stellate cells. miR-16 inhibits the expression of guanine nucleotide-binding -subunit 12 (G12) which is overexpressed during fibrogenesis and facilitates Atg12-5 formation, thus activating stellate cells[444]. Also miR-181-5p transferred to mouse HSCs *via* exosomes

from engineered adipose derived stem cells led to inhibition of fibrosis[445].

Several signals can induce autophagy in HSCs[180], including hypoxia-inducible factor-1alpha[446], transforming growth factor 1[447], as well as the danger-associated pattern molecule high-mobility group box-1 (HMGB-1)[448]. Additional signals like ROS-JNK1/2 and the XBP1 arm of the Unfolded Protein Response have also been identified as necessary requirements of HSCs activation through autophagy[449,450]. TGF-β1 has also been reported to mediate autophagy[440]. Similarly, HSCs in cell culture with depleted Atg2A fail to spontaneously trans-differentiate[451]. Quercetin attenuated hepatic fibrosis in mice through inhibition of hepatic HSC activation and autophagy[452].

Selective activation of mitophagy in HSCs also favors fibrosis. PM2.5 is an air pollutant that activates HSCs and initiates liver fibrosis. This is due to increased ROS production and induction of mitophagy through activation of the Pink1/Parkin pathway[453]. In contrast, inhibition of mitophagy was shown to promote inflammation[454] due to dissemination of inflammatory signals from HSCs production of inflammatory cytokines[455]. However very recently it was reported that selective inhibition of mitophagy in macrophages attenuates fibrosis. Mice Kupffer cells from CCL4-induced acute injury showed increased ROS production, activated mitophagy and increased TGF-β1 secretion. T-cell immunoglobulin domain and mucin domain-4 (TIM-4) interference in Kupffer cells inhibited Akt1-mediated ROS production and decreased mitophagy and TGF-β1 secretion through suppression of PINK1/Parkin, to ameliorate CCl4-induced hepatic fibrosis[456]. Seemingly in disagreement with this notion, is the finding that the autophagic proteinp62/SQSTM1, a negative controller of HSC activation is downregulated in trans-differentiating HSCs associated with hepatocellular carcinoma. P62 ablation increases fibrogenesis but this is not related to autophagy but rather to the reduction of p62-dependent activation of the vitamin D receptor (VDR) and the resultant loss of repression of HSC by VDR agonists [457,458].

Even in HSCs the characterization of autophagy as a double-edged sword has been justified. A novel molecular mechanism of selective autophagy in HSCs indicates that autophagy may also protect from liver fibrosis. The RNA-binding protein ELAVL1/HuR plays a crucial role in regulating ferroptosis in liver fibrosis. ELAV1 enhances ferritinophagy leading to ferroptosis of HSCs and attenuation of liver fibrosis[459]. Despite this report, most existing evidence indicate that activation of HSCs autophagy is pro-fibrogenic, therefore a selective block of autophagy in fibrogenic cells might be an attractive future anti-fibrotic therapy[90].

The opposite seems to happen in hepatic macrophages[55] where activation of autophagy is anti-fibrogenic[460]. Mice macrophages with specific deletion of atg5, secreted increased levels of ROS-induced IL-1A and IL-1B. In addition, liver myofibroblasts incubated with the conditioned medium of Atg5(-/-) macrophages expressed increased pro-fibrogenic genes. Attenuation of fibrosis was achieved after IL-1 neutralization indicating that IL1A/B are critical mediators of the profibrotic effects of autophagy inhibition in macrophages[461-463]. Autophagy in Kupffer cells is counteracted by the enzyme monoacylglycerol lipase catalyzing the production of arachidonic acid leading to inflammatory macrophage activation and fibrosis[464].

On the other hand deletion of Atg7 in sinusoidal endothelial cells (LSECs) demonstrated that the selective loss of their autophagy led to cellular dysfunction and decreased intrahepatic nitric oxide. Impairment of autophagy after CCL4-induced acute liver injury in rats, also impaired handling of oxidative stress by LSECs and amplified liver fibrosis[465].

Similarly, autophagy defective sinusoidal endothelial cells (LSECs) as demonstrated in patients with NASH favor advancement of fibrosis[282]. At the same time, even excessive autophagy activation may lead to caveolin-1 degradation, thus worsening the LSECs defenestration and ultimately promoting fibrosis[466]. Therefore, any dysregulation of autophagy in LSECs may aggravate liver fibrosis[467].

An elegant immunofluorescence study of cirrhotic livers linked autophagy with an additional population of fibrogenic cells other than HSCs, the reactive ductular cells (RDC) which were characterized as cholangiocyte-like epithelial cells positive for cytokeratin 19[85]. They are responsible for ductular reaction (DR), a common response to various insults of the liver implicated in the pathogenesis of cirrhosis[432]. Administration of chloroquine, reduced the expression of CK19 positive RDC and blunted liver fibrosis[86]. DR parallels HSC activation in many liver diseases[430]. Reactive ductular cells secrete soluble pro-fibrogenic factors targeting HSCs and myofibroblasts[468]. Recently it was demonstrated that in cirrhotic human livers, RDCs with activated autophagy also had upregulated expression of TGF and fibroblast specific protein-1[469] making autophagy a necessary requirement during the DR process. The role of autophagy in liver fibrosis is therefore complex and the end result depends on the cell population involved. In general, HSCs and RDCs have a pro-fibrogenic effect. On the contrary, autophagy counteracts fibrogenesis acting in hepatocytes, macrophages and LSECs[470].

HCC

The role of autophagy in tumor cell biology has not been fully elucidated. Autophagy has both pro-and anti-tumorigenic roles. For example, it can either inhibit inflammation acting as an anti-oncogen or protect tumor cells from ROS damage acting as a pro-oncogen[471,472].

Opposing effects have been reported. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis[473]. On the other hand, Ras-induced expression of two proteins Noxa and Beclin-1 promotes autophagic cell death, limiting thus the oncogenic potential of deregulated Ras signals[474]. Drugs like ursodexocycholic acid can efficiently eliminate resistant to other drugs cancer cells through induction of autophagic death[475].

HCC is one of the most common types of liver cancer[476]. Most of the HCC cases are accompanied by cirrhosis that results from long-standing chronic inflammation due to viral hepatitis or non-viral etiologies including heavy alcohol intake, NAFLD, autoimmune hepatitis, primary biliary cholangitis, and hemochromatosis[477].

Mice with impaired autophagy are unable to develop HCC even after of strong challenge. This was related to the induction of tumor suppressors like p53[478]. However, after initiation of HCC, the presence of autophagy is required to degrade tumor suppressors promoting thus the development of HCC[86]. Both macroautophagy and CMA are implicated as a double edge sword in liver tumorigenesis [479].

Autophagy has a dual role in hepatocellular carcinoma (HCC). It is an anti-cancer mechanism in the dysplastic stage of HCC initiation, while it favors HCC development and confers resistance to treatment [480,481]. This is possibly due to the maintenance of mitochondrial integrity and protection of cells against oxidative stress during HCC initiation, followed by the downregulation of tumor suppressors to promote the development of HCC[86,482].

In a study of 156 HCC patients increased levels of the autophagy marker LC3B are associated with a dismal prognosis[483]. Higher levels of LC3-II were associated with lymph nodes metastasis, higher vascular invasion and reduced 5-year survival[484].

Macroautophagy may also have an anti-oncogenic function, as reduction of either Atg5 or Atg7 Levels lead to appearance of multiple liver tumors[485]. Similarly, low levels of autophagic proteins and activity are associated with bad prognosis of human HCC[486,487]. Beclin-1 Levels are lower in HCC tissue samples compared to normal tissue from the same patient. Beclin-1 expression was studied in 300 HCC patients. A correlation with disease-free survival and overall survival was found only in the Bcl-xL+ve patients. It was suggested therefore that a synergy of defective autophagy and altered apoptotic activity lead to tumor progression and reduced survival[488].

Inhibition of autophagy leads to the accumulation of SQTSM1/p62. Accumulation of p62 on the one hand may protect from HCC initiation as it blocks the antioxidant functions of nuclear factor erythroid-2- related factor 2 (Nrf2)[489-492]. On the other hand, accumulation of p62 also contributes to carcinogenesis through persistent activation of Nrf2[493]. Nrf2 expression promotes the development of HCC [493]. Deletion of p62 in autophagy defective livers counteracts tumorigenesis. Therefore an accumulation of p62 is partly responsible for the increase in hepatic tumors, *via* the activation of Nrf2[492-494]. The activation of Nrf2 turns glucose and glutamine into anabolic pathways supporting tumor cell proliferation[176,495]. In addition, autophagy inhibits malignant transformation in the liver through Yes associated protein 1 (YAP1) degradation, a protein with a crucial role in hepatic oncogenesis[113,496].

Aberrant activation of the Wnt/β-catenin signaling is another critical pathway in the onset and development of HCC. A recent study reported that the Wnt/β-catenin inhibitors exert anti-tumor effects on HCC cells by regulating autophagy[497]. However this is in disagreement with a previous report where interfering with Wnt secretion in HCC cell lines does not affect autophagy or the level of β-catenin signaling despite cell growth suppression indicating that other mechanisms might underlie the growth-suppressive effect[498].

Furthermore, the activation of autophagy was shown to mediate inhibition of proliferation and induction of apoptosis of hepatoma cell through several mechanisms[499-506]. The induction of autophagy by concanavalin A or different chemotherapeutic drugs in murine livers inhibit hepatoma cell growth and prolongs survival[507-519]. On the other hand suppression of autophagy was reported to enhance the susceptibility of hepatocellular cancer cells towards a variety of chemotherapeutic agents [108,520-529].

Several microRNAs (mirRNAs) have been implicated in HCC tumorigenesis. miR-204 reduces tumor autophagy in HCC[530]. Moreover autophagy degradation of miRNA-224 suppressed the growth of HBV-related HCC[371], while miR-375 which is downregulated in HCC was reported to inhibit autophagy by decreasing the expression of Atg7 and autophagic flux. Up-regulation of miR-375 inhibits mitophagy of HCC cells, reduces the elimination of damaged mitochondria, and decreases cell viability [99]. miR-26 could inhibit autophagy and enhance chemosensitivity of HCC cells[531].

LncRNAs are another set of ncRNAs with a length exceeding 200 nucleotides without translation into proteins[109]. Several lncRNAs, like Hnf1a-as1, Hotair and Hulc promote autophagy and function as oncogenes in HCC[106-109].

The role of mitophagy and lipophagy is also important in HCC growth acting as a double edge sword. Increased mitophagy by concanavalin A, adriamycin or curcumin was shown to suppress hepatoma cell growth[507,510,517,530] while melatonin increased the sensitivity of human hepatoma cells to sorafenib by triggering mitophagy[532]. A recent study also demonstrated that inhibition of inflammasome activation and induction of mitophagy suppressed HCC growth[533]. On the other hand it has been demonstrated that increased mitophagy may facilitate HCC cell survival either through ROS production or attenuation of p53 activity[534,535].

Lipophagy can also act both ways. On the one hand, it can allow tumor cells to have access to a supply of energy critical to their growth[536] and on the other hand, lysosomal acid lipase, the lipase that facilitates lipophagy, exhibits tumor suppressor activity[537]. Lipophagy was also reported to induce apoptosis *in vitro*, *via* induction of ER and mitochondrial stress[538]. CCAAT enhancer binding protein a, a protein that is upregulated in HCC patients, increases resistance to energy starvation and favors carcinogenesis through lipophagy[539].

In addition to the general characteristics of autophagy implication in HCC, there are certain points to be mentioned in specific liver disease associated HCC. As mentioned before, autophagy is activated by the HBx protein[357,369] and this may be related to HBV carcinogenesis. Increased autophagosome formation by HBx was accompanied by decreased degradation of LC3 and SQSTM1/p62 and greatly impaired lysosomal acidification and accumulation of immature Cathepsin D. These data may indicate that repression of lysosomal function by HBx could be important for the initiation and progress of HBV-associated HCC[350].

CMA and cancer metabolism are also interconnected. Once malignant transformation occurs, CMA activity is significantly increased in cancer cells so that the new metabolic requirements are maintained [64]. Blockade of CMA which is upregulated in several cancers reduces progression and metastatic potential of solid tumors because the characteristic increased rates of aerobic glycolysis are reduced in a p53-dependent manner[540]. Macroautophagy and CMA seems to be interconnected and often substitute for one another as in the case of HCC. Under physiological conditions there is no expression of p62 in normal livers pointing to macroautophagy as the main mechanism facilitating cell survival. However in a recent study of 46 cirrhotic livers it was shown that p62 was increased indicating an impairment of macroautophagy, but LAMP-2A and heat shock protein 70 were uniformly increased indicating that an upregulated CMA was trying to compensate for the reduced macroautophagy and therefore promote HCC survival. Moreover, hydroxychloroquine inhibition of lysosomal degradation led to induction of the tumor suppressor p53 and promotion of apoptosis[541]. HCV is also an inducer of HCC. During HCV infection, increased cellular stress has been reported. Severe stress promotes Nrf2 transcription which in turn is responsible for CMA activation resulting in the suppression of hepatic innate immunity and possible degradation of tumor suppressors. The subsequent oncogenic cell programming initiated by a cytoplasmic virus like HCV, has been recently described in detail[542].

Defective autophagy is linked to MAFLD-related HCC, because the accumulated p62/SQSTM1, induces the oncogenic NF-κB activity while retained damaged mitochondria and produced ROS to damage cellular DNA[543]. A novel mechanism was recently reported in ethanol induced liver disease and HCC. Tumor necrosis factor-α-induced protein 8 (TNFAIP8) has been associated with tumor progression in several cancer types including the initiation of HCC. TNFAIP8 induced autophagy in liver cancer cells through blocking of AKT/mTOR signaling and direct interaction with ATtg3-Atg7 proteins. This mechanism is operative in alcohol related liver disease in mice and humans but not in high-fat-fed obese mice or patients with MAFLD[544]. Details of the molecular mechanisms of autophagy in both protection and promotion of HCC were recently published[545-547].

An additional aspect of HCC biology where autophagy plays an important role is the involvement of tumor-associated macrophages and tumor microenvironment. They are polarized after implication of sensing factors from tumor environment and autophagy[130,548]. Deficiency of TLR2 decreased the liver production of TNFα, IFN gamma and IL1a/b accompanied by reduction of autophagy flux and increase in oxidative stress and p62 aggregates in liver tissue. These changes were associated with increased carcinogenesis and progression of HCC[549]. Enhancement of autophagy in tumor-associated macrophages leads to M1 polarization which reduces tumor progression while M2 polarization is permissive for tumorigenesis[550]. The mTOR-TSC2 pathway, a key negative regulator of autophagy, is crucial for macrophage polarization since its activation leads to M2 phenotype. It was recently shown that the coagulants tissue factor (TF) and factor VII (FVII) produced in tumor microenvironment, are implicated in HCC growth promotion by suppression of autophagy mediated through mTOR activation and Atg7[551].

In view of the variable functions of autophagy, there should be an individualized approach of autophagy manipulations for HCC treatment. Thus, various lysosomal inhibitors including chloroquine and hydroxychloroquine have been used as treatment either as sole agents or in combinations with other treatment modalities in a variety of murine HCC models[523,552,553]. Interference with autophagy may be a sound therapeutic option for the treatment of HCC[554,555]. Based on the fact that autophagy is upregulated in metastatic HCC[556] use of autophagy inhibitors like chloroquine and hydrochloroquine in combination with other drugs may be a better option for treating metastatic HCC in humans. A combination of a number of drugs with autophagy inducers have been used to target cancer cells. A combination of percutaneous transarterial chemoembolization with chloroquine, was associated with increased tumor cell necrosis and apoptosis[557] and might counteract the presence of residual hepatocellular carcinoma cells[558,559]. Sorafenib, a multikinase inhibitor approved for HCC treatment, induces autophagy[560] and data show that a combination with autophagy inhibitors increase tumor response[537,561].

Cholangiocarcinoma

Xenografts in nude mice are widely used models of cholangiocarcinoma (CCA). Activated autophagy has been reported in tumor cells from such a model and in specimens from CCA patients[562]. LC3B, Beclin 1, and p62/SQSTM1 expressions were additionally found to be increased at the initial stage of the multistep cholangiocarcinogenesis[563]. However, a lower Beclin 1 expression was associated with metastatic lymph node disease and poor survival of patients with intrahepatic CCA[564,565]. Apoptosis was induced in cholangiocellular cell lines and tumor development was suppressed in a mice xenograft model after interference with autophagy[562]. Similarly, suppression of autophagy by chloroquine increased the chemosensitivity of cisplatin-treated CCA cells[566] and increased apoptosis of CCA cells through ER stress[567]. Chloroquine blockade of autophagy inhibited the tumor growth in Kras/p53 intrahepatic CCA[568,569]. CCA is extremely resistant to chemotherapy. 5-fluorouracil (5-FU) induced autophagy in CCA cells[570] while autophagy inhibition by capsaicin was followed by repression of malignant cell growth[570], indicating that autophagy may be implicated in the multidrug resistance of this tumor. Autophagy was also induced after incubation of CCA cells with the sphingosine kinase 2 inhibitor, ABC294640. Inhibition of autophagy by chloroquine potentiated ABC294640-induced apoptosis[571]. Modulation of autophagy therefore may be helpful in CCA treatment.

INHERITED METABOLIC DISEASES

A1 antitrypsin deficiency and fibrinogen storage disease

Autophagy is also implicated in other types of liver injury like the inherited metabolic diseases. Alpha 1-antitrypsin deficiency is the most extensively studied. Alpha 1-AT is a glycoprotein inhibitor of destructive neutrophil proteases[572,573]. Several naturally occurring mutants of alpha1-AT, have been shown to participate in the pathogenesis of human diseases, such as chronic liver-associated diseases [574-576]. The Z mutation resulting from a single G->A transition in codon 342, generates a mutant protein that forms aggregates in the hepatocytes[577]. Liver injury is caused by the retention of this polymerized mutant alpha1-ATZ molecule in the ER of hepatocytes followed by an induction of autophagic response. Removal of the insoluble alpha-1 anti-trypsin by the autophagosome is the mechanism by which the activation of autophagy protects the liver in alpha1-antitrypsin deficiency[578-581]. In earlier studies, liver injury was associated with mitophagy indicating that the ER retention of alpha(1)-ATZ led to involvement of the mitochondria, with specific patterns of mitochondrial dysfunction and mitochondrial injury[582,583].

Genetic studies in mice have shown that deletion of Atg5 led to an increased retention of alpha 1-ATZ [584] and that deficiency of Atg6 and Atg14 in yeasts inhibited alpha1-ATZ degradation[585]. Similarly, the induction of autophagy in mice by rapamycin reduced liver alpha1-ATZ aggregation and liver injury[582,586-589]. These findings have been repeated and verified when enhancement of autophagy [590] with either carbamazepine[591], gene transfer of the autophagy regulator TFEB[592] or an analog of glibenclamide[593] reduced the toxic protein. Recent preclinical studies have also demonstrated that an exogenous bile acid like norursodeoxycholicacid may be clinically useful in this condition[594,595].

Fibrinogen storage disease is a very rare autosomal-dominant ER storage disease presented with hypofibrinogenemia, elevated transaminases, accumulation of fibrinogen aggregates in the ER of hepatocytes and several fold increase of autophagocytic vacuoles. Some patients progress to cirrhosis similar to alpha-1-AT deficiency. A clinical study of eight patients has showed that administration of carbamazepine at low anticonvulsive dosage led to rapid normalization of alanine-aminotransferase indicating a critical role of autophagy in this disease[596].

Wilson's disease

Wilson disease is an inherited disease of copper metabolism linked to hundreds of mutations in the ATP7B gene[597]. Recent evidence based on studies from hepatocytes of patients and ATP7b deleted mice has shown the presence of an increased number of autophagosomes, indicating the activation of an autophagic response to prevent copper associated cell death[598]. Moreover, inhibition of autophagy accelerated hepatocyte death whereas increased autophagy by either starvation or TFEB overexpression had a cytoprotective effects[598]. Autophagy therefore seems to be a major protective mechanism for hepatocytes in copper accumulation. These findings may lead to the use of autophagy inducers like carbamazepine as a future potential treatment of Wilson's disease.

Glycogen storage disease

Glycogen storage disease type 1a (GSD1a) is an inherited hepatic disease associated with decreased autophagic flux as a consequence of defects in the glucose-6-phosphatase a, that converts glucose-6-phosphate into glucose. These abnormalities lead to glycogen and lipid accumulation in hepatocytes [599]. GSD1a is associated with the down regulation of several components of the autophagy machinery [600].

GSD1a has also been associated with defective sirtuin 1 (SIRT1) signaling leading to impairment of TFEB activity. As in other storage diseases, pharmacological or genetic activation of autophagy reduces the accumulation of glycogen and lipids in cellular and animal models[601].

VARIOUS DISEASES

Autophagy in the liver is implicated in other diseases as well. An important point that should always be remembered is that the liver is the site of almost 80% of body macrophages and therefore innate immunity can be deeply involved in liver and other organ abnormalities through impaired autophagy of Kupffer cells. Sepsis is the main paradigm of this notion.

Sepsis and liver autophagy

Infection can lead to a systemic multi-organ inflammatory response. Macrophages, play a critical role as they are the most important cells of the innate immunity. Autophagy induction is protective in sepsis through regulation of macrophage polarization. Negative regulation of macrophage activation inhibits inflammasome activation[602]. Autophagy also interferes with macrophage apoptosis. Uncontrolled autophagy however may lead to autophagy death of macrophages with additional aggravation of inflammation and the so called cytokine storm[603].A current example is possibly the SARS-Cov-2 pandemic[604]. Interestingly, autophagy-deficient macrophages after LPS stimulation over-secrete macrophage migration inhibitory factor and aggravate inflammation[605]. Other mechanisms are also involved including signaling pathways such as NF-κB, mTOR, and PI3K/AKT[603].

Mitophagy and mitochondrial dysfunction seem to be also a fundamental factor in multiple organ failure caused by sepsis[606]. It has been shown that mtDNA liberated from damaged mitochondria, induces a cascade of inflammatory responses[607-609]. Mitophagy therefore is of great importance for the protection against oxidative stress during sepsis. It should be noted however that mitophagy defects in the liver, are not the only cause of organ or cell damage during sepsis[610]. Nonetheless, the liver is the main organ responsible for sepsis-induced damage[611]. Autophagy is an important protective mechanism in septic liver injury. Increased autophagy can play a protective role in liver function in septic conditions where the activation of autophagy is mediated through activating transcription factor 4 (ATF4). ATF4 is inhibited 48 h after LPS-induced acute liver injury and reversed after obeticholic acid treatment[612]. Autophagy inhibitors or AMPK inhibitors administration reduced the protective mitochondrial function in LPS-induced human hepatocyte injury[613,614]. Mitophagy is also involved in apoptosis of CD4+ve T cells which is the main mechanism of immune inhibition during sepsis. Mitofusin 2 (Mfn2) is a mitochondrial outer membrane protein and a negative regulator of autophagy which is increased in sepsis leading to inhibition of autophagy and increase in apoptosis of CD4ve+ T cells[615]. Autophagy defects can affect antigen presentation by T cells leading to immunosuppression as in the case of Atg5 deficiency[616]. The role of autophagy in sepsis has been recently reviewed[617].

Acetaminophen liver damage

Autophagy is also implicated in acetaminophen induced liver disease. There is evidence that increased autophagy is protective against acetaminophen (APAP)-induced liver damage[618,619]. Pathogenetically, APAP was reported to form APAP-protein adducts in hepatocytes of mice and humans[620]. Adducts localized in mitochondria contribute to APAP-induced mitochondrial dysfunction and subsequent oxidant stress[621,622]. Therefore, it is plausible that removal of APAP-adducts will help to ameliorate APAP-induced mitochondrial damage and maintain hepatocyte integrity[41,623,624]. Experimental evidence indicates that autophagy is mostly responsible for the removal of APAP-adducts[625]. Moreover administration of adiponectin was found to attenuate APAP-induced injury activating AMPK mediated autophagy[626]. Activation of autophagy by rapamycin also attenuates APAP-induced liver injury, whereas inhibition of autophagy by chloroquine or deletion of Atg7 in hepatocytes deteriorates liver damage[153,627]. There is also evidence that autophagy is activated after APAP overdose in specific liver zones[53].

Somewhat different results were recently presented. Unc-51-like autophagy activating kinase 1 and 2 (Ulk1/2) are important autophagy initiation regulators. Unexpectedly, Ulk1/2 double knockout mice have normal autophagic activity after fasting, but are exceptionally resistant to APAP-induced liver injury possibly indicating that autophagy-dependent and independent ULK1/2 pathways have opposing effects in APAP-induced liver injury[628].

Reduction of ROS and repression of apoptosis by autophagy is also essential for hepatic regeneration after APAP-induced acute liver failure[520,627]. A very recent report confirmed that increased autophagy by rapamycin protects mice against APAP hepatotoxicity while chloroquine enhanced liver injury. Importantly it was demonstrated that APAP overdose activated PINK1/Parkin-mediated mitophagy and increased the expression of NF-kB and NLRP3 inflammasome signaling. These findings were reversed by rapamycin and augmented by chloroquine indicating the critical role of mitophagy in APAP hepatotoxicity[629].

Interestingly it was reported that infusion of human amniotic mesenchymal stromal cells ameliorated the APAP liver injury through promotion of Kupffer cell M2 polarization and reduction of Kupffer cell autophagy. These results suggest that Kupffer cell autophagy has an opposite effect on APAP hepatotoxicity compared to hepatocytes. This last observation may be useful for future therapeutic exploitation [630].

Acute liver failure

Acute liver failure (ALF) is a serious syndrome of different etiologies with high mortality[631]. HSCs implication is significant in ALF. Temporarily increased fibrosis in ALF is probably beneficial serving as scaffolding that maintains regenerating hepatocytes and hepatic integrity[437,632,633]. Data from a murine APAP induced ALF model have demonstrated that mortality was significantly increased in HSCs depleted animals[633]. HSCs cannot usually regenerate during ALF due to the submassive necrosis. Autophagy seems to be implicated[634]. The significance of HSCs survival has been verified in a study of patients with HBV induced acute liver failure. ALF was accompanied by fibrosis and HSCs activation and autophagy induction. It was shown for the first time that the High Mobility Group Box 1 (HMGB1) protein is a powerful inducer of autophagy responsible for HSCs survival[635].

As mentioned before, autophagy is crucial for HSCs activation which in turn maintains the liver architecture thus preventing the liver scaffold collapse during ALF.

Nitric oxide induces HSCs apoptosis through generation of ROS[636]. There is evidence however that nitric oxide is also involved in the regulation of autophagy in ALF. Observations in human liver tissue showed an inhibition of autophagy in HSCs while further *in vitro* experiments demonstrated that nitric oxide inhibited autophagy and increased apoptosis of HSCs. These findings were reproduced by chloroquine and reversed by the autophagy inducer rapamycin. Therefore, nitric oxide impairment of HSCs survival may be a decisive factor for the devastating effects of ALF[637].

An additional clinical and experimental study verified the significance of intact mitophagy in ALF. One of the measurements of oxidative stress is the level of superoxide dismutase (SOD). The serum superoxide dismutase was significantly increased in ALF patients, correlating with the MELD-Na score. SOD levels returned to normal in the remission stage of ALF. In liver tissue from ALF patients and mice models, manganese-dependent SOD was overexpressed and mitophagy in HSCs was inhibited by ROS. Inhibition of mitophagy promoted inflammation in HSCs which was reversed by a mitophagy inducer [454].

Acute liver damage

Autophagy also protects hepatocytes from acute liver injury, a characteristic of viral hepatitis and acute alcoholic and non-alcoholic steatohepatitis. Mechanisms and cells involved are different as both direct and indirect effects on hepatocytes and macrophages are implicated. Direct effects include autophagy dependent inhibition of caspase 8 in hepatocytes[638], while indirect effects on macrophages involve limitation of NF-kB-mediated inflammation and inflammasome-dependent IL-1b production through p62-dependent mitophagy[462,639]. Reduced macrophage autophagy can induce pro-inflammatory macrophage polarization and increase the immune mediated acute damage in obese mice[131]. The TAM family of RTKs (receptor tyrosine kinases), which is expressed in macrophages, has been reported to alleviate inflammation. AXL is the only member of the TAM family that induces autophagy in macrophages and ameliorates hepatic inflammatory responses inhibiting the NLRP3 inflammasome activation in murine macrophages[640].

The role of Kupffer cells (Kcs) is significant in the pathogenesis of acute liver injury. In a murine model of thioacetamide induced acute liver injury it was shown that hyperglycemia aggravated the liver lesions activating the NLRP3 inflammasome of Kupffer cells *via* inhibition of AMPK/mTOR-mediated autophagy. Interestingly, AMPK activation or mTOR signaling deletion restored autophagy and subsequently inhibited inflammasome activation in Kupffer cells[641]. Spermine is an anti-oxidative polyamine with autophagy induction properties. In a model of acute liver injury, spermine pretreatment ameliorated liver injury and intrahepatic inflammation by promoting M2 polarization of Kupffer cells.

Furthermore, spermine increased autophagy in KCs. Deletion of Atg5 in spermine treated KCs greatly increased pro-inflammatory cytokines and reduced the anti-inflammatory cytokine IL-10[642].

LSECs are also involved in acute liver injury. Selective impairment of autophagy in liver endothelial cells increases oxidative stress, thus leading to fibrosis in acute injury[465].

Ischemia/reperfusion injury

The central role of autophagy in ischemia/reperfusion injury (I/R) injury has been verified by the fact that pharmacological or genetic stimulation of autophagy ameliorate the liver reperfusion injury[643-645].

I/R impairs hepatocellular autophagy[646] through I/R-induced ATP depletion leading to energy shortage and malfunction of the autophagic machinery. Moreover Ca^{2+} overloading during I/R results in calpain overproduction and ultimate loss of key autophagy proteins like Atg7. Interestingly the autophagy suppressor chloroquine attenuated liver injury when administered in early phases of I/R but aggravated the lesions, as expected, when given in late phases[647].

Hepatic encephalopathy

Ammonia is an important mediator of hepatic encephalopathy. Increased ammonia levels rapidly induce an autophagic response that preferentially targets mitophagy[648-650]. Ammonia induced autophagy may in fact be a protective mechanism against encephalopathy as suggested by a recent report. Deletion of Atg7 or loss of functional TFEB deteriorated ammonia detoxification in mice. By contrast activation of liver autophagy either by rapamycin administration or genetic TFEB expression reduced ammonia levels in acquired hyperammonaemia[651].

Autoimmune hepatitis

The role of autophagy in autoimmune hepatitis (AIH) has not been adequately studied. It is suggested that autophagy is implicated in AIH through its involvement in antigen processing and presentation to T cells[652] and its well proven role in liver fibrosis[653], but the exact pathways have not been delineated.

Concanavalin A-induced hepatitis is an extensively used model for immune-mediated liver injury. Comparative proteomic results in this model have shown that the activation of immune system resulted in hepatitis with deregulation of autophagy as indicated by an increase in p62 and LC3B. Arctigenin is a biologically active lignan with antioxidant and anti-inflammatory properties. Pretreatment with arctigenin alleviated autophagy as well as apoptosis verifying that immunity and autophagy are interconnected in AIH pathogenesis[654].

A group of researchers recently used the same model of concanavalin (conA) induced experimental hepatitis to clarify the role of autophagy in AIH. Methyl prednisolone (MP) treatment significantly decreased inflammation in the liver and activated the Akt/mTOR pathway to inhibit hepatocyte apoptosis and autophagy. Reduced numbers of autophagosomes were present in the MP treated group compared to the conA group. It was further shown that MP attenuated the mitochondria-mediated autophagy and apoptosis[655]. In a second report on the same experimental model, accumulation of mature conventional dendritic cells (cDCs) was observed in the liver. *In vitro*, ConA treatment induced the expression of autophagy proteins and the formation of autophagosomes in dendritic cells. A further blockade of autophagy flux inhibited the maturation of DCs and the proliferation and differentiation of CD4+ T cells when ConA-induced DCs were co-cultured with CD4+ T cells. Taken together these studies elegantly showed that autophagy is critically implicated in AIH and aberrant autophagy and defective maturation of cDCs are involved in AIH immunopathogenesis[656].

A recent clinical study using immunohistochemistry in liver biopsy samples from chronic HCV and AIH patients confirmed the central role of autophagy in AIH. Activated but impaired autophagy and less efficient elimination of damaged mitochondria were demonstrated in AIH as compared with HCV. Increased p62 levels significantly correlated with necroinflammation in AIH[657].

Biliary disease

The mechanisms of liver damage in cholestasis are incompletely understood. Autophagy and protein degradation were shown to be impaired in cholestasis induced in bile duct ligated mice[658-660]. Moreover, defective autophagy after chloroquine inhibition or deletion of Atg7 and Atg5 led to increased cholestatic liver injury[661,662].

Accumulated toxic bile acids lead to ER stress, mitochondrial dysfunction with increased oxidative stress, inflammasome activation and apoptosis leading to liver fibrosis[663]. These events should in fact activate autophagy in cholestasis but instead, at least in mice, it appears that autophagy is inhibited in cholestasis[664,665]. Bile acids can inhibit autophagy in mice either *via* the farnesoid X receptor (FXR) during the feeding-fasting cycle[114,115] or independently of FXR[666]. How autophagy is affected in human cholestasis is under investigation.

In human disease autophagy was initially associated with the pathogenesis of primary biliary cholangitis (PBC)[667-669]. As mentioned before autophagy is also involved in the processing and presentation of various antigens. It is only logical therefore that an interesting hypothesis implicating deregulated cholangiocyte autophagy connected to cholangiocyte senescence has been proposed to explain not only the pathogenesis of PBC but of the other fibrosing cholangiopathies including primary sclerosing cholangitis (PSC) and biliary atresia as well[670].

An upregulation of autophagy was reported along with senescence in PBC[668,671]. LC3B and p62 proteins were accumulated in damaged bile ductular cells in association with senescence markers[68, 125] suggesting that autophagy could induce and facilitate cholangiocyte senescence[664,665,671-674]. Mitophagy may be specifically involved in PBC as granular expression of the mitochondrial protein PDC-E2 was co-localized with LC3[667].

Autophagy has also been implicated in the treatment of PBC. Ursodeoxycholic acid (UDCA) is still the first line treatment of PBC while obeticholic acid (OCA) is a second-line treatment[675-677]. Hydrophobic bile acids, such as glycochenodeoxycholic acid impair autophagy *in vitro* and induce abnormal expression of mitochondrial antigens and cellular senescence in cholangiocytes, possibly through induction of ER stress. Pretreatment with UDCA reduced ER stress and partially restored deregulated autophagy and cellular senescence[678]. It is not clear how UDCA stimulates autophagy. UDCA has been reported to be an FXR antagonist[679] but this may not be the explanation[680]. On the

contrary, OCA is a semi-synthetic FXR agonist with anti-cholestatic functions including the suppression of endogenous bile acid synthesis and interference with hepatocellular bile acid transporter systems [681]. OCA impairs autophagic flux *in vitro* and also *in vivo*. A favorable effect of treatment with OCA in a cholestatic disease like PBC would be incompatible with data, indicating that cholestasis progresses when autophagy is blocked[661,662]. However, the other potent, anti-cholestatic properties of OCA can overcome the negative effects of reduced autophagy.

A recent paper offers an interesting explanation. Autophagy seems to be also impaired in human cholestatic conditions where accumulated bile acids induce Rubicon in an FXR-dependent fashion. Rubicon induction suppresses autophagosome-lysosome fusion and inhibits proper autophagolytic breakdown. Rubicon was also induced after treatment with the FXR agonist OCA. Genetic inhibition of Rubicon reversed the impairment of autophagic flux. In contrast, UDCA reduced Rubicon levels, enhanced autophagic flux and autophagolysosome formation independently of FXR[680].

An overview of autophagy abnormalities is presented in Table 1.

CONCLUSION

Autophagy is an important process through which intracellular parts are degraded in the lysosomes. It is a fine example of effective cellular recycling mechanism, connecting cellular quality control with energy saves. There are three types of autophagy with various pathways of delivery to the lysosomes: Macroautophagy (which is further divided into non selective autophagy and selective macroautophagy targeting special organelles or specific compounds for degradation), microautophagy and chaperon-mediated autophagy. Autophagy is related to major physiologic processes as cell death, inflammation and immunity. It is increasingly recognized that it is implicated in almost every aspect of liver diseases, and this can be the basis for future pathophysiologically based and targeted management.

Table 1 Overview of autophagy abnormalities in liver disease

Disease	Abnormalities of autophagy	Results	Ref.
Obesity	↓Autophagy; Hepatocytes: ↓Mitophagy, ↓Lipophagy; HSCs: ↓Autophagy	↑ER stress, →↑Lipids, ↑Insuline resistance, → Anti-fibrotic	Liu *et al*[203], Lavallard *et al*[204], Gual *et al*[205], Tremblay *et al*[206]
NAFLD	↓Lipophagy; ↓CMA	Lipotoxicity, ↑Lipogenic enzymes	Madrigal-Matute *et al*[30], Zhou *et al*[234], Niso-Santano *et al*[235], Singh *et al*[236]
NASH	Hepatocytes: ↓Autophagy, ↓Mitophagy; Kupffer cells: ↓Autophagy; LSECs: ↓ Autophagy	↑Mallory-Denk bodies, ↑Inflammasome activation; ↑Cathepsins B,D, ↑ M1 polarization, ↓M2 polarization; ↑Inflammation, fibrosis	Xu *et al*[272], Noureddin *et al*[277], Zhang *et al* [285], Dey *et al*[287]
Alcoholic liver disease	Acute ETOH administration: ↑Autophagy, ↑Mitophagy, ↑Lipophagy, ↑Proteophagy; Chronic ETOH administration: ↑ Autophagy (low dose), ↓Autophagy (high dose); Kupffer cells: ↓Autophagy, ↑Autophagy; HSCs: ↓Autophagy, ↑Autophagy	Protection, protection, protection, →Clearance of Mallory-Denk bodies; →Protection, →Mitochondrial damage, Cell death; Liver damage, protection; Reduced fibrosis, increased fibrosis	Chao *et al*[308], Komatsu *et al*[311], Yan *et al*[314], Harada *et al*[318]
HBV	↑Autophagy, ↓Lysosomal acidification, ↑Mitophagy	↑Virus replication, ↓HBV degradation	Li *et al*[356], Tang *et al*[357], Luo *et al*[372], Wang *et al*[383]
HCV	↑Autophagy, ↓Lipophagy, ↑Mitophagy; ↑CMA	↑Virus replication, steatosis, ↑Virus replication, ↓Apoptosis, persitent infection, ↑Virus replication	Ferraris *et al*[387], Paul *et al*[395], Jassey *et al*[404], Ren *et al*[406]
Fibrosis-Cirrhosis	Hepatocytes: ↓Autophagy, ↓Lipophagy; Kupffer cells: ↓Mitophagy, or, ↑↑ Mitophagy; HSCs: ↓Mitophagy, ↓Lipophagy, or, ↑Lipophagy, ↑Mitophagy; LSECs: ↑ ↓Autophagy; Ductular reaction: ↑Autophagy	↑Fibrosis, ↑Lipotoxicity, ↓TGFb, ↓Fibrosis; ↑TGFb, ↑Fibrosis; Pro-inflammatory anti-fibrotic: →Pro-fibrotic, →Pro-fibrotic, ↑Fibrosis, ↑ Fibrosis	Zhang *et al*[437], Singh *et al*[438], Li *et al*[448], Sun *et al*[463]
HCC, "Double edge sword"	Induction stage: ↑CMA, ↑Autophagy; Late stages: ↑Autophagy, or, ↓Autophagy, ↑ Mitophagy, ↑Lipophagy	Anti-oncogenic: ↓YAP1, ↓proliferation, ↑Apoptosis→Anti-oncogenic, ↓ Tumor suppressors; ↑Tumor progression, ↓↑Progression↑↓Progression	Wang *et al*[558], Zhao *et al*[559], Prieto-Domínguez *et al*[560]; Niture *et al*[544], Yang *et al*[547]; Lin *et al*[549], Chen *et al*[550], Chen *et al*[551]
Cholangiocarcinoma	↑Autophagy	↑Tumor progression	Marciniak *et al*[580], Teckman *et al*[581]
A1 antitrypsin deficiency	↓Autophagy		Yamamura *et al*[590], Pastore *et al*[592]
Fibrinogen storage disease	↓Autophagy		Hu *et al*[609]
Wilson'S disease	↓Autophagy		Oami *et al*[611]
Glycogen storage disease	↓Autophagy		Xing *et al*[613]
Sepsis	Kupffer cells: ↑Autophagy, ↑↑Autophagy, ↓Mitophagy	M2 polarization, ↓Inflammasome activation; Kupffer cell apoptosis→Cytokine storm, ↓Apoptosis of CD4+ve T cells	Ying *et al*[615], Neumann *et al*[616], Sun *et al* [628], Shan *et al*[629]
Acetaminophene liver damage	↓Autophagy, ↓Mitophagy, ↑Kupffer cell autophagy	↑APAP-Protein adducts	Sydor *et al*[618], Kim *et al*[643], Biel *et al*[644]
Acute liver failure	↑Autophagy, ↓Autophagy, ↓HSCs Mitophagy	HMGB1→HSCs activation (protective); ↑NO,ROS→↓ HSCs→Devastation	Cheong *et al*[649], Sridhar *et al*[652]
Ischemia/reperfusion injury	↓Autophagy		Kwak *et al*[658], Huang *et al*[659]
Hepatic encephalopathy	↑Autophagy (NH4)	Protection	Woolbright *et al*[663], Manley *et al*[666]

Autoimmune hepatitis	↑Autophagy, ↓ Mitophagy	Defective maturation of dendritic cells	Sasaki *et al*[671], Sasaki *et al*[672], Young *et al*[673]
Biliary disease (experimental)	↓Autophagy	Possibly through increased bile acids	Sasaki *et al*[665], European Association for the Study of the Liver[675], Lindor *et al*[676], Panzitt *et al*[680]
Primary biliay cholangitis	Deregulated autophagy	Cholangiocyte senescence	Van de Graaf *et al*[669], Sasaki *et al*[665], Sasaki *et al*[674]

Note the double edge sword behaviour of autophagy, particularly evident in hepatocellular carcinoma. Autophagy refers to macroautophagy. HSCs: Hepatic stellate cells; LSECs: Liver sinusoidal endothelial cells; CMA: Chaperone mediated autophagy; ER: Enoplamic reticulum; ASH: Acute alcoholic hepatitis.

FOOTNOTES

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REFERENCES

1 **Choi AM**, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med* 2013; **368**: 651-662 [PMID: 23406030 DOI: 10.1056/NEJMra1205406]
2 **Levine B**, Kroemer G. Biological Functions of Autophagy Genes: A Disease Perspective. *Cell* 2019; **176**: 11-42 [PMID: 30633901 DOI: 10.1016/j.cell.2018.09.048]
3 **Schworer CM**, Shiffer KA, Mortimore GE. Quantitative relationship between autophagy and proteolysis during graded

amino acid deprivation in perfused rat liver. *J Biol Chem* 1981; **256**: 7652-7658 [PMID: 7019210 DOI: 10.1016/S0021-9258(19)69010-1]
4 **Mortimore GE**, Hutson NJ, Surmacz CA. Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. *Proc Natl Acad Sci USA* 1983; **80**: 2179-2183 [PMID: 6340116 DOI: 10.1073/pnas.80.8.2179]
5 **Ktistakis NT**. In praise of M. Anselmier who first used the term "autophagie" in 1859. *Autophagy* 2017; **13**: 2015-2017 [PMID: 28837378 DOI: 10.1080/15548627.2017.1367473]
6 **De Duve C**, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem J* 1955; **60**: 604-617 [PMID: 13249955 DOI: 10.1042/bj0600604]
7 **Novikoff AB**, Beaufay H, De Duve C. Electron microscopy of lysosomerich fractions from rat liver. *J Biophys Biochem Cytol* 1956; **2**: 179-184 [PMID: 13357540 DOI: 10.1083/jcb.2.4.179]
8 **Appelmans F**, Wattiaux R, De Duve C. Tissue fractionation studies. 5. The association of acid phosphatase with a special class of cytoplasmic granules in rat liver. *Biochem J* 1955; **59**: 438-445 [PMID: 14363114 DOI: 10.1042/bj0590438]
9 **Essner E**, Novikoff AB. Human hepatocellular pigments and lysosomes. *J Ultrastruct Res* 1960; **3**: 374-391 [PMID: 13820842 DOI: 10.1016/s0022-5320(60)90016-2]
10 **De Duve C**. The lysosome turns fifty. *Nat Cell Biol* 2005; **7**: 847-849 [PMID: 16136179 DOI: 10.1038/ncb0905-847]
11 **Arstila AU**, Trump BF. Studies on cellular autophagocytosis. The formation of autophagic vacuoles in the liver after glucagon administration. *Am J Pathol* 1968; **53**: 687-733 [PMID: 4300890]
12 **Takeshige K**, Baba M, Tsuboi S, Noda T, Ohsumi Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 1992; **119**: 301-311 [PMID: 1400575 DOI: 10.1083/jcb.119.2.301]
13 **Tsukada M**, Ohsumi Y. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. *FEBS Lett* 1993; **333**: 169-174 [PMID: 8224160 DOI: 10.1016/0014-5793(93)80398-e]
14 **Noda T**, Matsuura A, Wada Y, Ohsumi Y. Novel system for monitoring autophagy in the yeast Saccharomyces cerevisiae. *Biochem Biophys Res Commun* 1995; **210**: 126-132 [PMID: 7741731 DOI: 10.1006/bbrc.1995.1636]
15 **Klionsky DJ**, Cregg JM, Dunn WA Jr, Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, Ohsumi Y. A unified nomenclature for yeast autophagy-related genes. *Dev Cell* 2003; **5**: 539-545 [PMID: 14536056 DOI: 10.1016/s1534-5807(03)00296-x]
16 **Klionsky DJ**, Codogno P, Cuervo AM, Deretic V, Elazar Z, Fueyo-Margareto J, Gewirtz DA, Kroemer G, Levine B, Mizushima N, Rubinsztein DC, Thumm M, Tooze SA. A comprehensive glossary of autophagy-related molecules and processes. *Autophagy* 2010; **6**: 438-448 [PMID: 20484971 DOI: 10.4161/auto.6.4.12244]
17 **Klionsky DJ**, Baehrecke EH, Brumell JH, Chu CT, Codogno P, Cuervo AM, Debnath J, Deretic V, Elazar Z, Eskelinen EL, Finkbeiner S, Fueyo-Margareto J, Gewirtz D, Jäättelä M, Kroemer G, Levine B, Melia TJ, Mizushima N, Rubinsztein DC, Simonsen A, Thorburn A, Thumm M, Tooze SA. A comprehensive glossary of autophagy-related molecules and processes (2nd edition). *Autophagy* 2011; **7**: 1273-1294 [PMID: 21997368 DOI: 10.4161/auto.7.11.17661]
18 **Sheng R**, Qin ZH. History and Current Status of Autophagy Research. *Adv Exp Med Biol* 2019; **1206**: 3-37 [PMID: 31776978 DOI: 10.1007/978-981-15-0602-4_1]
19 **Harnett MM**, Pineda MA, Latré de Laté P, Eason RJ, Besteiro S, Harnett W, Langsley G. From Christian de Duve to Yoshinori Ohsumi: More to autophagy than just dining at home. *Biomed J* 2017; **40**: 9-22 [PMID: 28411887 DOI: 10.1016/j.bj.2016.12.004]
20 **Ohsumi Y**. Historical landmarks of autophagy research. *Cell Res* 2014; **24**: 9-23 [PMID: 24366340 DOI: 10.1038/cr.2013.169]
21 **Levine B**, Klionsky DJ. Autophagy wins the 2016 Nobel Prize in Physiology or Medicine: Breakthroughs in baker's yeast fuel advances in biomedical research. *Proc Natl Acad Sci USA* 2017; **114**: 201-205 [PMID: 28039434 DOI: 10.1073/pnas.1619876114]
22 **Mizushima N**. A brief history of autophagy from cell biology to physiology and disease. *Nat Cell Biol* 2018; **20**: 521-527 [PMID: 29686264 DOI: 10.1038/s41556-018-0092-5]
23 **Tooze SA**, Yoshimori T. The origin of the autophagosomal membrane. *Nat Cell Biol* 2010; **12**: 831-835 [PMID: 20811355 DOI: 10.1038/ncb0910-831]
24 **Li Y**, Ding WX. Adipose tissue autophagy and homeostasis in alcohol-induced liver injury. *Liver Res* 2017; **1**: 54-62 [PMID: 29109891 DOI: 10.1016/j.livres.2017.03.004]
25 **Yu L**, Chen Y, Tooze SA. Autophagy pathway: Cellular and molecular mechanisms. *Autophagy* 2018; **14**: 207-215 [PMID: 28933638 DOI: 10.1080/15548627.2017.1378838]
26 **Dikic I**, Elazar Z. Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol* 2018; **19**: 349-364 [PMID: 29618831 DOI: 10.1038/s41580-018-0003-4]
27 **Tanida I**. Autophagosome formation and molecular mechanism of autophagy. *Antioxid Redox Signal* 2011; **14**: 2201-2214 [PMID: 20712405 DOI: 10.1089/ars.2010.3482]
28 **Nakamura S**, Yoshimori T. New insights into autophagosome-lysosome fusion. *J Cell Sci* 2017; **130**: 1209-1216 [PMID: 28302910 DOI: 10.1242/jcs.196352]
29 **Sarkar C**, Zhao Z, Aungst S, Sabirzhanov B, Faden AI, Lipinski MM. Impaired autophagy flux is associated with neuronal cell death after traumatic brain injury. *Autophagy* 2014; **10**: 2208-2222 [PMID: 25484084 DOI: 10.4161/15548627.2014.981787]
30 **Madrigal-Matute J**, Cuervo AM. Regulation of Liver Metabolism by Autophagy. *Gastroenterology* 2016; **150**: 328-339 [PMID: 26453774 DOI: 10.1053/j.gastro.2015.09.042]
31 **Mizushima N**, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol Biol Cell* 2004; **15**: 1101-1111 [PMID: 14699058 DOI: 10.1091/mbc.e03-09-0704]
32 **Tanida I**, Minematsu-Ikeguchi N, Ueno T, Kominami E. Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy* 2005; **1**: 84-91 [PMID: 16874052 DOI: 10.4161/auto.1.2.1697]
33 **Mizushima N**, Yoshimori T, Levine B. Methods in mammalian autophagy research. *Cell* 2010; **140**: 313-326 [PMID:

20144757 DOI: 10.1016/j.cell.2010.01.028]

34 **Klionsky DJ**, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, Ahn HJ, Ait-Mohamed O, Ait-Si-Ali S, Akematsu T, Akira S, Al-Younes HM, Al-Zeer MA, Albert ML, Albin RL, Alegre-Abarrategui J, Aleo MF, Alirezaei M, Almasan A, Almonte-Becerril M, Amano A, Amaravadi R, Amarnath S, Amer AO, Andrieu-Abadie N, Anantharam V, Ann DK, Anoopkumar-Dukie S, Aoki H, Apostolova N, Arancia G, Aris JP, Asanuma K, Asare NY, Ashida H, Askanas V, Askew DS, Auberger P, Baba M, Backues SK, Baehrecke EH, Bahr BA, Bai XY, Bailly Y, Baiocchi R, Baldini G, Balduini W, Ballabio A, Bamber BA, Bampton ET, Bánhegyi G, Bartholomew CR, Bassham DC, Bast RC Jr, Batoko H, Bay BH, Beau I, Béchet DM, Begley TJ, Behl C, Behrends C, Bekri S, Bellaire B, Bendall LJ, Benetti L, Berliocchi L, Bernardi H, Bernassola F, Besteiro S, Bhatia-Kissova I, Bi X, Biard-Piechaczyk M, Blum JS, Boise LH, Bonaldo P, Boone DL, Bornhauser BC, Bortoluci KR, Bossis I, Bost F, Bourquin JP, Boya P, Boyer-Guittaut M, Bozhkov PV, Brady NR, Brancolini C, Brech A, Brenman JE, Brennand A, Bresnick EH, Brest P, Bridges D, Bristol ML, Brookes PS, Brown EJ, Brumell JH, Brunetti-Pierri N, Brunk UT, Bulman DE, Bultman SJ, Bultynck G, Burbulla LF, Bursch W, Butchar JP, Buzgariu W, Bydlowski SP, Cadwell K, Cahová M, Cai D, Cai J, Cai Q, Calabretta B, Calvo-Garrido J, Camougrand N, Campanella M, Campos-Salinas J, Candi E, Cao L, Caplan AB, Carding SR, Cardoso SM, Carew JS, Carlin CR, Carmignac V, Carneiro LA, Carra S, Caruso RA, Casari G, Casas C, Castino R, Cebollero E, Cecconi F, Celli J, Chaachouay H, Chae HJ, Chai CY, Chan DC, Chan EY, Chang RC, Che CM, Chen CC, Chen GC, Chen GQ, Chen M, Chen Q, Chen SS, Chen W, Chen X, Chen X, Chen X, Chen YG, Chen Y, Chen Y, Chen YJ, Chen Z, Cheng A, Cheng CH, Cheng Y, Cheong H, Cheong JH, Cherry S, Chess-Williams R, Cheung ZH, Chevet E, Chiang HL, Chiarelli R, Chiba T, Chin LS, Chiou SH, Chisari FV, Cho CH, Cho DH, Choi AM, Choi D, Choi KS, Choi ME, Chouaib S, Choubey D, Choubey V, Chu CT, Chuang TH, Chueh SH, Chun T, Chwae YJ, Chye ML, Ciarcia R, Ciriolo MR, Clague MJ, Clark RS, Clarke PG, Clarke R, Codogno P, Coller HA, Colombo MI, Comincini S, Condello M, Condorelli F, Cookson MR, Coombs GH, Coppens I, Corbalan R, Cossart P, Costelli P, Costes S, Coto-Montes A, Couve E, Coxon FP, Cregg JM, Crespo JL, Cronjé MJ, Cuervo AM, Cullen JJ, Czaja MJ, D'Amelio M, Darfeuille-Michaud A, Davids LM, Davies FE, De Felici M, de Groot JF, de Haan CA, De Martino L, De Milito A, De Tata V, Debnath J, Degterev A, Dehay B, Delbridge LM, Demarchi F, Deng YZ, Dengjel J, Dent P, Denton D, Deretic V, Desai SD, Devenish RJ, Di Gioacchino M, Di Paolo G, Di Pietro C, Díaz-Araya G, Díaz-Laviada I, Diaz-Meco MT, Diaz-Nido J, Dikic I, Dinesh-Kumar SP, Ding WX, Distelhorst CW, Diwan A, Djavaheri-Mergny M, Dokudovskaya S, Dong Z, Dorsey FC, Dosenko V, Dowling JJ, Doxsey S, Dreux M, Drew ME, Duan Q, Duchosal MA, Duff K, Dugail I, Durbeej M, Duszenko M, Edelstein CL, Edinger AL, Egea G, Eichinger L, Eissa NT, Ekmekcioglu S, El-Deiry WS, Elazar Z, Elgendy M, Ellerby LM, Eng KE, Engelbrecht AM, Engelender S, Erenpreisa J, Escalante R, Esclatine A, Eskelinen EL, Espert L, Espina V, Fan H, Fan J, Fan QW, Fan Z, Fang S, Fang Y, Fanto M, Fanzani A, Farkas T, Farré JC, Faure M, Fechheimer M, Feng CG, Feng J, Feng Q, Feng Y, Fésüs L, Feuer R, Figueiredo-Pereira ME, Fimia GM, Fingar DC, Finkbeiner S, Finkel T, Finley KD, Fiorito F, Fisher EA, Fisher PB, Flajolet M, Florez-McClure ML, Florio S, Fon EA, Fornai F, Fortunato F, Fotedar R, Fowler DH, Fox HS, Franco R, Frankel LB, Fransen M, Fuentes JM, Fueyo J, Fujii J, Fujisaki K, Fujita E, Fukuda M, Furukawa RH, Gaestel M, Gailly P, Gajewska M, Galliot B, Galy V, Ganesh S, Ganetzky B, Ganley IG, Gao FB, Gao GF, Gao J, Garcia L, Garcia-Manero G, Garcia-Marcos M, Garmyn M, Gartel AL, Gatti E, Gautel M, Gawriluk TR, Gegg ME, Geng J, Germain M, Gestwicki JE, Gewirtz DA, Ghavami S, Ghosh P, Giammarioli AM, Giatromanolaki AN, Gibson SB, Gilkerson RW, Ginger ML, Ginsberg HN, Golab J, Goligorsky MS, Golstein P, Gomez-Manzano C, Goncu E, Gongora C, Gonzalez CD, Gonzalez R, González-Estévez C, González-Polo RA, Gonzalez-Rey E, Gorbunov NV, Gorski S, Goruppi S, Gottlieb RA, Gozuacik D, Granato GE, Grant GD, Green KN, Gregorc A, Gros F, Grose C, Grunt TW, Gual P, Guan JL, Guan KL, Guichard SM, Gukovskaya AS, Gukovsky I, Gunst J, Gustafsson AB, Halayko AJ, Hale AN, Halonen SK, Hamasaki M, Han F, Han T, Hancock MK, Hansen M, Harada H, Harada M, Hardt SE, Harper JW, Harris AL, Harris J, Harris SD, Hashimoto M, Haspel JA, Hayashi S, Hazelhurst LA, He C, He YW, Hébert MJ, Heidenreich KA, Helfrich MH, Helgason GV, Henske EP, Herman B, Herman PK, Hetz C, Hilfiker S, Hill JA, Hocking LJ, Hofman P, Hofmann TG, Höhfeld J, Holyoake TL, Hong MH, Hood DA, Hotamisligil GS, Houwerzijl EJ, Høyer-Hansen M, Hu B, Hu CA, Hu HM, Hua Y, Huang C, Huang J, Huang S, Huang WP, Huber TB, Huh WK, Hung TH, Hupp TR, Hur GM, Hurley JB, Hussain SN, Hussey PJ, Hwang JJ, Hwang S, Ichihara A, Ilkhanizadeh S, Inoki K, Into T, Iovane V, Iovanna JL, Ip NY, Isaka Y, Ishida H, Isidoro C, Isobe K, Iwasaki A, Izquierdo M, Izumi Y, Jaakkola PM, Jäättelä M, Jackson GR, Jackson WT, Janji B, Jendrach M, Jeon JH, Jeung EB, Jiang H, Jiang H, Jiang JX, Jiang M, Jiang Q, Jiang X, Jiang X, Jiménez A, Jin M, Jin S, Joe CO, Johansen T, Johnson DE, Johnson GV, Jones NL, Joseph B, Joseph SK, Joubert AM, Juhász G, Juillerat-Jeanneret L, Jung CH, Jung YK, Kaarniranta K, Kaasik A, Kabuta T, Kadowaki M, Kagedal K, Kamada Y, Kaminskyy VO, Kampinga HH, Kanamori H, Kang C, Kang KB, Kang KI, Kang R, Kang YA, Kanki T, Kanneganti TD, Kanno H, Kanthasamy AG, Kanthasamy A, Karantza V, Kaushal GP, Kaushik S, Kawazoe Y, Ke PY, Kehrl JH, Kelekar A, Kerkhoff C, Kessel DH, Khalil H, Kiel JA, Kiger AA, Kihara A, Kim DR, Kim DH, Kim DH, Kim EK, Kim HR, Kim JS, Kim JH, Kim JC, Kim JK, Kim PK, Kim SW, Kim YS, Kim Y, Kimchi A, Kimmelman AC, King JS, Kinsella TJ, Kirkin V, Kirshenbaum LA, Kitamoto K, Kitazato K, Klein L, Klimecki WT, Klucken J, Knecht E, Ko BC, Koch JC, Koga H, Koh JY, Koh YH, Koike M, Komatsu M, Kominami E, Kong HJ, Kong WJ, Korolchuk VI, Kotake Y, Koukourakis MI, Kouri Flores JB, Kovács AL, Kraft C, Krainc D, Krämer H, Kretz-Remy C, Krichevsky AM, Kroemer G, Krüger R, Krut O, Ktistakis NT, Kuan CY, Kucharczyk R, Kumar A, Kumar R, Kumar S, Kundu M, Kung HJ, Kurz T, Kwon HJ, La Spada AR, Lafont F, Lamark T, Landry J, Lane JD, Lapaquette P, Laporte JF, László L, Lavandero S, Lavoie JN, Layfield R, Lazo PA, Le W, Le Cam L, Ledbetter DJ, Lee AJ, Lee BW, Lee GM, Lee J, Lee JH, Lee M, Lee MS, Lee SH, Leeuwenburgh C, Legembre P, Legouis R, Lehmann M, Lei HY, Lei QY, Leib DA, Leiro J, Lemasters JJ, Lemoine A, Lesniak MS, Lev D, Levenson VV, Levine B, Levy E, Li F, Li JL, Li L, Li S, Li W, Li XJ, Li YB, Li YP, Liang C, Liang Q, Liao YF, Liberski PP, Lieberman A, Lim HJ, Lim KL, Lim K, Lin CF, Lin FC, Lin J, Lin JD, Lin K, Lin WW, Lin WC, Lin YL, Linden R, Lingor P, Lippincott-Schwartz J, Lisanti MP, Liton PB, Liu B, Liu CF, Liu K, Liu L, Liu QA, Liu W, Liu YC, Liu Y, Lockshin RA, Lok CN, Lonial S, Loos B, Lopez-Berestein G, López-Otín C, Lossi L, Lotze MT, Lőw P, Lu B, Lu B, Lu B, Lu Z, Luciano F, Lukacs NW, Lund AH, Lynch-Day MA, Ma Y, Macian F, MacKeigan JP, Macleod KF, Madeo F, Maiuri L, Maiuri MC, Malagoli D, Malicdan MC, Malorni W, Man N, Mandelkow EM, Manon S, Manov I, Mao K, Mao

X, Mao Z, Marambaud P, Marazziti D, Marcel YL, Marchbank K, Marchetti P, Marciniak SJ, Marcondes M, Mardi M, Marfe G, Mariño G, Markaki M, Marten MR, Martin SJ, Martinand-Mari C, Martinet W, Martinez-Vicente M, Masini M, Matarrese P, Matsuo S, Matteoni R, Mayer A, Mazure NM, McConkey DJ, McConnell MJ, McDermott C, McDonald C, McInerney GM, McKenna SL, McLaughlin B, McLean PJ, McMaster CR, McQuibban GA, Meijer AJ, Meisler MH, Meléndez A, Melia TJ, Melino G, Mena MA, Menendez JA, Menna-Barreto RF, Menon MB, Menzies FM, Mercer CA, Merighi A, Merry DE, Meschini S, Meyer CG, Meyer TF, Miao CY, Miao JY, Michels PA, Michiels C, Mijaljica D, Milojkovic A, Minucci S, Miracco C, Miranti CK, Mitroulis I, Miyazawa K, Mizushima N, Mograbi B, Mohseni S, Molero X, Mollereau B, Mollinedo F, Momoi T, Monastyrska I, Monick MM, Monteiro MJ, Moore MN, Mora R, Moreau K, Moreira PI, Moriyasu Y, Moscat J, Mostowy S, Mottram JC, Motyl T, Moussa CE, Müller S, Muller S, Münger K, Münz C, Murphy LO, Murphy ME, Musarò A, Mysorekar I, Nagata E, Nagata K, Nahimana A, Nair U, Nakagawa T, Nakahira K, Nakano H, Nakatogawa H, Nanjundan M, Naqvi NI, Narendra DP, Narita M, Navarro M, Nawrocki ST, Nazarko TY, Nemchenko A, Netea MG, Neufeld TP, Ney PA, Nezis IP, Nguyen HP, Nie D, Nishino I, Nislow C, Nixon RA, Noda T, Noegel AA, Nogalska A, Noguchi S, Notterpek L, Novak I, Nozaki T, Nukina N, Nürnberger T, Nyfeler B, Obara K, Oberley TD, Oddo S, Ogawa M, Ohashi T, Okamoto K, Oleinick NL, Oliver FJ, Olsen LJ, Olsson S, Opota O, Osborne TF, Ostrander GK, Otsu K, Ou JH, Ouimet M, Overholtzer M, Ozpolat B, Paganetti P, Pagnini U, Pallet N, Palmer GE, Palumbo C, Pan T, Panaretakis T, Pandey UB, Papackova Z, Papassideri I, Paris I, Park J, Park OK, Parys JB, Parzych KR, Patschan S, Patterson C, Pattingre S, Pawelek JM, Peng J, Perlmutter DH, Perrotta I, Perry G, Pervaiz S, Peter M, Peters GJ, Petersen M, Petrovski G, Phang JM, Piacentini M, Pierre P, Pierrefite-Carle V, Pierron G, Pinkas-Kramarski R, Piras A, Piri N, Platanias LC, Pöggeler S, Poirot M, Poletti A, Poüs C, Pozuelo-Rubio M, Prætorius-Ibba M, Prasad A, Prescott M, Priault M, Produit-Zengaffinen N, Progulske-Fox A, Proikas-Cezanne T, Przedborski S, Przyklenk K, Puertollano R, Puyal J, Qian SB, Qin L, Qin ZH, Quaggin SE, Raben N, Rabinowich H, Rabkin SW, Rahman I, Rami A, Ramm G, Randall G, Randow F, Rao VA, Rathmell JC, Ravikumar B, Ray SK, Reed BH, Reed JC, Reggiori F, Régnier-Vigouroux A, Reichert AS, Reiners JJ Jr, Reiter RJ, Ren J, Revuelta JL, Rhodes CJ, Ritis K, Rizzo E, Robbins J, Roberge M, Roca H, Roccheri MC, Rocchi S, Rodemann HP, Rodríguez de Córdoba S, Rohrer B, Roninson IB, Rosen K, Rost-Roszkowska MM, Rouis M, Rouschop KM, Rovetta F, Rubin BP, Rubinsztein DC, Ruckdeschel K, Rucker EB 3rd, Rudich A, Rudolf E, Ruiz-Opazo N, Russo R, Rusten TE, Ryan KM, Ryter SW, Sabatini DM, Sadoshima J, Saha T, Saitoh T, Sakagami H, Sakai Y, Salekdeh GH, Salomoni P, Salvaterra PM, Salvesen G, Salvioli R, Sanchez AM, Sánchez-Alcázar JA, Sánchez-Prieto R, Sandri M, Sankar U, Sansanwal P, Santambrogio L, Saran S, Sarkar S, Sarwal M, Sasakawa C, Sasnauskiene A, Sass M, Sato K, Sato M, Schapira AH, Scharl M, Schätzl HM, Scheper W, Schiaffino S, Schneider C, Schneider ME, Schneider-Stock R, Schoenlein PV, Schorderet DF, Schüller C, Schwartz GK, Scorrano L, Sealy L, Seglen PO, Segura-Aguilar J, Seiliez I, Seleverstov O, Sell C, Seo JB, Separovic D, Setaluri V, Setoguchi T, Settembre C, Shacka JJ, Shanmugam M, Shapiro IM, Shaulian E, Shaw RJ, Shelhamer JH, Shen HM, Shen WC, Sheng ZH, Shi Y, Shibuya K, Shidoji Y, Shieh JJ, Shih CM, Shimada Y, Shimizu S, Shintani T, Shirihai OS, Shore GC, Sibirny AA, Sidhu SB, Sikorska B, Silva-Zacarin EC, Simmons A, Simon AK, Simon HU, Simone C, Simonsen A, Sinclair DA, Singh R, Sinha D, Sinicrope FA, Sirko A, Siu PM, Sivridis E, Skop V, Skulachev VP, Slack RS, Smaili SS, Smith DR, Soengas MS, Soldati T, Song X, Sood AK, Soong TW, Sotgia F, Spector SA, Spies CD, Springer W, Srinivasula SM, Stefanis L, Steffan JS, Stendel R, Stenmark H, Stephanou A, Stern ST, Sternberg C, Stork B, Strålfors P, Subauste CS, Sui X, Sulzer D, Sun J, Sun SY, Sun ZJ, Sung JJ, Suzuki K, Suzuki T, Swanson MS, Swanton C, Sweeney ST, Sy LK, Szabadkai G, Tabas I, Taegtmeyer H, Tafani M, Takács-Vellai K, Takano Y, Takegawa K, Takemura G, Takeshita F, Talbot NJ, Tan KS, Tanaka K, Tanaka K, Tang D, Tang D, Tanida I, Tannous BA, Tavernarakis N, Taylor GS, Taylor GA, Taylor JP, Terada LS, Terman A, Tettamanti G, Thevissen K, Thompson CB, Thorburn A, Thumm M, Tian F, Tian Y, Tocchini-Valentini G, Tolkovsky AM, Tomino Y, Tönges L, Tooze SA, Tournier C, Tower J, Towns R, Trajkovic V, Travassos LH, Tsai TF, Tschan MP, Tsubata T, Tsung A, Turk B, Turner LS, Tyagi SC, Uchiyama Y, Ueno T, Umekawa M, Umemiya-Shirafuji R, Unni VK, Vaccaro MI, Valente EM, Van den Berghe G, van der Klei IJ, van Doorn W, van Dyk LF, van Egmond M, van Grunsven LA, Vandenabeele P, Vandenberghe WP, Vanhorebeek I, Vaquero EC, Velasco G, Vellai T, Vicencio JM, Vierstra RD, Vila M, Vindis C, Viola G, Viscomi MT, Voitsekhovskaja OV, von Haefen C, Votruba M, Wada K, Wade-Martins R, Walker CL, Walsh CM, Walter J, Wan XB, Wang A, Wang C, Wang D, Wang F, Wang F, Wang G, Wang H, Wang HG, Wang HD, Wang J, Wang K, Wang M, Wang RC, Wang X, Wang X, Wang YJ, Wang Y, Wang Z, Wang ZC, Wang Z, Wansink DG, Ward DM, Watada H, Waters SL, Webster P, Wei L, Weihl CC, Weiss WA, Welford SM, Wen LP, Whitehouse CA, Whitton JL, Whitworth AJ, Wileman T, Wiley JW, Wilkinson S, Willbold D, Williams RL, Williamson PR, Wouters BG, Wu C, Wu DC, Wu WK, Wyttenbach A, Xavier RJ, Xi Z, Xia P, Xiao G, Xie Z, Xie Z, Xu DZ, Xu J, Xu L, Xu X, Yamamoto A, Yamamoto A, Yamashina S, Yamashita M, Yan X, Yanagida M, Yang DS, Yang E, Yang JM, Yang SY, Yang W, Yang WY, Yang Z, Yao MC, Yao TP, Yeganeh B, Yen WL, Yin JJ, Yin XM, Yoo OJ, Yoon G, Yoon SY, Yorimitsu T, Yoshikawa Y, Yoshimori T, Yoshimoto K, You HJ, Youle RJ, Younes A, Yu L, Yu L, Yu SW, Yu WH, Yuan ZM, Yue Z, Yun CH, Yuzaki M, Zabirnyk O, Silva-Zacarin E, Zacks D, Zacksenhaus E, Zaffaroni N, Zakeri Z, Zeh HJ 3rd, Zeitlin SO, Zhang H, Zhang HL, Zhang J, Zhang JP, Zhang L, Zhang L, Zhang MY, Zhang XD, Zhao M, Zhao YF, Zhao Y, Zhao ZJ, Zheng X, Zhivotovsky B, Zhong Q, Zhou CZ, Zhu C, Zhu WG, Zhu XF, Zhu X, Zhu Y, Zoladek T, Zong WX, Zorzano A, Zschocke J, Zuckerbraun B. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 2012; **8**: 445-544 [PMID: 22966490 DOI: 10.4161/auto.19496]

35 **Bjørkøy G**, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol* 2005; **171**: 603-614 [PMID: 16286508 DOI: 10.1083/jcb.200507002]

36 **Mizushima N**, Yoshimori T. How to interpret LC3 immunoblotting. *Autophagy* 2007; **3**: 542-545 [PMID: 17611390 DOI: 10.4161/auto.4600]

37 **Islam MA**, Sooro MA, Zhang P. Autophagic Regulation of p62 is Critical for Cancer Therapy. *Int J Mol Sci* 2018; **19** [PMID: 29738493 DOI: 10.3390/ijms19051405]

38 **Zhang NP**, Liu XJ, Xie L, Shen XZ, Wu J. Impaired mitophagy triggers NLRP3 inflammasome activation during the progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis. *Lab Invest* 2019; **99**: 749-763 [PMID: 30700851

DOI: 10.1038/s41374-018-0177-6]
39 **Mizushima N**, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 2011; **27**: 107-132 [PMID: 21801009 DOI: 10.1146/annurev-cellbio-092910-154005]
40 **Komatsu M**, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiyama Y, Kominami E, Tanaka K. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* 2007; **131**: 1149-1163 [PMID: 18083104 DOI: 10.1016/j.cell.2007.10.035]
41 **Czaja MJ**, Ding WX, Donohue TM Jr, Friedman SL, Kim JS, Komatsu M, Lemasters JJ, Lemoine A, Lin JD, Ou JH, Perlmutter DH, Randall G, Ray RB, Tsung A, Yin XM. Functions of autophagy in normal and diseased liver. *Autophagy* 2013; **9**: 1131-1158 [PMID: 23774882 DOI: 10.4161/auto.25063]
42 **Yin XM**, Ding WX, Gao W. Autophagy in the liver. *Hepatology* 2008; **47**: 1773-1785 [PMID: 18393362 DOI: 10.1002/hep.22146]
43 **Galluzzi L**, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, Choi AM, Chu CT, Codogno P, Colombo MI, Cuervo AM, Debnath J, Deretic V, Dikic I, Eskelinen EL, Fimia GM, Fulda S, Gewirtz DA, Green DR, Hansen M, Harper JW, Jäättelä M, Johansen T, Juhasz G, Kimmelman AC, Kraft C, Ktistakis NT, Kumar S, Levine B, Lopez-Otin C, Madeo F, Martens S, Martinez J, Melendez A, Mizushima N, Münz C, Murphy LO, Penninger JM, Piacentini M, Reggiori F, Rubinsztein DC, Ryan KM, Santambrogio L, Scorrano L, Simon AK, Simon HU, Simonsen A, Tavernarakis N, Tooze SA, Yoshimori T, Yuan J, Yue Z, Zhong Q, Kroemer G. Molecular definitions of autophagy and related processes. *EMBO J* 2017; **36**: 1811-1836 [PMID: 28596378 DOI: 10.15252/embj.201796697]
44 **Kim J**, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 2011; **13**: 132-141 [PMID: 21258367 DOI: 10.1038/ncb2152]
45 **Egan DF**, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, Shaw RJ. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 2011; **331**: 456-461 [PMID: 21205641 DOI: 10.1126/science.1196371]
46 **Angarola B**, Ferguson SM. Coordination of Rheb lysosomal membrane interactions with mTORC1 activation. *F1000Res* 2020; **9** [PMID: 32518628 DOI: 10.12688/f1000research.22367.1]
47 **Raben N**, Puertollano R. TFEB and TFE3: Linking Lysosomes to Cellular Adaptation to Stress. *Annu Rev Cell Dev Biol* 2016; **32**: 255-278 [PMID: 27298091 DOI: 10.1146/annurev-cellbio-111315-125407]
48 **Settembre C**, Ballabio A. TFEB regulates autophagy: an integrated coordination of cellular degradation and recycling processes. *Autophagy* 2011; **7**: 1379-1381 [PMID: 21785263 DOI: 10.4161/auto.7.11.17166]
49 **Settembre C**, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, Sardiello M, Rubinsztein DC, Ballabio A. TFEB links autophagy to lysosomal biogenesis. *Science* 2011; **332**: 1429-1433 [PMID: 21617040 DOI: 10.1126/science.1204592]
50 **Settembre C**, Fraldi A, Medina DL, Ballabio A. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat Rev Mol Cell Biol* 2013; **14**: 283-296 [PMID: 23609508 DOI: 10.1038/nrm3565]
51 **Martina JA**, Chen Y, Gucek M, Puertollano R. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* 2012; **8**: 903-914 [PMID: 22576015 DOI: 10.4161/auto.19653]
52 **Medina DL**, Di Paola S, Peluso I, Armani A, De Stefani D, Venditti R, Montefusco S, Scotto-Rosato A, Prezioso C, Forrester A, Settembre C, Wang W, Gao Q, Xu H, Sandri M, Rizzuto R, De Matteis MA, Ballabio A. Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat Cell Biol* 2015; **17**: 288-299 [PMID: 25720963 DOI: 10.1038/ncb3114]
53 **Chao X**, Wang H, Jaeschke H, Ding WX. Role and mechanisms of autophagy in acetaminophen-induced liver injury. *Liver Int* 2018; **38**: 1363-1374 [PMID: 29682868 DOI: 10.1111/liv.13866]
54 **Santambrogio L**, Cuervo AM. Chasing the elusive mammalian microautophagy. *Autophagy* 2011; **7**: 652-654 [PMID: 21460618 DOI: 10.4161/auto.7.6.15287]
55 **Olsvik HL**, Svenning S, Abudu YP, Brech A, Stenmark H, Johansen T, Mejlvang J. Endosomal microautophagy is an integrated part of the autophagic response to amino acid starvation. *Autophagy* 2019; **15**: 182-183 [PMID: 30295124 DOI: 10.1080/15548627.2018.1532265]
56 **Ke PY**. Diverse Functions of Autophagy in Liver Physiology and Liver Diseases. *Int J Mol Sci* 2019; **20** [PMID: 30642133 DOI: 10.3390/ijms20020300]
57 **Oku M**, Sakai Y. Three Distinct Types of Microautophagy Based on Membrane Dynamics and Molecular Machineries. *Bioessays* 2018; **40**: e1800008 [PMID: 29708272 DOI: 10.1002/bies.201800008]
58 **Kaushik S**, Cuervo AM. The coming of age of chaperone-mediated autophagy. *Nat Rev Mol Cell Biol* 2018; **19**: 365-381 [PMID: 29626215 DOI: 10.1038/s41580-018-0001-6]
59 **Tekirdag K**, Cuervo AM. Chaperone-mediated autophagy and endosomal microautophagy: Joint by a chaperone. *J Biol Chem* 2018; **293**: 5414-5424 [PMID: 29247007 DOI: 10.1074/jbc.R117.818237]
60 **Park C**, Suh Y, Cuervo AM. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. *Nat Commun* 2015; **6**: 6823 [PMID: 25880015 DOI: 10.1038/ncomms7823]
61 **Cuervo AM**, Knecht E, Terlecky SR, Dice JF. Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. *Am J Physiol* 1995; **269**: C1200-C1208 [PMID: 7491910 DOI: 10.1152/ajpcell.1995.269.5.C1200]
62 **Kiffin R**, Christian C, Knecht E, Cuervo AM. Activation of chaperone-mediated autophagy during oxidative stress. *Mol Biol Cell* 2004; **15**: 4829-4840 [PMID: 15331765 DOI: 10.1091/mbc.e04-06-0477]
63 **Dohi E**, Tanaka S, Seki T, Miyagi T, Hide I, Takahashi T, Matsumoto M, Sakai N. Hypoxic stress activates chaperone-mediated autophagy and modulates neuronal cell survival. *Neurochem Int* 2012; **60**: 431-442 [PMID: 22306777 DOI: 10.1016/j.neuint.2012.01.020]
64 **Finn PF**, Dice JF. Ketone bodies stimulate chaperone-mediated autophagy. *J Biol Chem* 2005; **280**: 25864-25870 [PMID: 15883160 DOI: 10.1074/jbc.M502456200]
65 **Ferreira JV**, Fôfo H, Bejarano E, Bento CF, Ramalho JS, Girão H, Pereira P. STUB1/CHIP is required for HIF1A

degradation by chaperone-mediated autophagy. *Autophagy* 2013; **9**: 1349-1366 [PMID: 23880665 DOI: 10.4161/auto.25190]

66 **Singh R**, Cuervo AM. Autophagy in the cellular energetic balance. *Cell Metab* 2011; **13**: 495-504 [PMID: 21531332 DOI: 10.1016/j.cmet.2011.04.004]

67 **Farré JC**, Subramani S. Mechanistic insights into selective autophagy pathways: lessons from yeast. *Nat Rev Mol Cell Biol* 2016; **17**: 537-552 [PMID: 27381245 DOI: 10.1038/nrm.2016.74]

68 **Kraft C**, Deplazes A, Sohrmann M, Peter M. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat Cell Biol* 2008; **10**: 602-610 [PMID: 18391941 DOI: 10.1038/ncb1723]

69 **Dunn WA Jr**, Cregg JM, Kiel JA, van der Klei IJ, Oku M, Sakai Y, Sibirny AA, Stasyk OV, Veenhuis M. Pexophagy: the selective autophagy of peroxisomes. *Autophagy* 2005; **1**: 75-83 [PMID: 16874024 DOI: 10.4161/auto.1.2.1737]

70 **Mancias JD**, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* 2014; **509**: 105-109 [PMID: 24695223 DOI: 10.1038/nature13148]

71 **Nakatogawa H**, Mochida K. Reticulophagy and nucleophagy: New findings and unsolved issues. *Autophagy* 2015; **11**: 2377-2378 [PMID: 26566146 DOI: 10.1080/15548627.2015.1106665]

72 **Singh R**, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. Autophagy regulates lipid metabolism. *Nature* 2009; **458**: 1131-1135 [PMID: 19339967 DOI: 10.1038/nature07976]

73 **Lee S**, Kim JS. Mitophagy: therapeutic potentials for liver disease and beyond. *Toxicol Res* 2014; **30**: 243-250 [PMID: 25584143 DOI: 10.5487/TR.2014.30.4.243]

74 **Khaminets A**, Heinrich T, Mari M, Grumati P, Huebner AK, Akutsu M, Liebmann L, Stolz A, Nietzsche S, Koch N, Mauthe M, Katona I, Qualmann B, Weis J, Reggiori F, Kurth I, Hübner CA, Dikic I. Regulation of endoplasmic reticulum turnover by selective autophagy. *Nature* 2015; **522**: 354-358 [PMID: 26040720 DOI: 10.1038/nature14498]

75 **Mochida K**, Oikawa Y, Kimura Y, Kirisako H, Hirano H, Ohsumi Y, Nakatogawa H. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature* 2015; **522**: 359-362 [PMID: 26040717 DOI: 10.1038/nature14506]

76 **Ellgaard L**, Sevier CS, Bulleid NJ. How Are Proteins Reduced in the Endoplasmic Reticulum? *Trends Biochem Sci* 2018; **43**: 32-43 [PMID: 29153511 DOI: 10.1016/j.tibs.2017.10.006]

77 **Grumati P**, Dikic I, Stolz A. ER-phagy at a glance. *J Cell Sci* 2018; **131** [PMID: 30177506 DOI: 10.1242/jcs.217364]

78 **Gatica D**, Lahiri V, Klionsky DJ. Cargo recognition and degradation by selective autophagy. *Nat Cell Biol* 2018; **20**: 233-242 [PMID: 29476151 DOI: 10.1038/s41556-018-0037-z]

79 **Smith MD**, Harley ME, Kemp AJ, Wills J, Lee M, Arends M, von Kriegsheim A, Behrends C, Wilkinson S. CCPG1 Is a Non-canonical Autophagy Cargo Receptor Essential for ER-Phagy and Pancreatic ER Proteostasis. *Dev Cell* 2018; **44**: 217-232. e11 [PMID: 29290589 DOI: 10.1016/j.devcel.2017.11.024]

80 **Ding WX**, Manley S, Ni HM. The emerging role of autophagy in alcoholic liver disease. *Exp Biol Med (Maywood)* 2011; **236**: 546-556 [PMID: 21478210 DOI: 10.1258/ebm.2011.010360]

81 **Wang L**, Khambu B, Zhang H, Yin XM. Autophagy in alcoholic liver disease, self-eating triggered by drinking. *Clin Res Hepatol Gastroenterol* 2015; **39** Suppl 1: S2-S6 [PMID: 26186877 DOI: 10.1016/j.clinre.2015.05.023]

82 **Zubiete-Franco I**, García-Rodríguez JL, Martínez-Uña M, Martínez-Lopez N, Woodhoo A, Juan VG, Beraza N, Lage-Medina S, Andrade F, Fernandez ML, Aldámiz-Echevarría L, Fernández-Ramos D, Falcon-Perez JM, Lopitz-Otsoa F, Fernandez-Tussy P, Barbier-Torres L, Luka Z, Wagner C, García-Monzón C, Lu SC, Aspichueta P, Mato JM, Martínez-Chantar ML, Varela-Rey M. Methionine and S-adenosylmethionine levels are critical regulators of PP2A activity modulating lipophagy during steatosis. *J Hepatol* 2016; **64**: 409-418 [PMID: 26394163 DOI: 10.1016/j.jhep.2015.08.037]

83 **Xiong J**, Wang K, He J, Zhang G, Zhang D, Chen F. TFE3 Alleviates Hepatic Steatosis through Autophagy-Induced Lipophagy and PGC1α-Mediated Fatty Acid β-Oxidation. *Int J Mol Sci* 2016; **17**: 387 [PMID: 26999124 DOI: 10.3390/ijms17030387]

84 **Chen K**, Yuan R, Zhang Y, Geng S, Li L. Tollip Deficiency Alters Atherosclerosis and Steatosis by Disrupting Lipophagy. *J Am Heart Assoc* 2017; **6** [PMID: 28396568 DOI: 10.1161/JAHA.116.004078]

85 **Hung TM**, Yuan RH, Huang WP, Chen YH, Lin YC, Lin CW, Lai HS, Lee PH. Increased Autophagy Markers Are Associated with Ductular Reaction during the Development of Cirrhosis. *Am J Pathol* 2015; **185**: 2454-2467 [PMID: 26158232 DOI: 10.1016/j.ajpath.2015.05.010]

86 **Tian Y**, Kuo CF, Sir D, Wang L, Govindarajan S, Petrovic LM, Ou JH. Autophagy inhibits oxidative stress and tumor suppressors to exert its dual effect on hepatocarcinogenesis. *Cell Death Differ* 2015; **22**: 1025-1034 [PMID: 25526090 DOI: 10.1038/cdd.2014.201]

87 **Zhang Z**, Zhao S, Yao Z, Wang L, Shao J, Chen A, Zhang F, Zheng S. Autophagy regulates turnover of lipid droplets *via* ROS-dependent Rab25 activation in hepatic stellate cell. *Redox Biol* 2017; **11**: 322-334 [PMID: 28038427 DOI: 10.1016/j.redox.2016.12.021]

88 **Martinez-Lopez N**, Singh R. Autophagy and Lipid Droplets in the Liver. *Annu Rev Nutr* 2015; **35**: 215-237 [PMID: 26076903 DOI: 10.1146/annurev-nutr-071813-105336]

89 **Schulze RJ**, Sathyanarayan A, Mashek DG. Breaking fat: The regulation and mechanisms of lipophagy. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017; **1862**: 1178-1187 [PMID: 28642194 DOI: 10.1016/j.bbalip.2017.06.008]

90 **Zhang Z**, Yao Z, Chen Y, Qian L, Jiang S, Zhou J, Shao J, Chen A, Zhang F, Zheng S. Lipophagy and liver disease: New perspectives to better understanding and therapy. *Biomed Pharmacother* 2018; **97**: 339-348 [PMID: 29091883 DOI: 10.1016/j.biopha.2017.07.168]

91 **Kounakis K**, Chaniotakis M, Markaki M, Tavernarakis N. Emerging Roles of Lipophagy in Health and Disease. *Front Cell Dev Biol* 2019; **7**: 185 [PMID: 31552248 DOI: 10.3389/fcell.2019.00185]

92 **Ding WX**, Ni HM, Li M, Liao Y, Chen X, Stolz DB, Dorn GW 2nd, Yin XM. Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. *J Biol Chem* 2010; **285**: 27879-27890 [PMID: 20573959 DOI: 10.1074/jbc.M110.119537]

93 **Ding WX**, Yin XM. Mitophagy: mechanisms, pathophysiological roles, and analysis. *Biol Chem* 2012; **393**: 547-564

[PMID: 22944659 DOI: 10.1515/hsz-2012-0119]
94 **Menzies RA**, Gold PH. The turnover of mitochondria in a variety of tissues of young adult and aged rats. *J Biol Chem* 1971; **246**: 2425-2429 [PMID: 5553400 DOI: 10.1016/S0021-9258(18)62305-1]
95 **Pfeifer U**. Inhibition by insulin of the formation of autophagic vacuoles in rat liver. A morphometric approach to the kinetics of intracellular degradation by autophagy. *J Cell Biol* 1978; **78**: 152-167 [PMID: 670291 DOI: 10.1083/jcb.78.1.152]
96 **Lemasters JJ**. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res* 2005; **8**: 3-5 [PMID: 15798367 DOI: 10.1089/rej.2005.8.3]
97 **Kim I**, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys* 2007; **462**: 245-253 [PMID: 17475204 DOI: 10.1016/j.abb.2007.03.034]
98 **Mizushima N**, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008; **451**: 1069-1075 [PMID: 18305538 DOI: 10.1038/nature06639]
99 **Youle RJ**, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* 2011; **12**: 9-14 [PMID: 21179058 DOI: 10.1038/nrm3028]
100 **Zhao L**, Li H, Wang Y, Zheng A, Cao L, Liu J. Autophagy Deficiency Leads to Impaired Antioxidant Defense *via* p62-FOXO1/3 Axis. *Oxid Med Cell Longev* 2019; **2019**: 2526314 [PMID: 31949875 DOI: 10.1155/2019/2526314]
101 **Lemasters JJ**. Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). *Redox Biol* 2014; **2**: 749-754 [PMID: 25009776 DOI: 10.1016/j.redox.2014.06.004]
102 **Flores-Toro JA**, Go KL, Leeuwenburgh C, Kim JS. Autophagy in the liver: cell's cannibalism and beyond. *Arch Pharm Res* 2016; **39**: 1050-1061 [PMID: 27515049 DOI: 10.1007/s12272-016-0807-8]
103 **Ke PY**. Mitophagy in the Pathogenesis of Liver Diseases. *Cells* 2020; **9** [PMID: 32235615 DOI: 10.3390/cells9040831]
104 **Panzitt K**, Tschernatsch MM, Guelly C, Moustafa T, Stradner M, Strohmaier HM, Buck CR, Denk H, Schroeder R, Trauner M, Zatloukal K. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. *Gastroenterology* 2007; **132**: 330-342 [PMID: 17241883 DOI: 10.1053/j.gastro.2006.08.026]
105 **Chen CL**, Tseng YW, Wu JC, Chen GY, Lin KC, Hwang SM, Hu YC. Suppression of hepatocellular carcinoma by baculovirus-mediated expression of long non-coding RNA PTENP1 and MicroRNA regulation. *Biomaterials* 2015; **44**: 71-81 [PMID: 25617127 DOI: 10.1016/j.biomaterials.2014.12.023]
106 **Liu Z**, Wei X, Zhang A, Li C, Bai J, Dong J. Long non-coding RNA HNF1A-AS1 functioned as an oncogene and autophagy promoter in hepatocellular carcinoma through sponging hsa-miR-30b-5p. *Biochem Biophys Res Commun* 2016; **473**: 1268-1275 [PMID: 27084450 DOI: 10.1016/j.bbrc.2016.04.054]
107 **Yang L**, Zhang X, Li H, Liu J. The long noncoding RNA HOTAIR activates autophagy by upregulating ATG3 and ATG7 in hepatocellular carcinoma. *Mol Biosyst* 2016; **12**: 2605-2612 [PMID: 27301338 DOI: 10.1039/c6mb00114a]
108 **Xiong H**, Ni Z, He J, Jiang S, Li X, He J, Gong W, Zheng L, Chen S, Li B, Zhang N, Lyu X, Huang G, Chen B, Zhang Y, He F. LncRNA HULC triggers autophagy *via* stabilizing Sirt1 and attenuates the chemosensitivity of HCC cells. *Oncogene* 2017; **36**: 3528-3540 [PMID: 28166203 DOI: 10.1038/onc.2016.521]
109 **Islam Khan MZ**, Tam SY, Law HKW. Autophagy-Modulating Long Non-coding RNAs (LncRNAs) and Their Molecular Events in Cancer. *Front Genet* 2018; **9**: 750 [PMID: 30693021 DOI: 10.3389/fgene.2018.00750]
110 **Horos R**, Büscher M, Kleinendorst R, Alleaume AM, Tarafder AK, Schwarzl T, Dziuba D, Tischer C, Zielonka EM, Adak A, Castello A, Huber W, Sachse C, Hentze MW. The Small Non-coding Vault RNA1-1 Acts as a Riboregulator of Autophagy. *Cell* 2019; **176**: 1054-1067. e12 [PMID: 30773316 DOI: 10.1016/j.cell.2019.01.030]
111 **Zhao Y**, Wang Z, Zhang W, Zhang L. MicroRNAs play an essential role in autophagy regulation in various disease phenotypes. *Biofactors* 2019; **45**: 844-856 [PMID: 31418958 DOI: 10.1002/biof.1555]
112 **Khambu B**, Huda N, Chen X, Antoine DJ, Li Y, Dai G, Köhler UA, Zong WX, Waguri S, Werner S, Oury TD, Dong Z, Yin XM. HMGB1 promotes ductular reaction and tumorigenesis in autophagy-deficient livers. *J Clin Invest* 2018; **128**: 2419-2435 [PMID: 29558368 DOI: 10.1172/JCI91814]
113 **Lee YA**, Noon LA, Akat KM, Ybanez MD, Lee TF, Berres ML, Fujiwara N, Goossens N, Chou HI, Parvin-Nejad FP, Khambu B, Kramer EGM, Gordon R, Pfleger C, Germain D, John GR, Campbell KN, Yue Z, Yin XM, Cuervo AM, Czaja MJ, Fiel MI, Hoshida Y, Friedman SL. Autophagy is a gatekeeper of hepatic differentiation and carcinogenesis by controlling the degradation of Yap. *Nat Commun* 2018; **9**: 4962 [PMID: 30470740 DOI: 10.1038/s41467-018-07338-z]
114 **Lee JM**, Wagner M, Xiao R, Kim KH, Feng D, Lazar MA, Moore DD. Nutrient-sensing nuclear receptors coordinate autophagy. *Nature* 2014; **516**: 112-115 [PMID: 25383539 DOI: 10.1038/nature13961]
115 **Seok S**, Fu T, Choi SE, Li Y, Zhu R, Kumar S, Sun X, Yoon G, Kang Y, Zhong W, Ma J, Kemper B, Kemper JK. Transcriptional regulation of autophagy by an FXR-CREB axis. *Nature* 2014; **516**: 108-111 [PMID: 25383523 DOI: 10.1038/nature13949]
116 **Kim H**, Williams D, Qiu Y, Song Z, Yang Z, Kimler V, Goldberg A, Zhang R, Yang Z, Chen X, Wang L, Fang D, Lin JD, Zhang K. Regulation of hepatic autophagy by stress-sensing transcription factor CREBH. *FASEB J* 2019; **33**: 7896-7914 [PMID: 30912978 DOI: 10.1096/fj.201802528R]
117 **Xu J**, Camfield R, Gorski SM. The interplay between exosomes and autophagy - partners in crime. *J Cell Sci* 2018; **131** [PMID: 30076239 DOI: 10.1242/jcs.215210]
118 **Zheng J**, Tan J, Miao YY, Zhang Q. Extracellular vesicles degradation pathway based autophagy lysosome pathway. *Am J Transl Res* 2019; **11**: 1170-1183 [PMID: 30972154]
119 **Yu L**, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, Mi N, Zhao Y, Liu Z, Wan F, Hailey DW, Oorschot V, Klumperman J, Baehrecke EH, Lenardo MJ. Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature* 2010; **465**: 942-946 [PMID: 20526321 DOI: 10.1038/nature09076]
120 **Chen Y**, Yu L. Recent progress in autophagic lysosome reformation. *Traffic* 2017; **18**: 358-361 [PMID: 28371052 DOI: 10.1111/tra.12484]
121 **Chen Y**, Yu L. Development of Research into Autophagic Lysosome Reformation. *Mol Cells* 2018; **41**: 45-49 [PMID: 29370688 DOI: 10.14348/molcells.2018.2265]

122 **Buratta S**, Tancini B, Sagini K, Delo F, Chiaradia E, Urbanelli L, Emiliani C. Lysosomal Exocytosis, Exosome Release and Secretory Autophagy: The Autophagic- and Endo-Lysosomal Systems Go Extracellular. *Int J Mol Sci* 2020; **21** [PMID: 32276321 DOI: 10.3390/ijms21072576]

123 **Galluzzi L**, Green DR. Autophagy-Independent Functions of the Autophagy Machinery. *Cell* 2019; **177**: 1682-1699 [PMID: 31199916 DOI: 10.1016/j.cell.2019.05.026]

124 **Virgin HW**, Levine B. Autophagy genes in immunity. *Nat Immunol* 2009; **10**: 461-470 [PMID: 19381141 DOI: 10.1038/ni.1726]

125 **Saitoh T**, Akira S. Regulation of innate immune responses by autophagy-related proteins. *J Cell Biol* 2010; **189**: 925-935 [PMID: 20548099 DOI: 10.1083/jcb.201002021]

126 **Levine B**, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* 2011; **469**: 323-335 [PMID: 21248839 DOI: 10.1038/nature09782]

127 **Mihalache CC**, Simon HU. Autophagy regulation in macrophages and neutrophils. *Exp Cell Res* 2012; **318**: 1187-1192 [PMID: 22245582 DOI: 10.1016/j.yexcr.2011.12.021]

128 **Shi CS**, Shenderov K, Huang NN, Kabat J, Abu-Asab M, Fitzgerald KA, Sher A, Kehrl JH. Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. *Nat Immunol* 2012; **13**: 255-263 [PMID: 22286270 DOI: 10.1038/ni.2215]

129 **Puleston DJ**, Simon AK. Autophagy in the immune system. *Immunology* 2014; **141**: 1-8 [PMID: 23991647 DOI: 10.1111/imm.12165]

130 **Chen P**, Cescon M, Bonaldo P. Autophagy-mediated regulation of macrophages and its applications for cancer. *Autophagy* 2014; **10**: 192-200 [PMID: 24300480 DOI: 10.4161/auto.26927]

131 **Liu K**, Zhao E, Ilyas G, Lalazar G, Lin Y, Haseeb M, Tanaka KE, Czaja MJ. Impaired macrophage autophagy increases the immune response in obese mice by promoting proinflammatory macrophage polarization. *Autophagy* 2015; **11**: 271-284 [PMID: 25650776 DOI: 10.1080/15548627.2015.1009787]

132 **Codogno P**, Mehrpour M, Proikas-Cezanne T. Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat Rev Mol Cell Biol* 2011; **13**: 7-12 [PMID: 22166994 DOI: 10.1038/nrm3249]

133 **Heckmann BL**, Boada-Romero E, Cunha LD, Magne J, Green DR. LC3-Associated Phagocytosis and Inflammation. *J Mol Biol* 2017; **429**: 3561-3576 [PMID: 28847720 DOI: 10.1016/j.jmb.2017.08.012]

134 **Martinez J**. LAP it up, fuzz ball: a short history of LC3-associated phagocytosis. *Curr Opin Immunol* 2018; **55**: 54-61 [PMID: 30286399 DOI: 10.1016/j.coi.2018.09.011]

135 **Münz C**. Non-canonical Functions of Macroautophagy Proteins During Endocytosis by Myeloid Antigen Presenting Cells. *Front Immunol* 2018; **9**: 2765 [PMID: 30542350 DOI: 10.3389/fimmu.2018.02765]

136 **Schneider JL**, Cuervo AM. Liver autophagy: much more than just taking out the trash. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 187-200 [PMID: 24192609 DOI: 10.1038/nrgastro.2013.211]

137 **Cuervo AM**, Macian F. Autophagy and the immune function in aging. *Curr Opin Immunol* 2014; **29**: 97-104 [PMID: 24929664 DOI: 10.1016/j.coi.2014.05.006]

138 **Gutierrez MG**, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 2004; **119**: 753-766 [PMID: 15607973 DOI: 10.1016/j.cell.2004.11.038]

139 **Czaja MJ**. Function of Autophagy in Nonalcoholic Fatty Liver Disease. *Dig Dis Sci* 2016; **61**: 1304-1313 [PMID: 26725058 DOI: 10.1007/s10620-015-4025-x]

140 **Wei J**, Long L, Yang K, Guy C, Shrestha S, Chen Z, Wu C, Vogel P, Neale G, Green DR, Chi H. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. *Nat Immunol* 2016; **17**: 277-285 [PMID: 26808230 DOI: 10.1038/ni.3365]

141 **Peral de Castro C**, Jones SA, Ní Cheallaigh C, Hearnden CA, Williams L, Winter J, Lavelle EC, Mills KH, Harris J. Autophagy regulates IL-23 secretion and innate T cell responses through effects on IL-1 secretion. *J Immunol* 2012; **189**: 4144-4153 [PMID: 22972933 DOI: 10.4049/jimmunol.1201946]

142 **Kroemer G**, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; **40**: 280-293 [PMID: 20965422 DOI: 10.1016/j.molcel.2010.09.023]

143 **Amelio I**, Melino G, Knight RA. Cell death pathology: cross-talk with autophagy and its clinical implications. *Biochem Biophys Res Commun* 2011; **414**: 277-281 [PMID: 21963447 DOI: 10.1016/j.bbrc.2011.09.080]

144 **Yonekawa T**, Thorburn A. Autophagy and cell death. *Essays Biochem* 2013; **55**: 105-117 [PMID: 24070475 DOI: 10.1042/bse0550105]

145 **Boya P**, González-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, Métivier D, Meley D, Souquere S, Yoshimori T, Pierron G, Codogno P, Kroemer G. Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol* 2005; **25**: 1025-1040 [PMID: 15657430 DOI: 10.1128/MCB.25.3.1025-1040.2005]

146 **Maiuri MC**, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007; **8**: 741-752 [PMID: 17717517 DOI: 10.1038/nrm2239]

147 **Eisenberg-Lerner A**, Bialik S, Simon HU, Kimchi A. Life and death partners: apoptosis, autophagy and the cross-talk between them. *Cell Death Differ* 2009; **16**: 966-975 [PMID: 19325568 DOI: 10.1038/cdd.2009.33]

148 **Nikoletopoulou V**, Markaki M, Palikaras K, Tavernarakis N. Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta* 2013; **1833**: 3448-3459 [PMID: 23770045 DOI: 10.1016/j.bbamcr.2013.06.001]

149 **Wang K**. Autophagy and apoptosis in liver injury. *Cell Cycle* 2015; **14**: 1631-1642 [PMID: 25927598 DOI: 10.1080/15384101.2015.1038685]

150 **Baehrecke EH**. Autophagy: dual roles in life and death? *Nat Rev Mol Cell Biol* 2005; **6**: 505-510 [PMID: 15928714 DOI: 10.1038/nrm1666]

151 **Levine B**, Yuan J. Autophagy in cell death: an innocent convict? *J Clin Invest* 2005; **115**: 2679-2688 [PMID: 16200202 DOI: 10.1172/JCI26390]

152 **Mariño G**, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2014; **15**: 81-94 [PMID: 24401948 DOI: 10.1038/nrm3735]

153 **Ni HM**, Bockus A, Boggess N, Jaeschke H, Ding WX. Activation of autophagy protects against acetaminophen-induced hepatotoxicity. *Hepatology* 2012; **55**: 222-232 [PMID: 21932416 DOI: 10.1002/hep.24690]

154 **Lockshin RA**, Zakeri Z. Cell death in health and disease. *J Cell Mol Med* 2007; **11**: 1214-1224 [PMID: 18031301 DOI: 10.1111/j.1582-4934.2007.00150.x]

155 **Strozyk E**, Kulms D. The role of AKT/mTOR pathway in stress response to UV-irradiation: implication in skin carcinogenesis by regulation of apoptosis, autophagy and senescence. *Int J Mol Sci* 2013; **14**: 15260-15285 [PMID: 23887651 DOI: 10.3390/ijms140815260]

156 **Liang Q**, Xiao Y, Liu K, Zhong C, Zeng M, Xiao F. Cr(VI)-Induced Autophagy Protects L-02 Hepatocytes from Apoptosis Through the ROS-AKT-mTOR Pathway. *Cell Physiol Biochem* 2018; **51**: 1863-1878 [PMID: 30504711 DOI: 10.1159/000495713]

157 **Zhou B**, Liu J, Kang R, Klionsky DJ, Kroemer G, Tang D. Ferroptosis is a type of autophagy-dependent cell death. *Semin Cancer Biol* 2020; **66**: 89-100 [PMID: 30880243 DOI: 10.1016/j.semcancer.2019.03.002]

158 **Liu J**, Kuang F, Kroemer G, Klionsky DJ, Kang R, Tang D. Autophagy-Dependent Ferroptosis: Machinery and Regulation. *Cell Chem Biol* 2020; **27**: 420-435 [PMID: 32160513 DOI: 10.1016/j.chembiol.2020.02.005]

159 **Gao M**, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. *Cell Res* 2016; **26**: 1021-1032 [PMID: 27514700 DOI: 10.1038/cr.2016.95]

160 **Hou W**, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ 3rd, Kang R, Tang D. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 2016; **12**: 1425-1428 [PMID: 27245739 DOI: 10.1080/15548627.2016.1187366]

161 **Kang R**, Tang D. Autophagy and Ferroptosis - What's the Connection? *Curr Pathobiol Rep* 2017; **5**: 153-159 [PMID: 29038744 DOI: 10.1007/s40139-017-0139-5]

162 **Bai Y**, Meng L, Han L, Jia Y, Zhao Y, Gao H, Kang R, Wang X, Tang D, Dai E. Lipid storage and lipophagy regulates ferroptosis. *Biochem Biophys Res Commun* 2019; **508**: 997-1003 [PMID: 30545638 DOI: 10.1016/j.bbrc.2018.12.039]

163 **Guo H**, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med* 2015; **21**: 677-687 [PMID: 26121197 DOI: 10.1038/nm.3893]

164 **Broz P**, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 2016; **16**: 407-420 [PMID: 27291964 DOI: 10.1038/nri.2016.58]

165 **Shibutani ST**, Saitoh T, Nowag H, Münz C, Yoshimori T. Autophagy and autophagy-related proteins in the immune system. *Nat Immunol* 2015; **16**: 1014-1024 [PMID: 26382870 DOI: 10.1038/ni.3273]

166 **de Lavera I**, Pavon AD, Paz MV, Oropesa-Avila M, de la Mata M, Alcocer-Gomez E, Garrido-Maraver J, Cotan D, Alvarez-Cordoba M, Sanchez-Alcazar JA. The Connections Among Autophagy, Inflammasome and Mitochondria. *Curr Drug Targets* 2017; **18**: 1030-1038 [PMID: 27231105 DOI: 10.2174/1389450117666160527143143]

167 **Sanjuan MA**, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, Komatsu M, Tanaka K, Cleveland JL, Withoff S, Green DR. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 2007; **450**: 1253-1257 [PMID: 18097414 DOI: 10.1038/nature06421]

168 **Yordy B**, Iwasaki A. Autophagy in the control and pathogenesis of viral infection. *Curr Opin Virol* 2011; **1**: 196-203 [PMID: 21927636 DOI: 10.1016/j.coviro.2011.05.016]

169 **Oh JE**, Lee HK. Autophagy as an innate immune modulator. *Immune Netw* 2013; **13**: 1-9 [PMID: 23559894 DOI: 10.4110/in.2013.13.1.1]

170 **Schroder K**, Tschopp J. The inflammasomes. *Cell* 2010; **140**: 821-832 [PMID: 20303873 DOI: 10.1016/j.cell.2010.01.040]

171 **Wang Z**, Zhang S, Xiao Y, Zhang W, Wu S, Qin T, Yue Y, Qian W, Li L. NLRP3 Inflammasome and Inflammatory Diseases. *Oxid Med Cell Longev* 2020; **2020**: 4063562 [PMID: 32148650 DOI: 10.1155/2020/4063562]

172 **Thorburn A**. Autophagy and its effects: making sense of double-edged swords. *PLoS Biol* 2014; **12**: e1001967 [PMID: 25313680 DOI: 10.1371/journal.pbio.1001967]

173 **White E**. Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer* 2012; **12**: 401-410 [PMID: 22534666 DOI: 10.1038/nrc3262]

174 **Amaravadi R**, Kimmelman AC, White E. Recent insights into the function of autophagy in cancer. *Genes Dev* 2016; **30**: 1913-1930 [PMID: 27664235 DOI: 10.1101/gad.287524.116]

175 **Deretic V**, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* 2013; **13**: 722-737 [PMID: 24064518 DOI: 10.1038/nri3532]

176 **Codogno P**, Meijer AJ. Autophagy in the liver. *J Hepatol* 2013; **59**: 389-391 [PMID: 23669287 DOI: 10.1016/j.jhep.2013.02.031]

177 **Gual P**, Gilgenkrantz H, Lotersztajn S. Autophagy in chronic liver diseases: the two faces of Janus. *Am J Physiol Cell Physiol* 2017; **312**: C263-C273 [PMID: 27903585 DOI: 10.1152/ajpcell.00295.2016]

178 **Ueno T**, Komatsu M. Autophagy in the liver: functions in health and disease. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 170-184 [PMID: 28053338 DOI: 10.1038/nrgastro.2016.185]

179 **Weiskirchen R**, Tacke F. Relevance of Autophagy in Parenchymal and Non-Parenchymal Liver Cells for Health and Disease. *Cells* 2019; **8** [PMID: 30609663 DOI: 10.3390/cells8010016]

180 **Allaire M**, Rautou PE, Codogno P, Lotersztajn S. Autophagy in liver diseases: Time for translation? *J Hepatol* 2019; **70**: 985-998 [PMID: 30711404 DOI: 10.1016/j.jhep.2019.01.026]

181 **Yan S**, Huda N, Khambu B, Yin XM. Relevance of autophagy to fatty liver diseases and potential therapeutic applications. *Amino Acids* 2017; **49**: 1965-1979 [PMID: 28478585 DOI: 10.1007/s00726-017-2429-y]

182 **Khambu B**, Yan S, Huda N, Liu G, Yin XM. Autophagy in non-alcoholic fatty liver disease and alcoholic liver disease. *Liver Res* 2018; **2**: 112-119 [PMID: 31123622 DOI: 10.1016/j.livres.2018.09.004]

183 **Khambu B**, Yan S, Huda N, Liu G, Yin XM. Homeostatic Role of Autophagy in Hepatocytes. *Semin Liver Dis* 2018; **38**: 308-319 [PMID: 30357768 DOI: 10.1055/s-0038-1669939]

184 **Hazari Y**, Bravo-San Pedro JM, Hetz C, Galluzzi L, Kroemer G. Autophagy in hepatic adaptation to stress. *J Hepatol* 2020; **72**: 183-196 [PMID: 31849347 DOI: 10.1016/j.jhep.2019.08.026]

185 **Deretic V**, Levine B. Autophagy balances inflammation in innate immunity. *Autophagy* 2018; **14**: 243-251 [PMID: 29165043 DOI: 10.1080/15548627.2017.1402992]

186 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014. e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

187 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

188 **Marra F**, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. *J Hepatol* 2018; **68**: 280-295 [PMID: 29154964 DOI: 10.1016/j.jhep.2017.11.014]

189 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9]

190 **Hammoutene A**, Rautou PE. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *J Hepatol* 2019; **70**: 1278-1291 [PMID: 30797053 DOI: 10.1016/j.jhep.2019.02.012]

191 **Cingolani F**, Czaja MJ. Regulation and Functions of Autophagic Lipolysis. *Trends Endocrinol Metab* 2016; **27**: 696-705 [PMID: 27365163 DOI: 10.1016/j.tem.2016.06.003]

192 **Zechner R**, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *J Lipid Res* 2009; **50**: 3-21 [PMID: 18952573 DOI: 10.1194/jlr.R800031-JLR200]

193 **Lee IH**, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, Tsokos M, Alt FW, Finkel T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci USA* 2008; **105**: 3374-3379 [PMID: 18296641 DOI: 10.1073/pnas.0712145105]

194 **Tomaipitinca L**, Mandatori S, Mancinelli R, Giulitti F, Petrungaro S, Moresi V, Facchiano A, Ziparo E, Gaudio E, Giampietri C. The Role of Autophagy in Liver Epithelial Cells and Its Impact on Systemic Homeostasis. *Nutrients* 2019; **11** [PMID: 30979078 DOI: 10.3390/nu11040827]

195 **Kurahashi T**, Hamashima S, Shirato T, Lee J, Homma T, Kang ES, Fujii J. An SOD1 deficiency enhances lipid droplet accumulation in the fasted mouse liver by aborting lipophagy. *Biochem Biophys Res Commun* 2015; **467**: 866-871 [PMID: 26474701 DOI: 10.1016/j.bbrc.2015.10.052]

196 **Rodriguez-Navarro JA**, Kaushik S, Koga H, Dall'Armi C, Shui G, Wenk MR, Di Paolo G, Cuervo AM. Inhibitory effect of dietary lipids on chaperone-mediated autophagy. *Proc Natl Acad Sci USA* 2012; **109**: E705-E714 [PMID: 22331875 DOI: 10.1073/pnas.1113036109]

197 **Liu HY**, Han J, Cao SY, Hong T, Zhuo D, Shi J, Liu Z, Cao W. Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia: inhibition of FoxO1-dependent expression of key autophagy genes by insulin. *J Biol Chem* 2009; **284**: 31484-31492 [PMID: 19758991 DOI: 10.1074/jbc.M109.033936]

198 **Koga H**, Kaushik S, Cuervo AM. Altered lipid content inhibits autophagic vesicular fusion. *FASEB J* 2010; **24**: 3052-3065 [PMID: 20375270 DOI: 10.1096/fj.09-144519]

199 **Liu K**, Lou J, Wen T, Yin J, Xu B, Ding W, Wang A, Liu D, Zhang C, Chen D, Li N. Depending on the stage of hepatosteatosis, p53 causes apoptosis primarily through either DRAM-induced autophagy or BAX. *Liver Int* 2013; **33**: 1566-1574 [PMID: 23875779 DOI: 10.1111/Liv.12238]

200 **Kovsan J**, Blüher M, Tarnovscki T, Klöting N, Kirshtein B, Madar L, Shai I, Golan R, Harman-Boehm I, Schön MR, Greenberg AS, Elazar Z, Bashan N, Rudich A. Altered autophagy in human adipose tissues in obesity. *J Clin Endocrinol Metab* 2011; **96**: E268-E277 [PMID: 21047928 DOI: 10.1210/jc.2010-1681]

201 **Yang L**, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab* 2010; **11**: 467-478 [PMID: 20519119 DOI: 10.1016/j.cmet.2010.04.005]

202 **Nuñez CE**, Rodrigues VS, Gomes FS, Moura RF, Victorio SC, Bombassaro B, Chaim EA, Pareja JC, Geloneze B, Velloso LA, Araujo EP. Defective regulation of adipose tissue autophagy in obesity. *Int J Obes (Lond)* 2013; **37**: 1473-1480 [PMID: 23478428 DOI: 10.1038/ijo.2013.27]

203 **Liu Y**, Palanivel R, Rai E, Park M, Gabor TV, Scheid MP, Xu A, Sweeney G. Adiponectin stimulates autophagy and reduces oxidative stress to enhance insulin sensitivity during high-fat diet feeding in mice. *Diabetes* 2015; **64**: 36-48 [PMID: 25071026 DOI: 10.2337/db14-0267]

204 **Lavallard VJ**, Meijer AJ, Codogno P, Gual P. Autophagy, signaling and obesity. *Pharmacol Res* 2012; **66**: 513-525 [PMID: 22982482 DOI: 10.1016/j.phrs.2012.09.003]

205 **Gual P**, Le Marchand-Brustel Y, Tanti JF. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 2005; **87**: 99-109 [PMID: 15733744 DOI: 10.1016/j.biochi.2004.10.019]

206 **Tremblay F**, Krebs M, Dombrowski L, Brehm A, Bernroider E, Roth E, Nowotny P, Waldhäusl W, Marette A, Roden M. Overactivation of S6 kinase 1 as a cause of human insulin resistance during increased amino acid availability. *Diabetes* 2005; **54**: 2674-2684 [PMID: 16123357 DOI: 10.2337/diabetes.54.9.2674]

207 **Hotamisligil GS**. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 2010; **140**: 900-917 [PMID: 20303879 DOI: 10.1016/j.cell.2010.02.034]

208 **Codogno P**, Meijer AJ. Autophagy: a potential link between obesity and insulin resistance. *Cell Metab* 2010; **11**: 449-451 [PMID: 20519116 DOI: 10.1016/j.cmet.2010.05.006]

209 **Inami Y**, Yamashina S, Izumi K, Ueno T, Tanida I, Ikejima K, Watanabe S. Hepatic steatosis inhibits autophagic proteolysis *via* impairment of autophagosomal acidification and cathepsin expression. *Biochem Biophys Res Commun* 2011; **412**: 618-625 [PMID: 21856284 DOI: 10.1016/j.bbrc.2011.08.012]

210 **Fukuo Y**, Yamashina S, Sonoue H, Arakawa A, Nakadera E, Aoyama T, Uchiyama A, Kon K, Ikejima K, Watanabe S. Abnormality of autophagic function and cathepsin expression in the liver from patients with non-alcoholic fatty liver

disease. *Hepatol Res* 2014; **44**: 1026-1036 [PMID: 24299564 DOI: 10.1111/hepr.12282]

211 **Wang X**, Zhang X, Chu ESH, Chen X, Kang W, Wu F, To KF, Wong VWS, Chan HLY, Chan MTV, Sung JJY, Wu WKK, Yu J. Defective lysosomal clearance of autophagosomes and its clinical implications in nonalcoholic steatohepatitis. *FASEB J* 2018; **32**: 37-51 [PMID: 28842428 DOI: 10.1096/fj.201601393R]

212 **Mizunoe Y**, Sudo Y, Okita N, Hiraoka H, Mikami K, Narahara T, Negishi A, Yoshida M, Higashibata R, Watanabe S, Kaneko H, Natori D, Furuichi T, Yasukawa H, Kobayashi M, Higami Y. Involvement of lysosomal dysfunction in autophagosome accumulation and early pathologies in adipose tissue of obese mice. *Autophagy* 2017; **13**: 642-653 [PMID: 28121218 DOI: 10.1080/15548627.2016.1274850]

213 **Ju L**, Han J, Zhang X, Deng Y, Yan H, Wang C, Li X, Chen S, Alimujiang M, Li X, Fang Q, Yang Y, Jia W. Obesity-associated inflammation triggers an autophagy-lysosomal response in adipocytes and causes degradation of perilipin 1. *Cell Death Dis* 2019; **10**: 121 [PMID: 30741926 DOI: 10.1038/s41419-019-1393-8]

214 **Mizunoe Y**, Kobayashi M, Tagawa R, Nakagawa Y, Shimano H, Higami Y. Association between Lysosomal Dysfunction and Obesity-Related Pathology: A Key Knowledge to Prevent Metabolic Syndrome. *Int J Mol Sci* 2019; **20**: 3688 [PMID: 31357643 DOI: 10.3390/ijms20153688]

215 **Qian Q**, Zhang Z, Li M, Savage K, Cheng D, Rauckhorst AJ, Ankrum JA, Taylor EB, Ding WX, Xiao Y, Cao HJ, Yang L. Hepatic Lysosomal iNOS Activity Impairs Autophagy in Obesity. *Cell Mol Gastroenterol Hepatol* 2019; **8**: 95-110 [PMID: 30926581 DOI: 10.1016/j.jcmgh.2019.03.005]

216 **Wu H**, Wang Y, Li W, Chen H, Du L, Liu D, Wang X, Xu T, Liu L, Chen Q. Deficiency of mitophagy receptor FUNDC1 impairs mitochondrial quality and aggravates dietary-induced obesity and metabolic syndrome. *Autophagy* 2019; **15**: 1882-1898 [PMID: 30898010 DOI: 10.1080/15548627.2019.1596482]

217 **Su Z**, Nie Y, Huang X, Zhu Y, Feng B, Tang L, Zheng G. Mitophagy in Hepatic Insulin Resistance: Therapeutic Potential and Concerns. *Front Pharmacol* 2019; **10**: 1193 [PMID: 31649547 DOI: 10.3389/fphar.2019.01193]

218 **Kim KH**, Jeong YT, Oh H, Kim SH, Cho JM, Kim YN, Kim SS, Kim DH, Hur KY, Kim HK, Ko T, Han J, Kim HL, Kim J, Back SH, Komatsu M, Chen H, Chan DC, Konishi M, Itoh N, Choi CS, Lee MS. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat Med* 2013; **19**: 83-92 [PMID: 23202295 DOI: 10.1038/nm.3014]

219 **Zhu S**, Wu Y, Ye X, Ma L, Qi J, Yu D, Wei Y, Lin G, Ren G, Li D. FGF21 ameliorates nonalcoholic fatty liver disease by inducing autophagy. *Mol Cell Biochem* 2016; **420**: 107-119 [PMID: 27435856 DOI: 10.1007/s11010-016-2774-2]

220 **Shen J**, Chan HL, Wong GL, Choi PC, Chan AW, Chan HY, Chim AM, Yeung DK, Chan FK, Woo J, Yu J, Chu WC, Wong VW. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. *J Hepatol* 2012; **56**: 1363-1370 [PMID: 22314419 DOI: 10.1016/j.jhep.2011.12.025]

221 **Adams AC**, Halstead CA, Hansen BC, Irizarry AR, Martin JA, Myers SR, Reynolds VL, Smith HW, Wroblewski VJ, Kharitonenkov A. LY2405319, an Engineered FGF21 Variant, Improves the Metabolic Status of Diabetic Monkeys. *PLoS One* 2013; **8**: e65763 [PMID: 23823755 DOI: 10.1371/journal.pone.0065763]

222 **Kharitonenkov A**, Beals JM, Micanovic R, Strifler BA, Rathnachalam R, Wroblewski VJ, Li S, Koester A, Ford AM, Coskun T, Dunbar JD, Cheng CC, Frye CC, Bumol TF, Moller DE. Rational design of a fibroblast growth factor 21-based clinical candidate, LY2405319. *PLoS One* 2013; **8**: e58575 [PMID: 23536797 DOI: 10.1371/journal.pone.0058575]

223 **Gaich G**, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. *Cell Metab* 2013; **18**: 333-340 [PMID: 24011069 DOI: 10.1016/j.cmet.2013.08.005]

224 **Romero M**, Zorzano A. Role of autophagy in the regulation of adipose tissue biology. *Cell Cycle* 2019; **18**: 1435-1445 [PMID: 31135269 DOI: 10.1080/15384101.2019.1624110]

225 **Wang L**, Liu X, Nie J, Zhang J, Kimball SR, Zhang H, Zhang WJ, Jefferson LS, Cheng Z, Ji Q, Shi Y. ALCAT1 controls mitochondrial etiology of fatty liver diseases, linking defective mitophagy to steatosis. *Hepatology* 2015; **61**: 486-496 [PMID: 25203315 DOI: 10.1002/hep.27420]

226 **Sinha RA**, Yen PM. Thyroid hormone-mediated autophagy and mitochondrial turnover in NAFLD. *Cell Biosci* 2016; **6**: 46 [PMID: 27437098 DOI: 10.1186/s13578-016-0113-7]

227 **Chi HC**, Tsai CY, Tsai MM, Yeh CT, Lin KH. Molecular functions and clinical impact of thyroid hormone-triggered autophagy in liver-related diseases. *J Biomed Sci* 2019; **26**: 24 [PMID: 30849993 DOI: 10.1186/s12929-019-0517-x]

228 **Pang L**, Liu K, Liu D, Lv F, Zang Y, Xie F, Yin J, Shi Y, Wang Y, Chen D. Differential effects of reticulophagy and mitophagy on nonalcoholic fatty liver disease. *Cell Death Dis* 2018; **9**: 90 [PMID: 29367738 DOI: 10.1038/s41419-017-0136-y]

229 **Yamada T**, Murata D, Adachi Y, Itoh K, Kameoka S, Igarashi A, Kato T, Araki Y, Huganir RL, Dawson TM, Yanagawa T, Okamoto K, Iijima M, Sesaki H. Mitochondrial Stasis Reveals p62-Mediated Ubiquitination in Parkin-Independent Mitophagy and Mitigates Nonalcoholic Fatty Liver Disease. *Cell Metab* 2018; **28**: 588-604. e5 [PMID: 30017357 DOI: 10.1016/j.cmet.2018.06.014]

230 **Lee K**, Haddad A, Osme A, Kim C, Borzou A, Ilchenko S, Allende D, Dasarathy S, McCullough A, Sadygov RG, Kasumov T. Hepatic Mitochondrial Defects in a Nonalcoholic Fatty Liver Disease Mouse Model Are Associated with Increased Degradation of Oxidative Phosphorylation Subunits. *Mol Cell Proteomics* 2018; **17**: 2371-2386 [PMID: 30171159 DOI: 10.1074/mcp.RA118.000961]

231 **Shao N**, Yu XY, Ma XF, Lin WJ, Hao M, Kuang HY. Exenatide Delays the Progression of Nonalcoholic Fatty Liver Disease in C57BL/6 Mice, Which May Involve Inhibition of the NLRP3 Inflammasome through the Mitophagy Pathway. *Gastroenterol Res Pract* 2018; **2018**: 1864307 [PMID: 29849583 DOI: 10.1155/2018/1864307]

232 **Li R**, Xin T, Li D, Wang C, Zhu H, Zhou H. Therapeutic effect of Sirtuin 3 on ameliorating nonalcoholic fatty liver disease: The role of the ERK-CREB pathway and Bnip3-mediated mitophagy. *Redox Biol* 2018; **18**: 229-243 [PMID: 30056271 DOI: 10.1016/j.redox.2018.07.011]

233 **Yu X**, Hao M, Liu Y, Ma X, Lin W, Xu Q, Zhou H, Shao N, Kuang H. Liraglutide ameliorates non-alcoholic steatohepatitis by inhibiting NLRP3 inflammasome and pyroptosis activation *via* mitophagy. *Eur J Pharmacol* 2019; **864**:

172715 [PMID: 31593687 DOI: 10.1016/j.ejphar.2019.172715]
234 **Zhou T**, Chang L, Luo Y, Zhou Y, Zhang J. Mst1 inhibition attenuates non-alcoholic fatty liver disease *via* reversing Parkin-related mitophagy. *Redox Biol* 2019; **21**: 101120 [PMID: 30708325 DOI: 10.1016/j.redox.2019.101120]
235 **Niso-Santano M**, Malik SA, Pietrocola F, Bravo-San Pedro JM, Mariño G, Cianfanelli V, Ben-Younès A, Troncoso R, Markaki M, Sica V, Izzo V, Chaba K, Bauvy C, Dupont N, Kepp O, Rockenfeller P, Wolinski H, Madeo F, Lavandero S, Codogno P, Harper F, Pierron G, Tavernarakis N, Cecconi F, Maiuri MC, Galluzzi L, Kroemer G. Unsaturated fatty acids induce non-canonical autophagy. *EMBO J* 2015; **34**: 1025-1041 [PMID: 25586377 DOI: 10.15252/embj.201489363]
236 **Singh R**, Xiang Y, Wang Y, Baikati K, Cuervo AM, Luu YK, Tang Y, Pessin JE, Schwartz GJ, Czaja MJ. Autophagy regulates adipose mass and differentiation in mice. *J Clin Invest* 2009; **119**: 3329-3339 [PMID: 19855132 DOI: 10.1172/JCI39228]
237 **Mei S**, Ni HM, Manley S, Bockus A, Kassel KM, Luyendyk JP, Copple BL, Ding WX. Differential roles of unsaturated and saturated fatty acids on autophagy and apoptosis in hepatocytes. *J Pharmacol Exp Ther* 2011; **339**: 487-498 [PMID: 21856859 DOI: 10.1124/jpet.111.184341]
238 **Deng X**, Pan X, Cheng C, Liu B, Zhang H, Zhang Y, Xu K. Regulation of SREBP-2 intracellular trafficking improves impaired autophagic flux and alleviates endoplasmic reticulum stress in NAFLD. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017; **1862**: 337-350 [PMID: 28011404 DOI: 10.1016/j.bbalip.2016.12.007]
239 **Maus M**, Cuk M, Patel B, Lian J, Ouimet M, Kaufmann U, Yang J, Horvath R, Hornig-Do HT, Chrzanowska-Lightowlers ZM, Moore KJ, Cuervo AM, Feske S. Store-Operated Ca^{2+} Entry Controls Induction of Lipolysis and the Transcriptional Reprogramming to Lipid Metabolism. *Cell Metab* 2017; **25**: 698-712 [PMID: 28132808 DOI: 10.1016/j.cmet.2016.12.021]
240 **Li S**, Dou X, Ning H, Song Q, Wei W, Zhang X, Shen C, Li J, Sun C, Song Z. Sirtuin 3 acts as a negative regulator of autophagy dictating hepatocyte susceptibility to lipotoxicity. *Hepatology* 2017; **66**: 936-952 [PMID: 28437863 DOI: 10.1002/hep.29229]
241 **Sinha RA**, Farah BL, Singh BK, Siddique MM, Li Y, Wu Y, Ilkayeva OR, Gooding J, Ching J, Zhou J, Martinez L, Xie S, Bay BH, Summers SA, Newgard CB, Yen PM. Caffeine stimulates hepatic lipid metabolism by the autophagy-lysosomal pathway in mice. *Hepatology* 2014; **59**: 1366-1380 [PMID: 23929677 DOI: 10.1002/hep.26667]
242 **Ding WX**. Drinking coffee burns hepatic fat by inducing lipophagy coupled with mitochondrial β-oxidation. *Hepatology* 2014; **59**: 1235-1238 [PMID: 24114874 DOI: 10.1002/hep.26736]
243 **Molloy JW**, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. *Hepatology* 2012; **55**: 429-436 [PMID: 21987293 DOI: 10.1002/hep.24731]
244 **Anty R**, Marjoux S, Iannelli A, Patouraux S, Schneck AS, Bonnafous S, Gire C, Amzolini A, Ben-Amor I, Saint-Paul MC, Mariné-Barjoan E, Pariente A, Gugenheim J, Gual P, Tran A. Regular coffee but not espresso drinking is protective against fibrosis in a cohort mainly composed of morbidly obese European women with NAFLD undergoing bariatric surgery. *J Hepatol* 2012; **57**: 1090-1096 [PMID: 22820478 DOI: 10.1016/j.jhep.2012.07.014]
245 **Chen R**, Wang Q, Song S, Liu F, He B, Gao X. Protective role of autophagy in methionine-choline deficient diet-induced advanced nonalcoholic steatohepatitis in mice. *Eur J Pharmacol* 2016; **770**: 126-133 [PMID: 26593434 DOI: 10.1016/j.ejphar.2015.11.012]
246 **Ji G**, Wang Y, Deng Y, Li X, Jiang Z. Resveratrol ameliorates hepatic steatosis and inflammation in methionine/choline-deficient diet-induced steatohepatitis through regulating autophagy. *Lipids Health Dis* 2015; **14**: 134 [PMID: 26498332 DOI: 10.1186/s12944-015-0139-6]
247 **Ding S**, Jiang J, Zhang G, Bu Y, Zhang G, Zhao X. Resveratrol and caloric restriction prevent hepatic steatosis by regulating SIRT1-autophagy pathway and alleviating endoplasmic reticulum stress in high-fat diet-fed rats. *PLoS One* 2017; **12**: e0183541 [PMID: 28817690 DOI: 10.1371/journal.pone.0183541]
248 **Xiao L**, Liang S, Ge L, Qiu S, Wan H, Wu S, Fei J, Peng S, Zeng X. Si-Wei-Qing-Gan-Tang Improves Non-Alcoholic Steatohepatitis by Modulating the Nuclear Factor-κB Signal Pathway and Autophagy in Methionine and Choline Deficient Diet-Fed Rats. *Front Pharmacol* 2020; **11**: 530 [PMID: 32425782 DOI: 10.3389/fphar.2020.00530]
249 **Wang X**, Zhang ZF, Zheng GH, Wang AM, Sun CH, Qin SP, Zhuang J, Lu J, Ma DF, Zheng YL. The Inhibitory Effects of Purple Sweet Potato Color on Hepatic Inflammation Is Associated with Restoration of NAD^+ Levels and Attenuation of NLRP3 Inflammasome Activation in High-Fat-Diet-Treated Mice. *Molecules* 2017; **22** [PMID: 28786950 DOI: 10.3390/molecules22081315]
250 **Kim HM**, Kim Y, Lee ES, Huh JH, Chung CH. Caffeic acid ameliorates hepatic steatosis and reduces ER stress in high fat diet-induced obese mice by regulating autophagy. *Nutrition* 2018; **55-56**: 63-70 [PMID: 29960159 DOI: 10.1016/j.nut.2018.03.010]
251 **Elmansi AM**, El-Karef AA, Shishtawy MMEl, Eissa LA. Hepatoprotective Effect of Curcumin on Hepatocellular Carcinoma Through Autophagic and Apoptic Pathways. *Ann Hepatol* 2017; **16**: 607-618 [PMID: 28611265 DOI: 10.5604/01.3001.0010.0307]
252 **Liu C**, Liu L, Zhu HD, Sheng JQ, Wu XL, He XX, Tian DA, Liao JZ, Li PY. Celecoxib alleviates nonalcoholic fatty liver disease by restoring autophagic flux. *Sci Rep* 2018; **8**: 4108 [PMID: 29515134 DOI: 10.1038/s41598-018-22339-0]
253 **Alexaki A**, Gupta SD, Majumder S, Kono M, Tuymetova G, Harmon JM, Dunn TM, Proia RL. Autophagy regulates sphingolipid levels in the liver. *J Lipid Res* 2014; **55**: 2521-2531 [PMID: 25332431 DOI: 10.1194/jlr.M051862]
254 **Menikdiwela KR**, Ramalingam L, Rasha F, Wang S, Dufour JM, Kalupahana NS, Sunahara KKS, Martins JO, Moustaid-Moussa N. Autophagy in metabolic syndrome: breaking the wheel by targeting the renin-angiotensin system. *Cell Death Dis* 2020; **11**: 87 [PMID: 32015340 DOI: 10.1038/s41419-020-2275-9]
255 **Tanaka S**, Hikita H, Tatsumi T, Sakamori R, Nozaki Y, Sakane S, Shiode Y, Nakabori T, Saito Y, Hiramatsu N, Tabata K, Kawabata T, Hamasaki M, Eguchi H, Nagano H, Yoshimori T, Takehara T. Rubicon inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in nonalcoholic fatty liver disease in mice. *Hepatology* 2016; **64**: 1994-2014 [PMID: 27637015 DOI: 10.1002/hep.28820]
256 **Yan H**, Gao Y, Zhang Y. Inhibition of JNK suppresses autophagy and attenuates insulin resistance in a rat model of

nonalcoholic fatty liver disease. *Mol Med Rep* 2017; **15**: 180-186 [PMID: 27909723 DOI: 10.3892/mmr.2016.5966]
257 **Lavallard VJ**, Gual P. Autophagy and non-alcoholic fatty liver disease. *Biomed Res Int* 2014; **2014**: 120179 [PMID: 25295245 DOI: 10.1155/2014/120179]
258 **Mao Y**, Yu F, Wang J, Guo C, Fan X. Autophagy: a new target for nonalcoholic fatty liver disease therapy. *Hepat Med* 2016; **8**: 27-37 [PMID: 27099536 DOI: 10.2147/HMER.S98120]
259 **Schneider JL**, Suh Y, Cuervo AM. Deficient chaperone-mediated autophagy in liver leads to metabolic dysregulation. *Cell Metab* 2014; **20**: 417-432 [PMID: 25043815 DOI: 10.1016/j.cmet.2014.06.009]
260 **Kaushik S**, Cuervo AM. Degradation of lipid droplet-associated proteins by chaperone-mediated autophagy facilitates lipolysis. *Nat Cell Biol* 2015; **17**: 759-770 [PMID: 25961502 DOI: 10.1038/ncb3166]
261 **Schneider JL**, Villarroya J, Diaz-Carretero A, Patel B, Urbanska AM, Thi MM, Villarroya F, Santambrogio L, Cuervo AM. Loss of hepatic chaperone-mediated autophagy accelerates proteostasis failure in aging. *Aging Cell* 2015; **14**: 249-264 [PMID: 25620427 DOI: 10.1111/acel.12310]
262 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]
263 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
264 **Bedossa P**, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, Tordjman J, Clement K. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 2012; **56**: 1751-1759 [PMID: 22707395 DOI: 10.1002/hep.25889]
265 **Harada M**, Hanada S, Toivola DM, Ghori N, Omary MB. Autophagy activation by rapamycin eliminates mouse Mallory-Denk bodies and blocks their proteasome inhibitor-mediated formation. *Hepatology* 2008; **47**: 2026-2035 [PMID: 18454506 DOI: 10.1002/hep.22294]
266 **González-Rodríguez A**, Mayoral R, Agra N, Valdecantos MP, Pardo V, Miquilena-Colina ME, Vargas-Castrillón J, Lo Iacono O, Corazzari M, Fimia GM, Piacentini M, Muntané J, Boscá L, García-Monzón C, Martín-Sanz P, Valverde ÁM. Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. *Cell Death Dis* 2014; **5**: e1179 [PMID: 24743734 DOI: 10.1038/cddis.2014.162]
267 **Li P**, He K, Li J, Liu Z, Gong J. The role of Kupffer cells in hepatic diseases. *Mol Immunol* 2017; **85**: 222-229 [PMID: 28314211 DOI: 10.1016/j.molimm.2017.02.018]
268 **Tacke F**. Targeting hepatic macrophages to treat liver diseases. *J Hepatol* 2017; **66**: 1300-1312 [PMID: 28267621 DOI: 10.1016/j.jhep.2017.02.026]
269 **Thomas H**. NAFLD: A critical role for the NLRP3 inflammasome in NASH. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 197 [PMID: 28223701 DOI: 10.1038/nrgastro.2017.21]
270 **Feldstein AE**, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression *via* a lysosomal pathway. *Hepatology* 2004; **40**: 185-194 [PMID: 15239102 DOI: 10.1002/hep.20283]
271 **Tang Y**, Cao G, Min X, Wang T, Sun S, Du X, Zhang W. Cathepsin B inhibition ameliorates the non-alcoholic steatohepatitis through suppressing caspase-1 activation. *J Physiol Biochem* 2018; **74**: 503-510 [PMID: 30019185 DOI: 10.1007/s13105-018-0644-y]
272 **Xu X**, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AW Jr. Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. *Cell Metab* 2013; **18**: 816-830 [PMID: 24315368 DOI: 10.1016/j.cmet.2013.11.001]
273 **Grijalva A**, Xu X, Ferrante AW Jr. Autophagy Is Dispensable for Macrophage-Mediated Lipid Homeostasis in Adipose Tissue. *Diabetes* 2016; **65**: 967-980 [PMID: 26868294 DOI: 10.2337/db15-1219]
274 **Bieghs V**, Hendrikx T, van Gorp PJ, Verheyen F, Guichot YD, Walenbergh SM, Jeurissen ML, Gijbels M, Rensen SS, Bast A, Plat J, Kalhan SC, Koek GH, Leitersdorf E, Hofker MH, Lütjohann D, Shiri-Sverdlov R. The cholesterol derivative 27-hydroxycholesterol reduces steatohepatitis in mice. *Gastroenterology* 2013; **144**: 167-178. e1 [PMID: 23041327 DOI: 10.1053/j.gastro.2012.09.062]
275 **Houben T**, Oligschlaeger Y, Hendrikx T, Bitorina AV, Walenbergh SMA, van Gorp PJ, Gijbels MJJ, Friedrichs S, Plat J, Schaap FG, Lütjohann D, Hofker MH, Shiri-Sverdlov R. Cathepsin D regulates lipid metabolism in murine steatohepatitis. *Sci Rep* 2017; **7**: 3494 [PMID: 28615690 DOI: 10.1038/s41598-017-03796-5]
276 **Ford ES**, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002; **287**: 356-359 [PMID: 11790215 DOI: 10.1001/jama.287.3.356]
277 **Noureddin M**, Yates KP, Vaughn IA, Neuschwander-Tetri BA, Sanyal AJ, McCullough A, Merriman R, Hameed B, Doo E, Kleiner DE, Behling C, Loomba R; NASH CRN. Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. *Hepatology* 2013; **58**: 1644-1654 [PMID: 23686698 DOI: 10.1002/hep.26465]
278 **Li Z**, Berk M, McIntyre TM, Gores GJ, Feldstein AE. The lysosomal-mitochondrial axis in free fatty acid-induced hepatic lipotoxicity. *Hepatology* 2008; **47**: 1495-1503 [PMID: 18220271 DOI: 10.1002/hep.22183]
279 **Sunny NE**, Bril F, Cusi K. Mitochondrial Adaptation in Nonalcoholic Fatty Liver Disease: Novel Mechanisms and Treatment Strategies. *Trends Endocrinol Metab* 2017; **28**: 250-260 [PMID: 27986466 DOI: 10.1016/j.tem.2016.11.006]
280 **Simões ICM**, Fontes A, Pinton P, Zischka H, Wieckowski MR. Mitochondria in non-alcoholic fatty liver disease. *Int J Biochem Cell Biol* 2018; **95**: 93-99 [PMID: 29288054 DOI: 10.1016/j.biocel.2017.12.019]
281 **Hammoutene A**, Lasselin J, Vion A, Colnot N, Paradis V, Lotersztajn S, Boulanger C, Rautou P. Defective autophagy in liver sinusoidal endothelial cells promotes non alcoholic steatohepatitis and fibrosis development. *J Hepatol* 2018; **68**: S29 [DOI: 10.1016/S0168-8278(18)30276-9]
282 **Hammoutene A**, Biquard L, Lasselin J, Kheloufi M, Tanguy M, Vion AC, Mérian J, Colnot N, Loyer X, Tedgui A,

Codogno P, Lotersztajn S, Paradis V, Boulanger CM, Rautou PE. A defect in endothelial autophagy occurs in patients with non-alcoholic steatohepatitis and promotes inflammation and fibrosis. *J Hepatol* 2020; **72**: 528-538 [PMID: 31726115 DOI: 10.1016/j.jhep.2019.10.028]

283 **Scita G**, Di Fiore PP. The endocytic matrix. *Nature* 2010; **463**: 464-473 [PMID: 20110990 DOI: 10.1038/nature08910]

284 **Zhao GN**, Zhang P, Gong J, Zhang XJ, Wang PX, Yin M, Jiang Z, Shen LJ, Ji YX, Tong J, Wang Y, Wei QF, Wang Y, Zhu XY, Zhang X, Fang J, Xie Q, She ZG, Wang Z, Huang Z, Li H. Tmbim1 is a multivesicular body regulator that protects against non-alcoholic fatty liver disease in mice and monkeys by targeting the lysosomal degradation of Tlr4. *Nat Med* 2017; **23**: 742-752 [PMID: 28481357 DOI: 10.1038/nm.4334]

285 **Zhang X**, Wu WK, Xu W, Man K, Wang X, Han J, Leung WY, Wu R, Liu K, Yu J. C-X-C Motif Chemokine 10 Impairs Autophagy and Autolysosome Formation in Non-alcoholic Steatohepatitis. *Theranostics* 2017; **7**: 2822-2836 [PMID: 28824718 DOI: 10.7150/thno.19068]

286 **Zakhari S**. Overview: how is alcohol metabolized by the body? *Alcohol Res Health* 2006; **29**: 245-254 [PMID: 17718403 DOI: 10.1159/000095013]

287 **Dey A**, Cederbaum AI. Alcohol and oxidative liver injury. *Hepatology* 2006; **43**: S63-S74 [PMID: 16447273 DOI: 10.1002/hep.20957]

288 **Ding WX**, Li M, Chen X, Ni HM, Lin CW, Gao W, Lu B, Stolz DB, Clemens DL, Yin XM. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology* 2010; **139**: 1740-1752 [PMID: 20659474 DOI: 10.1053/j.gastro.2010.07.041]

289 **Kong X**, Yang Y, Ren L, Shao T, Li F, Zhao C, Liu L, Zhang H, McClain CJ, Feng W. Activation of autophagy attenuates EtOH-LPS-induced hepatic steatosis and injury through MD2 associated TLR4 signaling. *Sci Rep* 2017; **7**: 9292 [PMID: 28839246 DOI: 10.1038/s41598-017-09045-z]

290 **Thomes PG**, Ehlers RA, Trambly CS, Clemens DL, Fox HS, Tuma DJ, Donohue TM. Multilevel regulation of autophagosome content by ethanol oxidation in HepG2 cells. *Autophagy* 2013; **9**: 63-73 [PMID: 23090141 DOI: 10.4161/auto.22490]

291 **Wu D**, Wang X, Zhou R, Yang L, Cederbaum AI. Alcohol steatosis and cytotoxicity: the role of cytochrome P4502E1 and autophagy. *Free Radic Biol Med* 2012; **53**: 1346-1357 [PMID: 22819980 DOI: 10.1016/j.freeradbiomed.2012.07.005]

292 **Sid B**, Verrax J, Calderon PB. Role of AMPK activation in oxidative cell damage: Implications for alcohol-induced liver disease. *Biochem Pharmacol* 2013; **86**: 200-209 [PMID: 23688501 DOI: 10.1016/j.bcp.2013.05.007]

293 **Ding WX**, Li M, Yin XM. Selective taste of ethanol-induced autophagy for mitochondria and lipid droplets. *Autophagy* 2011; **7**: 248-249 [PMID: 21150309 DOI: 10.4161/auto.7.2.14347]

294 **Scherz-Shouval R**, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J* 2007; **26**: 1749-1760 [PMID: 17347651 DOI: 10.1038/sj.emboj.7601623]

295 **Thomes PG**, Trambly CS, Thiele GM, Duryee MJ, Fox HS, Haorah J, Donohue TM Jr. Proteasome activity and autophagosome content in liver are reciprocally regulated by ethanol treatment. *Biochem Biophys Res Commun* 2012; **417**: 262-267 [PMID: 22142844 DOI: 10.1016/j.bbrc.2011.11.097]

296 **Yeon JE**, Califano S, Xu J, Wands JR, De La Monte SM. Potential role of PTEN phosphatase in ethanol-impaired survival signaling in the liver. *Hepatology* 2003; **38**: 703-714 [PMID: 12939597 DOI: 10.1053/jhep.2003.50368]

297 **Shulga N**, Hoek JB, Pastorino JG. Elevated PTEN levels account for the increased sensitivity of ethanol-exposed cells to tumor necrosis factor-induced cytotoxicity. *J Biol Chem* 2005; **280**: 9416-9424 [PMID: 15623531 DOI: 10.1074/jbc.M409505200]

298 **Liuzzi JP**, Yoo C. Role of zinc in the regulation of autophagy during ethanol exposure in human hepatoma cells. *Biol Trace Elem Res* 2013; **156**: 350-356 [PMID: 24061963 DOI: 10.1007/s12011-013-9816-3]

299 **Wu D**, Wang X, Zhou R, Cederbaum A. CYP2E1 enhances ethanol-induced lipid accumulation but impairs autophagy in HepG2 E47 cells. *Biochem Biophys Res Commun* 2010; **402**: 116-122 [PMID: 20932821 DOI: 10.1016/j.bbrc.2010.09.127]

300 **Guo R**, Xu X, Babcock SA, Zhang Y, Ren J. Aldehyde dedydrogenase-2 plays a beneficial role in ameliorating chronic alcohol-induced hepatic steatosis and inflammation through regulation of autophagy. *J Hepatol* 2015; **62**: 647-656 [PMID: 25457208 DOI: 10.1016/j.jhep.2014.10.009]

301 **Denaës T**, Lodder J, Chobert MN, Ruiz I, Pawlotsky JM, Lotersztajn S, Teixeira-Clerc F. The Cannabinoid Receptor 2 Protects Against Alcoholic Liver Disease Via a Macrophage Autophagy-Dependent Pathway. *Sci Rep* 2016; **6**: 28806 [PMID: 27346657 DOI: 10.1038/srep28806]

302 **Thomes PG**, Trambly CS, Fox HS, Tuma DJ, Donohue TM Jr. Acute and Chronic Ethanol Administration Differentially Modulate Hepatic Autophagy and Transcription Factor EB. *Alcohol Clin Exp Res* 2015; **39**: 2354-2363 [PMID: 26556759 DOI: 10.1111/acer.12904]

303 **Ni HM**, Du K, You M, Ding WX. Critical role of FoxO3a in alcohol-induced autophagy and hepatotoxicity. *Am J Pathol* 2013; **183**: 1815-1825 [PMID: 24095927 DOI: 10.1016/j.ajpath.2013.08.011]

304 **Huang H**, Tindall DJ. Dynamic FoxO transcription factors. *J Cell Sci* 2007; **120**: 2479-2487 [PMID: 17646672 DOI: 10.1242/jcs.001222]

305 **Eid N**, Ito Y, Maemura K, Otsuki Y. Elevated autophagic sequestration of mitochondria and lipid droplets in steatotic hepatocytes of chronic ethanol-treated rats: an immunohistochemical and electron microscopic study. *J Mol Histol* 2013; **44**: 311-326 [PMID: 23371376 DOI: 10.1007/s10735-013-9483-x]

306 **Lin CW**, Zhang H, Li M, Xiong X, Chen X, Chen X, Dong XC, Yin XM. Pharmacological promotion of autophagy alleviates steatosis and injury in alcoholic and non-alcoholic fatty liver conditions in mice. *J Hepatol* 2013; **58**: 993-999 [PMID: 23339953 DOI: 10.1016/j.jhep.2013.01.011]

307 **Kharbanda KK**, McVicker DL, Zetterman RK, Donohue TM Jr. Ethanol consumption reduces the proteolytic capacity and protease activities of hepatic lysosomes. *Biochim Biophys Acta* 1995; **1245**: 421-429 [PMID: 8541322 DOI: 10.1016/0304-4165(95)00121-2]

308 **Chao X**, Wang S, Zhao K, Li Y, Williams JA, Li T, Chavan H, Krishnamurthy P, He XC, Li L, Ballabio A, Ni HM, Ding

WX. Impaired TFEB-Mediated Lysosome Biogenesis and Autophagy Promote Chronic Ethanol-Induced Liver Injury and Steatosis in Mice. *Gastroenterology* 2018; **155**: 865-879. e12 [PMID: 29782848 DOI: 10.1053/j.gastro.2018.05.027]

309 **Rautou PE**, Mansouri A, Lebrec D, Durand F, Valla D, Moreau R. Autophagy in liver diseases. *J Hepatol* 2010; **53**: 1123-1134 [PMID: 20810185 DOI: 10.1016/j.jhep.2010.07.006]

310 **You M**, Matsumoto M, Pacold CM, Cho WK, Crabb DW. The role of AMP-activated protein kinase in the action of ethanol in the liver. *Gastroenterology* 2004; **127**: 1798-1808 [PMID: 15578517 DOI: 10.1053/j.gastro.2004.09.049]

311 **Komatsu M**, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, Sou YS, Ueno I, Sakamoto A, Tong KI, Kim M, Nishito Y, Iemura S, Natsume T, Ueno T, Kominami E, Motohashi H, Tanaka K, Yamamoto M. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 2010; **12**: 213-223 [PMID: 20173742 DOI: 10.1038/ncb2021]

312 **Lu Y**, Cederbaum AI. Autophagy Protects against CYP2E1/Chronic Ethanol-Induced Hepatotoxicity. *Biomolecules* 2015; **5**: 2659-2674 [PMID: 26501338 DOI: 10.3390/biom5042659]

313 **Liu L**, Xie P, Li W, Wu Y, An W. Augmenter of Liver Regeneration Protects against Ethanol-Induced Acute Liver Injury by Promoting Autophagy. *Am J Pathol* 2019; **189**: 552-567 [PMID: 30553838 DOI: 10.1016/j.ajpath.2018.11.006]

314 **Yan S**, Zhou J, Chen X, Dong Z, Yin XM. Diverse Consequences in Liver Injury in Mice with Different Autophagy Functional Status Treated with Alcohol. *Am J Pathol* 2019; **189**: 1744-1762 [PMID: 31199920 DOI: 10.1016/j.ajpath.2019.05.011]

315 **Williams JA**, Ding WX. A Mechanistic Review of Mitophagy and Its Role in Protection against Alcoholic Liver Disease. *Biomolecules* 2015; **5**: 2619-2642 [PMID: 26501336 DOI: 10.3390/biom5042619]

316 **Eid N**, Ito Y, Otsuki Y. Triggering of Parkin Mitochondrial Translocation in Mitophagy: Implications for Liver Diseases. *Front Pharmacol* 2016; **7**: 100 [PMID: 27199746 DOI: 10.3389/fphar.2016.00100]

317 **Xiong X**, Tao R, DePinho RA, Dong XC. The autophagy-related gene 14 (Atg14) is regulated by forkhead box O transcription factors and circadian rhythms and plays a critical role in hepatic autophagy and lipid metabolism. *J Biol Chem* 2012; **287**: 39107-39114 [PMID: 22992773 DOI: 10.1074/jbc.M112.412569]

318 **Harada M**. Autophagy is involved in the elimination of intracellular inclusions, Mallory-Denk bodies, in hepatocytes. *Med Mol Morphol* 2010; **43**: 13-18 [PMID: 20340001 DOI: 10.1007/s00795-009-0476-5]

319 **Strnad P**, Zatloukal K, Stumptner C, Kulaksiz H, Denk H. Mallory-Denk-bodies: lessons from keratin-containing hepatic inclusion bodies. *Biochim Biophys Acta* 2008; **1782**: 764-774 [PMID: 18805482 DOI: 10.1016/j.bbadis.2008.08.008]

320 **Schulze RJ**, Rasineni K, Weller SG, Schott MB, Schroeder B, Casey CA, McNiven MA. Ethanol exposure inhibits hepatocyte lipophagy by inactivating the small guanosine triphosphatase Rab7. *Hepatol Commun* 2017; **1**: 140-152 [PMID: 29404450 DOI: 10.1002/hep4.1021]

321 **Rasineni K**, Donohue TM Jr, Thomes PG, Yang L, Tuma DJ, McNiven MA, Casey CA. Ethanol-induced steatosis involves impairment of lipophagy, associated with reduced Dynamin2 activity. *Hepatol Commun* 2017; **1**: 501-512 [PMID: 29152606 DOI: 10.1002/hep4.1063]

322 **Eid N**, Ito Y, Horibe A, Otsuki Y. Ethanol-induced mitophagy in liver is associated with activation of the PINK1-Parkin pathway triggered by oxidative DNA damage. *Histol Histopathol* 2016; **31**: 1143-1159 [PMID: 26935412 DOI: 10.14670/HH-11-747]

323 **Eid N**, Ito Y, Horibe A, Otsuki Y, Kondo Y. Ethanol-Induced Mitochondrial Damage in Sertoli Cells is Associated with Parkin Overexpression and Activation of Mitophagy. *Cells* 2019; **8** [PMID: 30934625 DOI: 10.3390/cells8030283]

324 **Williams JA**, Ni HM, Ding Y, Ding WX. Parkin regulates mitophagy and mitochondrial function to protect against alcohol-induced liver injury and steatosis in mice. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G324-G340 [PMID: 26159696 DOI: 10.1152/ajpgi.00108.2015]

325 **Eid N**, Ito Y, Otsuki Y. Mitophagy in steatotic hepatocytes of ethanol-treated wild-type and Parkin knockout mice. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G513-G514 [PMID: 26374875 DOI: 10.1152/ajpgi.00254.2015]

326 **Yu X**, Xu Y, Zhang S, Sun J, Liu P, Xiao L, Tang Y, Liu L, Yao P. Quercetin Attenuates Chronic Ethanol-Induced Hepatic Mitochondrial Damage through Enhanced Mitophagy. *Nutrients* 2016; **8** [PMID: 26742072 DOI: 10.3390/nu8010027]

327 **Zhou H**, Wang S, Hu S, Chen Y, Ren J. ER-Mitochondria Microdomains in Cardiac Ischemia-Reperfusion Injury: A Fresh Perspective. *Front Physiol* 2018; **9**: 755 [PMID: 29962971 DOI: 10.3389/fphys.2018.00755]

328 **Zhou H**, Zhu P, Wang J, Toan S, Ren J. DNA-PKcs promotes alcohol-related liver disease by activating Drp1-related mitochondrial fission and repressing FUNDC1-required mitophagy. *Signal Transduct Target Ther* 2019; **4**: 56 [PMID: 31839999 DOI: 10.1038/s41392-019-0094-1]

329 **Ilyas G**, Cingolani F, Zhao E, Tanaka K, Czaja MJ. Decreased Macrophage Autophagy Promotes Liver Injury and Inflammation from Alcohol. *Alcohol Clin Exp Res* 2019; **43**: 1403-1413 [PMID: 30964198 DOI: 10.1111/acer.14041]

330 **Liang S**, Zhong Z, Kim SY, Uchiyama R, Roh YS, Matsushita H, Gottlieb RA, Seki E. Murine macrophage autophagy protects against alcohol-induced liver injury by degrading interferon regulatory factor 1 (IRF1) and removing damaged mitochondria. *J Biol Chem* 2019; **294**: 12359-12369 [PMID: 31235522 DOI: 10.1074/jbc.RA119.007409]

331 **Xie ZY**, Xiao ZH, Wang FF. Inhibition of autophagy reverses alcohol-induced hepatic stellate cells activation through activation of Nrf2-Keap1-ARE signaling pathway. *Biochimie* 2018; **147**: 55-62 [PMID: 29305174 DOI: 10.1016/j.biochi.2017.12.013]

332 **Szabo G**, Kamath PS, Shah VH, Thursz M, Mathurin P; EASL-AASLD Joint Meeting. Alcohol-Related Liver Disease: Areas of Consensus, Unmet Needs and Opportunities for Further Study. *Hepatology* 2019; **69**: 2271-2283 [PMID: 30645002 DOI: 10.1002/hep.30369]

333 **You Y**, Li WZ, Zhang S, Hu B, Li YX, Li HD, Tang HH, Li QW, Guan YY, Liu LX, Bao WL, Shen X. SNX10 mediates alcohol-induced liver injury and steatosis by regulating the activation of chaperone-mediated autophagy. *J Hepatol* 2018; **69**: 129-141 [PMID: 29452206 DOI: 10.1016/j.jhep.2018.01.038]

334 **Cai Y**, Jogasuria A, Yin H, Xu MJ, Hu X, Wang J, Kim C, Wu J, Lee K, Gao B, You M. The Detrimental Role Played by Lipocalin-2 in Alcoholic Fatty Liver in Mice. *Am J Pathol* 2016; **186**: 2417-2428 [PMID: 27427417 DOI: 10.1016/j.ajpath.2016.05.006]

335 **Han W**, Fu X, Xie J, Meng Z, Gu Y, Wang X, Li L, Pan H, Huang W. MiR-26a enhances autophagy to protect against ethanol-induced acute liver injury. *J Mol Med (Berl)* 2015; **93**: 1045-1055 [PMID: 25877859 DOI: 10.1007/s00109-015-1282-2]

336 **Babuta M**, Furi I, Bala S, Bukong TN, Lowe P, Catalano D, Calenda C, Kodys K, Szabo G. Dysregulated Autophagy and Lysosome Function Are Linked to Exosome Production by Micro-RNA 155 in Alcoholic Liver Disease. *Hepatology* 2019; **70**: 2123-2141 [PMID: 31090940 DOI: 10.1002/hep.30766]

337 **Thomes PG**, Rasineni K, Yang L, Donohue TM Jr, Kubik JL, McNiven MA, Casey CA. Ethanol withdrawal mitigates fatty liver by normalizing lipid catabolism. *Am J Physiol Gastrointest Liver Physiol* 2019; **316**: G509-G518 [PMID: 30714813 DOI: 10.1152/ajpgi.00376.2018]

338 **Kirchgesner T**, Danse E. Drink responsibly! *JBR-BTR* 2014; **97**: 44 [PMID: 24765774 DOI: 10.5334/jbr-btr.13]

339 **Thiele M**, Rausch V, Fluhr G, Kjærgaard M, Piecha F, Mueller J, Straub BK, Lupșor-Platon M, De-Ledinghen V, Seitz HK, Detlefsen S, Madsen B, Krag A, Mueller S. Controlled attenuation parameter and alcoholic hepatic steatosis: Diagnostic accuracy and role of alcohol detoxification. *J Hepatol* 2018; **68**: 1025-1032 [PMID: 29343427 DOI: 10.1016/j.jhep.2017.12.029]

340 **Yan S**, Khambu B, Hong H, Liu G, Huda N, Yin XM. Autophagy, Metabolism, and Alcohol-Related Liver Disease: Novel Modulators and Functions. *Int J Mol Sci* 2019; **20** [PMID: 31614437 DOI: 10.3390/ijms20205029]

341 **Yang L**, Yang C, Thomes PG, Kharbanda KK, Casey CA, McNiven MA, Donohue TM Jr. Lipophagy and Alcohol-Induced Fatty Liver. *Front Pharmacol* 2019; **10**: 495 [PMID: 31143122 DOI: 10.3389/fphar.2019.00495]

342 **Chao X**, Ding WX. Role and mechanisms of autophagy in alcohol-induced liver injury. *Adv Pharmacol* 2019; **85**: 109-131 [PMID: 31307584 DOI: 10.1016/bs.apha.2019.01.008]

343 **Tang L**, Yang F, Fang Z, Hu C. Resveratrol Ameliorates Alcoholic Fatty Liver by Inducing Autophagy. *Am J Chin Med* 2016; **44**: 1207-1220 [PMID: 27627919 DOI: 10.1142/S0192415X16500671]

344 **Atef MM**, Hafez YM, Alshenawy HA, Emam MN. Ameliorative effects of autophagy inducer, simvastatin on alcohol-induced liver disease in a rat model. *J Cell Biochem* 2018 [PMID: 30417426 DOI: 10.1002/jcb.28042]

345 **Guo X**, Cui R, Zhao J, Mo R, Peng L, Yan M. Corosolic acid protects hepatocytes against ethanol-induced damage by modulating mitogen-activated protein kinases and activating autophagy. *Eur J Pharmacol* 2016; **791**: 578-588 [PMID: 27663281 DOI: 10.1016/j.ejphar.2016.09.031]

346 **Li Y**, Chen M, Wang J, Guo X, Xiao L, Liu P, Liu L, Tang Y, Yao P. Quercetin ameliorates autophagy in alcohol liver disease associated with lysosome through mTOR-TFEB pathway. *J Funct Foods* 2019; **52**: 177-185 [DOI: 10.1016/j.jff.2018.10.033]

347 **Shi X**, Sun R, Zhao Y, Fu R, Wang R, Zhao H, Wang Z, Tang F, Zhang N, Tian X, Yao J. Promotion of autophagosome–lysosome fusion *via* salvianolic acid A-mediated SIRT1 upregulation ameliorates alcoholic liver disease. *RSC Adv* 2018; **8**: 20411-20422 [DOI: 10.1039/C8RA00798E]

348 **Lemasters JJ**, Zhong Z. Mitophagy in hepatocytes: Types, initiators and role in adaptive ethanol metabolism. *Liver Res* 2018; **2**: 125-132 [PMID: 31157120 DOI: 10.1016/j.livres.2018.09.005]

349 **Mao Y**, Da L, Tang H, Yang J, Lei Y, Tiollais P, Li T, Zhao M. Hepatitis B virus X protein reduces starvation-induced cell death through activation of autophagy and inhibition of mitochondrial apoptotic pathway. *Biochem Biophys Res Commun* 2011; **415**: 68-74 [PMID: 22020078 DOI: 10.1016/j.bbrc.2011.10.013]

350 **Liu B**, Fang M, Hu Y, Huang B, Li N, Chang C, Huang R, Xu X, Yang Z, Chen Z, Liu W. Hepatitis B virus X protein inhibits autophagic degradation by impairing lysosomal maturation. *Autophagy* 2014; **10**: 416-430 [PMID: 24401568 DOI: 10.4161/auto.27286]

351 **Cho HK**, Cheong KJ, Kim HY, Cheong J. Endoplasmic reticulum stress induced by hepatitis B virus X protein enhances cyclo-oxygenase 2 expression *via* activating transcription factor 4. *Biochem J* 2011; **435**: 431-439 [PMID: 21244365 DOI: 10.1042/BJ20102071]

352 **Li B**, Gao B, Ye L, Han X, Wang W, Kong L, Fang X, Zeng Y, Zheng H, Li S, Wu Z, Ye L. Hepatitis B virus X protein (HBx) activates ATF6 and IRE1-XBP1 pathways of unfolded protein response. *Virus Res* 2007; **124**: 44-49 [PMID: 17092596 DOI: 10.1016/j.virusres.2006.09.011]

353 **Xu Z**, Jensen G, Yen TS. Activation of hepatitis B virus S promoter by the viral large surface protein *via* induction of stress in the endoplasmic reticulum. *J Virol* 1997; **71**: 7387-7392 [PMID: 9311817 DOI: 10.1128/JVI.71.10.7387-7392.1997]

354 **Hsieh YH**, Su IJ, Wang HC, Chang WW, Lei HY, Lai MD, Chang WT, Huang W. Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. *Carcinogenesis* 2004; **25**: 2023-2032 [PMID: 15180947 DOI: 10.1093/carcin/bgh207]

355 **Na B**, Huang Z, Wang Q, Qi Z, Tian Y, Lu CC, Yu J, Hanes MA, Kakar S, Huang EJ, Ou JH, Liu L, Yen TS. Transgenic expression of entire hepatitis B virus in mice induces hepatocarcinogenesis independent of chronic liver injury. *PLoS One* 2011; **6**: e26240 [PMID: 22022578 DOI: 10.1371/journal.pone.0026240]

356 **Li J**, Liu Y, Wang Z, Liu K, Wang Y, Liu J, Ding H, Yuan Z. Subversion of cellular autophagy machinery by hepatitis B virus for viral envelopment. *J Virol* 2011; **85**: 6319-6333 [PMID: 21507968 DOI: 10.1128/JVI.02627-10]

357 **Tang H**, Da L, Mao Y, Li Y, Li D, Xu Z, Li F, Wang Y, Tiollais P, Li T, Zhao M. Hepatitis B virus X protein sensitizes cells to starvation-induced autophagy *via* up-regulation of beclin 1 expression. *Hepatology* 2009; **49**: 60-71 [PMID: 19065679 DOI: 10.1002/hep.22581]

358 **Zhong L**, Shu W, Dai W, Gao B, Xiong S. Reactive Oxygen Species-Mediated c-Jun NH_2-Terminal Kinase Activation Contributes to Hepatitis B Virus X Protein-Induced Autophagy *via* Regulation of the Beclin-1/Bcl-2 Interaction. *J Virol* 2017; **91** [PMID: 28515304 DOI: 10.1128/JVI.00001-17]

359 **Fu S**, Wang J, Hu X, Zhou RR, Fu Y, Tang D, Kang R, Huang Y, Sun L, Li N, Fan XG. Crosstalk between hepatitis B virus X and high-mobility group box 1 facilitates autophagy in hepatocytes. *Mol Oncol* 2018; **12**: 322-338 [PMID: 29316268 DOI: 10.1002/1878-0261.12165]

360 **Cheng LS**, Li J, Liu Y, Wang FP, Wang SQ, She WM, Wu SD, Qi XL, Zhou YP, Jiang W. HMGB1-induced autophagy: a new pathway to maintain Treg function during chronic hepatitis B virus infection. *Clin Sci (Lond)* 2017; **131**: 381-394

[PMID: 28082516 DOI: 10.1042/CS20160704]
361 **Kim SJ**, Khan M, Quan J, Till A, Subramani S, Siddiqui A. Hepatitis B virus disrupts mitochondrial dynamics: induces fission and mitophagy to attenuate apoptosis. *PLoS Pathog* 2013; **9**: e1003722 [PMID: 24339771 DOI: 10.1371/journal.ppat.1003722]
362 **Huang XY**, Li D, Chen ZX, Huang YH, Gao WY, Zheng BY, Wang XZ. Hepatitis B Virus X protein elevates Parkin-mediated mitophagy through Lon Peptidase in starvation. *Exp Cell Res* 2018; **368**: 75-83 [PMID: 29689279 DOI: 10.1016/j.yexcr.2018.04.016]
363 **Wang J**, Shi Y, Yang H. [Infection with hepatitis B virus enhances basal autophagy]. *Wei Sheng Wu Xue Bao* 2010; **50**: 1651-1656 [PMID: 21365919]
364 **Lin CL**, Kao JH. Hepatitis B viral factors and clinical outcomes of chronic hepatitis B. *J Biomed Sci* 2008; **15**: 137-145 [PMID: 18058038 DOI: 10.1007/s11373-007-9225-8]
365 **Xie M**, Yang Z, Liu Y, Zheng M. The role of HBV-induced autophagy in HBV replication and HBV related-HCC. *Life Sci* 2018; **205**: 107-112 [PMID: 29709654 DOI: 10.1016/j.lfs.2018.04.051]
366 **Choi Y**, Bowman JW, Jung JU. Autophagy during viral infection - a double-edged sword. *Nat Rev Microbiol* 2018; **16**: 341-354 [PMID: 29556036 DOI: 10.1038/s41579-018-0003-6]
367 **Sir D**, Ou JH. Autophagy in viral replication and pathogenesis. *Mol Cells* 2010; **29**: 1-7 [PMID: 20077024 DOI: 10.1007/s10059-010-0014-2]
368 **Sir D**, Ann DK, Ou JH. Autophagy by hepatitis B virus and for hepatitis B virus. *Autophagy* 2010; **6**: 548-549 [PMID: 20305390 DOI: 10.4161/auto.6.4.11669]
369 **Sir D**, Tian Y, Chen WL, Ann DK, Yen TS, Ou JH. The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. *Proc Natl Acad Sci USA* 2010; **107**: 4383-4388 [PMID: 20142477 DOI: 10.1073/pnas.0911373107]
370 **Tian Y**, Sir D, Kuo CF, Ann DK, Ou JH. Autophagy required for hepatitis B virus replication in transgenic mice. *J Virol* 2011; **85**: 13453-13456 [PMID: 21957292 DOI: 10.1128/JVI.06064-11]
371 **Lan SH**, Wu SY, Zuchini R, Lin XZ, Su IJ, Tsai TF, Lin YJ, Wu CT, Liu HS. Autophagy suppresses tumorigenesis of hepatitis B virus-associated hepatocellular carcinoma through degradation of microRNA-224. *Hepatology* 2014; **59**: 505-517 [PMID: 23913306 DOI: 10.1002/hep.26659]
372 **Luo MX**, Wong SH, Chan MT, Yu L, Yu SS, Wu F, Xiao Z, Wang X, Zhang L, Cheng AS, Ng SS, Chan FK, Cho CH, Yu J, Sung JJ, Wu WK. Autophagy Mediates HBx-Induced Nuclear Factor-κB Activation and Release of IL-6, IL-8, and CXCL2 in Hepatocytes. *J Cell Physiol* 2015; **230**: 2382-2389 [PMID: 25708728 DOI: 10.1002/jcp.24967]
373 **Hu S**, Liu X, Gao Y, Zhou R, Wei M, Dong J, Yan H, Zhao Y. Hepatitis B Virus Inhibits Neutrophil Extracellular Trap Release by Modulating Reactive Oxygen Species Production and Autophagy. *J Immunol* 2019; **202**: 805-815 [PMID: 30567731 DOI: 10.4049/jimmunol.1800871]
374 **Lin Y**, Wu C, Wang X, Liu S, Kemper T, Li F, Squire A, Zhu Y, Zhang J, Chen X, Lu M. Synaptosomal-associated protein 29 is required for the autophagic degradation of hepatitis B virus. *FASEB J* 2019; **33**: 6023-6034 [PMID: 30742775 DOI: 10.1096/fj.201801995RR]
375 **Lin Y**, Wu C, Wang X, Liu S, Zhao K, Kemper T, Yu H, Li M, Zhang J, Chen M, Zhu Y, Chen X, Lu M. Glucosamine promotes hepatitis B virus replication through its dual effects in suppressing autophagic degradation and inhibiting MTORC1 signaling. *Autophagy* 2020; **16**: 548-561 [PMID: 31204557 DOI: 10.1080/15548627.2019.1632104]
376 **Gracia-Sancho J**, Guixé-Muntet S, Hide D, Bosch J. Modulation of autophagy for the treatment of liver diseases. *Expert Opin Investig Drugs* 2014; **23**: 965-977 [PMID: 24749698 DOI: 10.1517/13543784.2014.912274]
377 **Döring T**, Zeyen L, Bartusch C, Prange R. Hepatitis B Virus Subverts the Autophagy Elongation Complex Atg5-12/16L1 and Does Not Require Atg8/LC3 Lipidation for Viral Maturation. *J Virol* 2018; **92** [PMID: 29367244 DOI: 10.1128/JVI.01513-17]
378 **Shin GC**, Kang HS, Lee AR, Kim KH. Hepatitis B virus-triggered autophagy targets TNFRSF10B/death receptor 5 for degradation to limit TNFSF10/TRAIL response. *Autophagy* 2016; **12**: 2451-2466 [PMID: 27740879 DOI: 10.1080/15548627.2016.1239002]
379 **Khabir M**, Aliche AZ, Sureau C, Blanchet M, Labonté P. Hepatitis Delta Virus Alters the Autophagy Process To Promote Its Genome Replication. *J Virol* 2020; **94** [PMID: 31748400 DOI: 10.1128/jvi.01936-19]
380 **Khan M**, Imam H, Siddiqui A. Subversion of cellular autophagy during virus infection: Insights from hepatitis B and hepatitis C viruses. *Liver Res* 2018; **2**: 146-156 [PMID: 31803515 DOI: 10.1016/j.livres.2018.09.002]
381 **Wang L**, Ou JH. Hepatitis C virus and autophagy. *Biol Chem* 2015; **396**: 1215-1222 [PMID: 26024249 DOI: 10.1515/hsz-2015-0172]
382 **Chan ST**, Ou JJ. Hepatitis C Virus-Induced Autophagy and Host Innate Immune Response. *Viruses* 2017; **9** [PMID: 28805674 DOI: 10.3390/v9080224]
383 **Wang J**, Kang R, Huang H, Xi X, Wang B, Wang J, Zhao Z. Hepatitis C virus core protein activates autophagy through EIF2AK3 and ATF6 UPR pathway-mediated MAP1LC3B and ATG12 expression. *Autophagy* 2014; **10**: 766-784 [PMID: 24589849 DOI: 10.4161/auto.27954]
384 **Huang H**, Kang R, Wang J, Luo G, Yang W, Zhao Z. Hepatitis C virus inhibits AKT-tuberous sclerosis complex (TSC), the mechanistic target of rapamycin (MTOR) pathway, through endoplasmic reticulum stress to induce autophagy. *Autophagy* 2013; **9**: 175-195 [PMID: 23169238 DOI: 10.4161/auto.22791]
385 **Kim JY**, Wang L, Lee J, Ou JJ. Hepatitis C Virus Induces the Localization of Lipid Rafts to Autophagosomes for Its RNA Replication. *J Virol* 2017; **91** [PMID: 28747506 DOI: 10.1128/JVI.00541-17]
386 **Sir D**, Kuo CF, Tian Y, Liu HM, Huang EJ, Jung JU, Machida K, Ou JH. Replication of hepatitis C virus RNA on autophagosomal membranes. *J Biol Chem* 2012; **287**: 18036-18043 [PMID: 22496373 DOI: 10.1074/jbc.M111.320085]
387 **Ferraris P**, Blanchard E, Roingeard P. Ultrastructural and biochemical analyses of hepatitis C virus-associated host cell membranes. *J Gen Virol* 2010; **91**: 2230-2237 [PMID: 20484561 DOI: 10.1099/vir.0.022186-0]
388 **Wang L**, Ou JJ. Regulation of Autophagy by Hepatitis C Virus for Its Replication. *DNA Cell Biol* 2018; **37**: 287-290 [PMID: 29350547 DOI: 10.1089/dna.2017.4115]

389 **Wang L**, Kim JY, Liu HM, Lai MMC, Ou JJ. HCV-induced autophagosomes are generated *via* homotypic fusion of phagophores that mediate HCV RNA replication. *PLoS Pathog* 2017; **13**: e1006609 [PMID: 28931085 DOI: 10.1371/journal.ppat.1006609]

390 **Shrivastava S**, Devhare P, Sujijantarat N, Steele R, Kwon YC, Ray R, Ray RB. Knockdown of Autophagy Inhibits Infectious Hepatitis C Virus Release by the Exosomal Pathway. *J Virol* 2016; **90**: 1387-1396 [PMID: 26581990 DOI: 10.1128/JVI.02383-15]

391 **Mori H**, Fukuhara T, Ono C, Tamura T, Sato A, Fauzyah Y, Wada M, Okamoto T, Noda T, Yoshimori T, Matsuura Y. Induction of selective autophagy in cells replicating hepatitis C virus genome. *J Gen Virol* 2018; **99**: 1643-1657 [PMID: 30311874 DOI: 10.1099/jgv.0.001161]

392 **Mohl BP**, Tedbury PR, Griffin S, Harris M. Hepatitis C virus-induced autophagy is independent of the unfolded protein response. *J Virol* 2012; **86**: 10724-10732 [PMID: 22837205 DOI: 10.1128/JVI.01667-12]

393 **Dreux M**, Gastaminza P, Wieland SF, Chisari FV. The autophagy machinery is required to initiate hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 14046-14051 [PMID: 19666601 DOI: 10.1073/pnas.0907344106]

394 **Romero-Brey I**, Merz A, Chiramel A, Lee JY, Chlanda P, Haselman U, Santarella-Mellwig R, Habermann A, Hoppe S, Kallis S, Walther P, Antony C, Krijnse-Locker J, Bartenschlager R. Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis C virus replication. *PLoS Pathog* 2012; **8**: e1003056 [PMID: 23236278 DOI: 10.1371/journal.ppat.1003056]

395 **Paul D**, Hoppe S, Saher G, Krijnse-Locker J, Bartenschlager R. Morphological and biochemical characterization of the membranous hepatitis C virus replication compartment. *J Virol* 2013; **87**: 10612-10627 [PMID: 23885072 DOI: 10.1128/JVI.01370-13]

396 **Ke PY**, Chen SS. Activation of the unfolded protein response and autophagy after hepatitis C virus infection suppresses innate antiviral immunity in vitro. *J Clin Invest* 2011; **121**: 37-56 [PMID: 21135505 DOI: 10.1172/JCI41474]

397 **Chan ST**, Lee J, Narula M, Ou JJ. Suppression of Host Innate Immune Response by Hepatitis C Virus *via* Induction of Autophagic Degradation of TRAF6. *J Virol* 2016; **90**: 10928-10935 [PMID: 27681126 DOI: 10.1128/JVI.01365-16]

398 **Sir D**, Chen WL, Choi J, Wakita T, Yen TS, Ou JH. Induction of incomplete autophagic response by hepatitis C virus *via* the unfolded protein response. *Hepatology* 2008; **48**: 1054-1061 [PMID: 18688877 DOI: 10.1002/hep.22464]

399 **Dash S**, Chava S, Aydin Y, Chandra PK, Ferraris P, Chen W, Balart LA, Wu T, Garry RF. Hepatitis C Virus Infection Induces Autophagy as a Prosurvival Mechanism to Alleviate Hepatic ER-Stress Response. *Viruses* 2016; **8** [PMID: 27223299 DOI: 10.3390/v8050150]

400 **Taguwa S**, Kambara H, Fujita N, Noda T, Yoshimori T, Koike K, Moriishi K, Matsuura Y. Dysfunction of autophagy participates in vacuole formation and cell death in cells replicating hepatitis C virus. *J Virol* 2011; **85**: 13185-13194 [PMID: 21994453 DOI: 10.1128/JVI.06099-11]

401 **Vescovo T**, Romagnoli A, Perdomo AB, Corazzari M, Ciccosanti F, Alonzi T, Nardacci R, Ippolito G, Tripodi M, Garcia-Monzon C, Lo Iacono O, Piacentini M, Fimia GM. Autophagy protects cells from HCV-induced defects in lipid metabolism. *Gastroenterology* 2012; **142**: 644-653. e3 [PMID: 22155365 DOI: 10.1053/j.gastro.2011.11.033]

402 **Chu VC**, Bhattacharya S, Nomoto A, Lin J, Zaidi SK, Oberley TD, Weinman SA, Azhar S, Huang TT. Persistent expression of hepatitis C virus non-structural proteins leads to increased autophagy and mitochondrial injury in human hepatoma cells. *PLoS One* 2011; **6**: e28551 [PMID: 22164304 DOI: 10.1371/journal.pone.0028551]

403 **Kim SJ**, Syed GH, Khan M, Chiu WW, Sohail MA, Gish RG, Siddiqui A. Hepatitis C virus triggers mitochondrial fission and attenuates apoptosis to promote viral persistence. *Proc Natl Acad Sci USA* 2014; **111**: 6413-6418 [PMID: 24733894 DOI: 10.1073/pnas.1321114111]

404 **Jassey A**, Liu CH, Changou CA, Richardson CD, Hsu HY, Lin LT. Hepatitis C Virus Non-Structural Protein 5A (NS5A) Disrupts Mitochondrial Dynamics and Induces Mitophagy. *Cells* 2019; **8** [PMID: 30934919 DOI: 10.3390/cells8040290]

405 **Hara Y**, Yanatori I, Ikeda M, Kiyokage E, Nishina S, Tomiyama Y, Toida K, Kishi F, Kato N, Imamura M, Chayama K, Hino K. Hepatitis C virus core protein suppresses mitophagy by interacting with parkin in the context of mitochondrial depolarization. *Am J Pathol* 2014; **184**: 3026-3039 [PMID: 25244949 DOI: 10.1016/j.ajpath.2014.07.024]

406 **Ren H**, Elgner F, Jiang B, Himmelsbach K, Medvedev R, Ploen D, Hildt E. The Autophagosomal SNARE Protein Syntaxin 17 Is an Essential Factor for the Hepatitis C Virus Life Cycle. *J Virol* 2016; **90**: 5989-6000 [PMID: 27099307 DOI: 10.1128/JVI.00551-16]

407 **Kurt R**, Chandra PK, Aboulnasr F, Panigrahi R, Ferraris P, Aydin Y, Reiss K, Wu T, Balart LA, Dash S. Chaperone-Mediated Autophagy Targets IFNAR1 for Lysosomal Degradation in Free Fatty Acid Treated HCV Cell Culture. *PLoS One* 2015; **10**: e0125962 [PMID: 25961570 DOI: 10.1371/journal.pone.0125962]

408 **Matsui C**, Deng L, Minami N, Abe T, Koike K, Shoji I. Hepatitis C Virus NS5A Protein Promotes the Lysosomal Degradation of Hepatocyte Nuclear Factor 1α *via* Chaperone-Mediated Autophagy. *J Virol* 2018; **92** [PMID: 29695419 DOI: 10.1128/JVI.00639-18]

409 **Zhang MQ**, Li JR, Peng ZG, Zhang JP. Differential Effects of Autophagy-Related 10 Protein on HCV Replication and Autophagy Flux Are Mediated by Its Cysteine^{44} and Cysteine^{135}. *Front Immunol* 2018; **9**: 2176 [PMID: 30319633 DOI: 10.3389/fimmu.2018.02176]

410 **Zhao Q**, Hu ZY, Zhang JP, Jiang JD, Ma YY, Li JR, Peng ZG, Chen JH. Dual Roles of Two Isoforms of Autophagy-related Gene ATG10 in HCV-Subgenomic replicon Mediated Autophagy Flux and Innate Immunity. *Sci Rep* 2017; **7**: 11250 [PMID: 28900156 DOI: 10.1038/s41598-017-11105-3]

411 **Mizui T**, Yamashina S, Tanida I, Takei Y, Ueno T, Sakamoto N, Ikejima K, Kitamura T, Enomoto N, Sakai T, Kominami E, Watanabe S. Inhibition of hepatitis C virus replication by chloroquine targeting virus-associated autophagy. *J Gastroenterol* 2010; **45**: 195-203 [PMID: 19760134 DOI: 10.1007/s00535-009-0132-9]

412 **Kim JY**, Ou JJ. Regulation of Apolipoprotein E Trafficking by Hepatitis C Virus-Induced Autophagy. *J Virol* 2018; **92** [PMID: 29695434 DOI: 10.1128/JVI.00211-18]

413 **Zhang L**. Autophagy in hepatitis B or C virus infection: An incubator and a potential therapeutic target. *Life Sci* 2020; **242**: 117206 [PMID: 31866520 DOI: 10.1016/j.lfs.2019.117206]

414 **Lazar C**, Uta M, Branza-Nichita N. Modulation of the unfolded protein response by the human hepatitis B virus. *Front*

Microbiol 2014; **5**: 433 [PMID: 25191311 DOI: 10.3389/fmicb.2014.00433]

415 **Ait-Goughoulte M**, Kanda T, Meyer K, Ryerse JS, Ray RB, Ray R. Hepatitis C virus genotype 1a growth and induction of autophagy. *J Virol* 2008; **82**: 2241-2249 [PMID: 18077704 DOI: 10.1128/JVI.02093-07]

416 **Rautou PE**, Cazals-Hatem D, Feldmann G, Mansouri A, Grodet A, Barge S, Martinot-Peignoux M, Duces A, Bièche I, Lebrec D, Bedossa P, Paradis V, Marcellin P, Valla D, Asselah T, Moreau R. Changes in autophagic response in patients with chronic hepatitis C virus infection. *Am J Pathol* 2011; **178**: 2708-2715 [PMID: 21641393 DOI: 10.1016/j.ajpath.2011.02.021]

417 **Aweya JJ**, Mak TM, Lim SG, Tan YJ. The p7 protein of the hepatitis C virus induces cell death differently from the influenza A virus viroporin M2. *Virus Res* 2013; **172**: 24-34 [PMID: 23246447 DOI: 10.1016/j.virusres.2012.12.005]

418 **Ríos-Ocampo WA**, Daemen T, Buist-Homan M, Faber KN, Navas MC, Moshage H. Hepatitis C virus core or NS3/4A protein expression preconditions hepatocytes against oxidative stress and endoplasmic reticulum stress. *Redox Rep* 2019; **24**: 17-26 [PMID: 30909829 DOI: 10.1080/13510002.2019.1596431]

419 **Su WC**, Chao TC, Huang YL, Weng SC, Jeng KS, Lai MM. Rab5 and class III phosphoinositide 3-kinase Vps34 are involved in hepatitis C virus NS4B-induced autophagy. *J Virol* 2011; **85**: 10561-10571 [PMID: 21835792 DOI: 10.1128/JVI.00173-11]

420 **Tanida I**, Fukasawa M, Ueno T, Kominami E, Wakita T, Hanada K. Knockdown of autophagy-related gene decreases the production of infectious hepatitis C virus particles. *Autophagy* 2009; **5**: 937-945 [PMID: 19625776 DOI: 10.4161/auto.5.7.9243]

421 **Tsuchida T**, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 397-411 [PMID: 28487545 DOI: 10.1038/nrgastro.2017.38]

422 **Williams MJ**, Clouston AD, Forbes SJ. Links between hepatic fibrosis, ductular reaction, and progenitor cell expansion. *Gastroenterology* 2014; **146**: 349-356 [PMID: 24315991 DOI: 10.1053/j.gastro.2013.11.034]

423 **Sato K**, Marzioni M, Meng F, Francis H, Glaser S, Alpini G. Ductular Reaction in Liver Diseases: Pathological Mechanisms and Translational Significances. *Hepatology* 2019; **69**: 420-430 [PMID: 30070383 DOI: 10.1002/hep.30150]

424 **Gouw AS**, Clouston AD, Theise ND. Ductular reactions in human liver: diversity at the interface. *Hepatology* 2011; **54**: 1853-1863 [PMID: 21983984 DOI: 10.1002/hep.24613]

425 **Clouston AD**, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. *Hepatology* 2005; **41**: 809-818 [PMID: 15793848 DOI: 10.1002/hep.20650]

426 **Richardson MM**, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, Purdie DM, Demetris AJ, Clouston AD. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. *Gastroenterology* 2007; **133**: 80-90 [PMID: 17631134 DOI: 10.1053/j.gastro.2007.05.012]

427 **Wood MJ**, Gadd VL, Powell LW, Ramm GA, Clouston AD. Ductular reaction in hereditary hemochromatosis: the link between hepatocyte senescence and fibrosis progression. *Hepatology* 2014; **59**: 848-857 [PMID: 24037895 DOI: 10.1002/hep.26706]

428 **Ni HM**, Bockus A, Wozniak AL, Jones K, Weinman S, Yin XM, Ding WX. Dissecting the dynamic turnover of GFP-LC3 in the autolysosome. *Autophagy* 2011; **7**: 188-204 [PMID: 21107021 DOI: 10.4161/auto.7.2.14181]

429 **Song Y**, Zhao Y, Wang F, Tao L, Xiao J, Yang C. Autophagy in hepatic fibrosis. *Biomed Res Int* 2014; **2014**: 436242 [PMID: 24779010 DOI: 10.1155/2014/436242]

430 **Kwanten WJ**, Martinet W, Michielsen PP, Francque SM. Role of autophagy in the pathophysiology of nonalcoholic fatty liver disease: a controversial issue. *World J Gastroenterol* 2014; **20**: 7325-7338 [PMID: 24966603 DOI: 10.3748/wjg.v20.i23.7325]

431 **Li J**, Chen K, Li S, Feng J, Liu T, Wang F, Zhang R, Xu S, Zhou Y, Zhou S, Xia Y, Lu J, Zhou Y, Guo C. Protective effect of fucoidan from Fucus vesiculosus on liver fibrosis *via* the TGF-β1/Smad pathway-mediated inhibition of extracellular matrix and autophagy. *Drug Des Devel Ther* 2016; **10**: 619-630 [PMID: 26929597 DOI: 10.2147/DDDT.S98740]

432 **Gordy C**, He YW. The crosstalk between autophagy and apoptosis: where does this lead? *Protein Cell* 2012; **3**: 17-27 [PMID: 22314807 DOI: 10.1007/s13238-011-1127-x]

433 **Mao YQ**, Fan XM. Autophagy: A new therapeutic target for liver fibrosis. *World J Hepatol* 2015; **7**: 1982-1986 [PMID: 26261688 DOI: 10.4254/wjh.v7.i16.1982]

434 **Miyamae Y**, Nishito Y, Nakai N, Nagumo Y, Usui T, Masuda S, Kambe T, Nagao M. Tetrandrine induces lipid accumulation through blockade of autophagy in a hepatic stellate cell line. *Biochem Biophys Res Commun* 2016; **477**: 40-46 [PMID: 27270032 DOI: 10.1016/j.bbrc.2016.06.018]

435 **He W**, Wang B, Yang J, Zhuang Y, Wang L, Huang X, Chen J. Chloroquine improved carbon tetrachloride-induced liver fibrosis through its inhibition of the activation of hepatic stellate cells: role of autophagy. *Biol Pharm Bull* 2014; **37**: 1505-1509 [PMID: 25177034 DOI: 10.1248/bpb.b14-00297]

436 **Lee AY**, Lee JW, Kim JE, Mock HJ, Park S, Kim S, Hong SH, Kim JY, Park EJ, Kang KS, Kim KP, Cho MH. Dihydroceramide is a key metabolite that regulates autophagy and promotes fibrosis in hepatic steatosis model. *Biochem Biophys Res Commun* 2017; **494**: 460-469 [PMID: 29066349 DOI: 10.1016/j.bbrc.2017.10.110]

437 **Zhang XW**, Mi S, Li Z, Zhou JC, Xie J, Hua F, Li K, Cui B, Lv XX, Yu JJ, Hu ZW. Antagonism of Interleukin-17A ameliorates experimental hepatic fibrosis by restoring the IL-10/STAT3-suppressed autophagy in hepatocytes. *Oncotarget* 2017; **8**: 9922-9934 [PMID: 28039485 DOI: 10.18632/oncotarget.14266]

438 **Singh R**, Cuervo AM. Lipophagy: connecting autophagy and lipid metabolism. *Int J Cell Biol* 2012; **2012**: 282041 [PMID: 22536247 DOI: 10.1155/2012/282041]

439 **Hernández-Gea V**, Ghiassi-Nejad Z, Rozenfeld R, Gordon R, Fiel MI, Yue Z, Czaja MJ, Friedman SL. Autophagy releases lipid that promotes fibrogenesis by activated hepatic stellate cells in mice and in human tissues. *Gastroenterology* 2012; **142**: 938-946 [PMID: 22240484 DOI: 10.1053/j.gastro.2011.12.044]

440 **Thoen LF**, Guimarães EL, Dollé L, Mannaerts I, Najimi M, Sokal E, van Grunsven LA. A role for autophagy during

hepatic stellate cell activation. *J Hepatol* 2011; **55**: 1353-1360 [PMID: 21803012 DOI: 10.1016/j.jhep.2011.07.010]

441 **Thoen LF**, Guimarães EL, Grunsven LA. Autophagy: a new player in hepatic stellate cell activation. *Autophagy* 2012; **8**: 126-128 [PMID: 22082960 DOI: 10.4161/auto.8.1.18105]

442 **Hernández-Gea V**, Hilscher M, Rozenfeld R, Lim MP, Nieto N, Werner S, Devi LA, Friedman SL. Endoplasmic reticulum stress induces fibrogenic activity in hepatic stellate cells through autophagy. *J Hepatol* 2013; **59**: 98-104 [PMID: 23485523 DOI: 10.1016/j.jhep.2013.02.016]

443 **Vere CC**, Streba CT, Streba L, Rogoveanu I. Lipid serum profile in patients with viral liver cirrhosis. *Med Princ Pract* 2012; **21**: 566-568 [PMID: 22722340 DOI: 10.1159/000339206]

444 **Kim KM**, Han CY, Kim JY, Cho SS, Kim YS, Koo JH, Lee JM, Lim SC, Kang KW, Kim JS, Hwang SJ, Ki SH, Kim SG. $G\alpha_{12}$ overexpression induced by miR-16 dysregulation contributes to liver fibrosis by promoting autophagy in hepatic stellate cells. *J Hepatol* 2018; **68**: 493-504 [PMID: 29080810 DOI: 10.1016/j.jhep.2017.10.011]

445 **Qu Y**, Zhang Q, Cai X, Li F, Ma Z, Xu M, Lu L. Exosomes derived from miR-181-5p-modified adipose-derived mesenchymal stem cells prevent liver fibrosis *via* autophagy activation. *J Cell Mol Med* 2017; **21**: 2491-2502 [PMID: 28382720 DOI: 10.1111/jcmm.13170]

446 **Deng J**, Huang Q, Wang Y, Shen P, Guan F, Li J, Huang H, Shi C. Hypoxia-inducible factor-1alpha regulates autophagy to activate hepatic stellate cells. *Biochem Biophys Res Commun* 2014; **454**: 328-334 [PMID: 25450397 DOI: 10.1016/j.bbrc.2014.10.076]

447 **Fu MY**, He YJ, Lv X, Liu ZH, Shen Y, Ye GR, Deng YM, Shu JC. Transforming growth factorβ1 reduces apoptosis *via* autophagy activation in hepatic stellate cells. *Mol Med Rep* 2014; **10**: 1282-1288 [PMID: 25059289 DOI: 10.3892/mmr.2014.2383]

448 **Li J**, Zeng C, Zheng B, Liu C, Tang M, Jiang Y, Chang Y, Song W, Wang Y, Yang C. HMGB1-induced autophagy facilitates hepatic stellate cells activation: a new pathway in liver fibrosis. *Clin Sci (Lond)* 2018; **132**: 1645-1667 [PMID: 29907694 DOI: 10.1042/CS20180177]

449 **Zhang Z**, Guo M, Zhao S, Shao J, Zheng S. ROS-JNK1/2-dependent activation of autophagy is required for the induction of anti-inflammatory effect of dihydroartemisinin in liver fibrosis. *Free Radic Biol Med* 2016; **101**: 272-283 [PMID: 27989749 DOI: 10.1016/j.freeradbiomed.2016.10.498]

450 **Kim RS**, Hasegawa D, Goossens N, Tsuchida T, Athwal V, Sun X, Robinson CL, Bhattacharya D, Chou HI, Zhang DY, Fuchs BC, Lee Y, Hoshida Y, Friedman SL. The XBP1 Arm of the Unfolded Protein Response Induces Fibrogenic Activity in Hepatic Stellate Cells Through Autophagy. *Sci Rep* 2016; **6**: 39342 [PMID: 27996033 DOI: 10.1038/srep39342]

451 **Hong Y**, Li S, Wang J, Li Y. In vitro inhibition of hepatic stellate cell activation by the autophagy-related lipid droplet protein ATG2A. *Sci Rep* 2018; **8**: 9232 [PMID: 29915313 DOI: 10.1038/s41598-018-27686-6]

452 **Wu L**, Zhang Q, Mo W, Feng J, Li S, Li J, Liu T, Xu S, Wang W, Lu X, Yu Q, Chen K, Xia Y, Lu J, Xu L, Zhou Y, Fan X, Guo C. Quercetin prevents hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing autophagy *via* the TGF-β1/Smads and PI3K/Akt pathways. *Sci Rep* 2017; **7**: 9289 [PMID: 28839277 DOI: 10.1038/s41598-017-09673-5]

453 **Qiu YN**, Wang GH, Zhou F, Hao JJ, Tian L, Guan LF, Geng XK, Ding YC, Wu HW, Zhang KZ. PM2.5 induces liver fibrosis *via* triggering ROS-mediated mitophagy. *Ecotoxicol Environ Saf* 2019; **167**: 178-187 [PMID: 30336408 DOI: 10.1016/j.ecoenv.2018.08.050]

454 **Tian Z**, Chen Y, Yao N, Hu C, Wu Y, Guo D, Liu J, Yang Y, Chen T, Zhao Y, He Y. Role of mitophagy regulation by ROS in hepatic stellate cells during acute liver failure. *Am J Physiol Gastrointest Liver Physiol* 2018; **315**: G374-G384 [PMID: 29648877 DOI: 10.1152/ajpgi.00032.2018]

455 **Li J**, Zhao YR, Tian Z. Roles of hepatic stellate cells in acute liver failure: From the perspective of inflammation and fibrosis. *World J Hepatol* 2019; **11**: 412-420 [PMID: 31183002 DOI: 10.4254/wjh.v11.i5.412]

456 **Wu H**, Chen G, Wang J, Deng M, Yuan F, Gong J. TIM-4 interference in Kupffer cells against CCL4-induced liver fibrosis by mediating Akt1/Mitophagy signalling pathway. *Cell Prolif* 2020; **53**: e12731 [PMID: 31755616 DOI: 10.1111/cpr.12731]

457 **Ding N**, Yu RT, Subramaniam N, Sherman MH, Wilson C, Rao R, Leblanc M, Coulter S, He M, Scott C, Lau SL, Atkins AR, Barish GD, Gunton JE, Liddle C, Downes M, Evans RM. A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. *Cell* 2013; **153**: 601-613 [PMID: 23622244 DOI: 10.1016/j.cell.2013.03.028]

458 **Duran A**, Hernandez ED, Reina-Campos M, Castilla EA, Subramaniam S, Raghunandan S, Roberts LR, Kisseleva T, Karin M, Diaz-Meco MT, Moscat J. p62/SQSTM1 by Binding to Vitamin D Receptor Inhibits Hepatic Stellate Cell Activity, Fibrosis, and Liver Cancer. *Cancer Cell* 2016; **30**: 595-609 [PMID: 27728806 DOI: 10.1016/j.ccell.2016.09.004]

459 **Zhang Z**, Yao Z, Wang L, Ding H, Shao J, Chen A, Zhang F, Zheng S. Activation of ferritinophagy is required for the RNA-binding protein ELAVL1/HuR to regulate ferroptosis in hepatic stellate cells. *Autophagy* 2018; **14**: 2083-2103 [PMID: 30081711 DOI: 10.1080/15548627.2018.1503146]

460 **Krenkel O**, Tacke F. Liver macrophages in tissue homeostasis and disease. *Nat Rev Immunol* 2017; **17**: 306-321 [PMID: 28317925 DOI: 10.1038/nri.2017.11]

461 **Lodder J**, Denaës T, Chobert MN, Wan J, El-Benna J, Pawlotsky JM, Lotersztajn S, Teixeira-Clerc F. Macrophage autophagy protects against liver fibrosis in mice. *Autophagy* 2015; **11**: 1280-1292 [PMID: 26061908 DOI: 10.1080/15548627.2015.1058473]

462 **Ilyas G**, Zhao E, Liu K, Lin Y, Tesfa L, Tanaka KE, Czaja MJ. Macrophage autophagy limits acute toxic liver injury in mice through down regulation of interleukin-1β. *J Hepatol* 2016; **64**: 118-127 [PMID: 26325539 DOI: 10.1016/j.jhep.2015.08.019]

463 **Sun K**, Xu L, Jing Y, Han Z, Chen X, Cai C, Zhao P, Zhao X, Yang L, Wei L. Autophagy-deficient Kupffer cells promote tumorigenesis by enhancing mtROS-NF-κB-IL1α/β-dependent inflammation and fibrosis during the preneoplastic stage of hepatocarcinogenesis. *Cancer Lett* 2017; **388**: 198-207 [PMID: 28011320 DOI: 10.1016/j.canlet.2016.12.004]

464 **Habib A**, Chokr D, Wan J, Hegde P, Mabire M, Siebert M, Ribeiro-Parenti L, Le Gall M, Lettéron P, Pilard N, Mansouri A, Brouillet A, Tardelli M, Weiss E, Le Faouder P, Guillou H, Cravatt BF, Moreau R, Trauner M, Lotersztajn S. Inhibition of monoacylglycerol lipase, an anti-inflammatory and antifibrogenic strategy in the liver. *Gut* 2019; **68**: 522-

532 [PMID: 30301768 DOI: 10.1136/gutjnl-2018-316137]

465 **Ruart M**, Chavarria L, Campreciós G, Suárez-Herrera N, Montironi C, Guixé-Muntet S, Bosch J, Friedman SL, Garcia-Pagán JC, Hernández-Gea V. Impaired endothelial autophagy promotes liver fibrosis by aggravating the oxidative stress response during acute liver injury. *J Hepatol* 2019; **70**: 458-469 [PMID: 30367898 DOI: 10.1016/j.jhep.2018.10.015]

466 **Luo X**, Wang D, Zhu X, Wang G, You Y, Ning Z, Li Y, Jin S, Huang Y, Hu Y, Chen T, Meng Y, Li X. Autophagic degradation of caveolin-1 promotes liver sinusoidal endothelial cells defenestration. *Cell Death Dis* 2018; **9**: 576 [PMID: 29760379 DOI: 10.1038/s41419-018-0567-0]

467 **Boteon YL**, Laing R, Mergental H, Reynolds GM, Mirza DF, Afford SC, Bhogal RH. Mechanisms of autophagy activation in endothelial cell and their targeting during normothermic machine liver perfusion. *World J Gastroenterol* 2017; **23**: 8443-8451 [PMID: 29358854 DOI: 10.3748/wjg.v23.i48.8443]

468 **Pozniak KN**, Pearen MA, Pereira TN, Kramer CSM, Kalita-De Croft P, Nawaratna SK, Fernandez-Rojo MA, Gobert GN, Tirnitz-Parker JEE, Olynyk JK, Shepherd RW, Lewindon PJ, Ramm GA. Taurocholate Induces Biliary Differentiation of Liver Progenitor Cells Causing Hepatic Stellate Cell Chemotaxis in the Ductular Reaction: Role in Pediatric Cystic Fibrosis Liver Disease. *Am J Pathol* 2017; **187**: 2744-2757 [PMID: 28935574 DOI: 10.1016/j.ajpath.2017.08.024]

469 **Hung TM**, Huang YJ, Lin YC, Chen YH, Wu YM, Lee PH. A critical role of autophagy in regulating the mesenchymal transition of ductular cells in liver cirrhosis. *Sci Rep* 2019; **9**: 10673 [PMID: 31337842 DOI: 10.1038/s41598-019-46764-x]

470 **Hung TM**, Hsiao CC, Lin CW, Lee PH. Complex Cell Type-Specific Roles of Autophagy in Liver Fibrosis and Cirrhosis. *Pathogens* 2020; **9** [PMID: 32197543 DOI: 10.3390/pathogens9030225]

471 **Degenhardt K**, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gélinas C, Fan Y, Nelson DA, Jin S, White E. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* 2006; **10**: 51-64 [PMID: 16843265 DOI: 10.1016/j.ccr.2006.06.001]

472 **Yazdani HO**, Huang H, Tsung A. Autophagy: Dual Response in the Development of Hepatocellular Carcinoma. *Cells* 2019; **8** [PMID: 30695997 DOI: 10.3390/cells8020091]

473 **Guo JY**, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V, Coller HA, Dipaola RS, Gelinas C, Rabinowitz JD, White E. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* 2011; **25**: 460-470 [PMID: 21317241 DOI: 10.1101/gad.2016311]

474 **Elgendy M**, Sheridan C, Brumatti G, Martin SJ. Oncogenic Ras-induced expression of Noxa and Beclin-1 promotes autophagic cell death and limits clonogenic survival. *Mol Cell* 2011; **42**: 23-35 [PMID: 21353614 DOI: 10.1016/j.molcel.2011.02.009]

475 **Lim SC**, Han SI. Ursodeoxycholic acid effectively kills drug-resistant gastric cancer cells through induction of autophagic death. *Oncol Rep* 2015; **34**: 1261-1268 [PMID: 26133914 DOI: 10.3892/or.2015.4076]

476 **White DL**, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of Hepatocellular Carcinoma in All 50 United States, From 2000 Through 2012. *Gastroenterology* 2017; **152**: 812-820. e5 [PMID: 27889576 DOI: 10.1053/j.gastro.2016.11.020]

477 **West J**, Card TR, Aithal GP, Fleming KM. Risk of hepatocellular carcinoma among individuals with different aetiologies of cirrhosis: a population-based cohort study. *Aliment Pharmacol Ther* 2017; **45**: 983-990 [PMID: 28144999 DOI: 10.1111/apt.13961]

478 **Chang Y**, Yan W, He X, Zhang L, Li C, Huang H, Nace G, Geller DA, Lin J, Tsung A. miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. *Gastroenterology* 2012; **143**: 177-87. e8 [PMID: 22504094 DOI: 10.1053/j.gastro.2012.04.009]

479 **Ni HM**, Chao X, Yang H, Deng F, Wang S, Bai Q, Qian H, Cui Y, Cui W, Shi Y, Zong WX, Wang Z, Yang L, Ding WX. Dual Roles of Mammalian Target of Rapamycin in Regulating Liver Injury and Tumorigenesis in Autophagy-Defective Mouse Liver. *Hepatology* 2019; **70**: 2142-2155 [PMID: 31095752 DOI: 10.1002/hep.30770]

480 **Cui J**, Gong Z, Shen HM. The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets. *Biochim Biophys Acta* 2013; **1836**: 15-26 [PMID: 23428608 DOI: 10.1016/j.bbcan.2013.02.003]

481 **Sun K**, Guo XL, Zhao QD, Jing YY, Kou XR, Xie XQ, Zhou Y, Cai N, Gao L, Zhao X, Zhang SS, Song JR, Li D, Deng WJ, Li R, Wu MC, Wei LX. Paradoxical role of autophagy in the dysplastic and tumor-forming stages of hepatocarcinoma development in rats. *Cell Death Dis* 2013; **4**: e501 [PMID: 23429287 DOI: 10.1038/cddis.2013.35]

482 **Taniguchi K**, Yamachika S, He F, Karin M. p62/SQSTM1-Dr. Jekyll and Mr. Hyde that prevents oxidative stress but promotes liver cancer. *FEBS Lett* 2016; **590**: 2375-2397 [PMID: 27404485 DOI: 10.1002/1873-3468.12301]

483 **Lazova R**, Camp RL, Klump V, Siddiqui SF, Amaravadi RK, Pawelek JM. Punctate LC3B expression is a common feature of solid tumors and associated with proliferation, metastasis, and poor outcome. *Clin Cancer Res* 2012; **18**: 370-379 [PMID: 22080440 DOI: 10.1158/1078-0432.CCR-11-1282]

484 **Wu DH**, Jia CC, Chen J, Lin ZX, Ruan DY, Li X, Lin Q, Min-Dong, Ma XK, Wan XB, Cheng N, Chen ZH, Xing YF, Wu XY, Wen JY. Autophagic LC3B overexpression correlates with malignant progression and predicts a poor prognosis in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 12225-12233 [PMID: 25256671 DOI: 10.1007/s13277-014-2531-7]

485 **Takamura A**, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* 2011; **25**: 795-800 [PMID: 21498569 DOI: 10.1101/gad.2016211]

486 **Bao L**, Chandra PK, Moroz K, Zhang X, Thung SN, Wu T, Dash S. Impaired autophagy response in human hepatocellular carcinoma. *Exp Mol Pathol* 2014; **96**: 149-154 [PMID: 24369267 DOI: 10.1016/j.yexmp.2013.12.002]

487 **Shi YH**, Ding ZB, Zhou J, Qiu SJ, Fan J. Prognostic significance of Beclin 1-dependent apoptotic activity in hepatocellular carcinoma. *Autophagy* 2009; **5**: 380-382 [PMID: 19145109 DOI: 10.4161/auto.5.3.7658]

488 **Ding ZB**, Shi YH, Zhou J, Qiu SJ, Xu Y, Dai Z, Shi GM, Wang XY, Ke AW, Wu B, Fan J. Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 9167-9175 [PMID: 19010888 DOI: 10.1158/0008-5472.CAN-08-1573]

489 **DeNicola GM**, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA, Tuveson DA. Oncogene-induced Nrf2

transcription promotes ROS detoxification and tumorigenesis. *Nature* 2011; **475**: 106-109 [PMID: 21734707 DOI: 10.1038/nature10189]

490 **Jain A**, Lamark T, Sjøttem E, Larsen KB, Awuh JA, Øvervatn A, McMahon M, Hayes JD, Johansen T. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem* 2010; **285**: 22576-22591 [PMID: 20452972 DOI: 10.1074/jbc.M110.118976]

491 **Lau A**, Wang XJ, Zhao F, Villeneuve NF, Wu T, Jiang T, Sun Z, White E, Zhang DD. A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. *Mol Cell Biol* 2010; **30**: 3275-3285 [PMID: 20421418 DOI: 10.1128/MCB.00248-10]

492 **Inami Y**, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, Watanabe S, Ando J, Iwadate M, Yamamoto M, Lee MS, Tanaka K, Komatsu M. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J Cell Biol* 2011; **193**: 275-284 [PMID: 21482715 DOI: 10.1083/jcb.201102031]

493 **Ni HM**, Woolbright BL, Williams J, Copple B, Cui W, Luyendyk JP, Jaeschke H, Ding WX. Nrf2 promotes the development of fibrosis and tumorigenesis in mice with defective hepatic autophagy. *J Hepatol* 2014; **61**: 617-625 [PMID: 24815875 DOI: 10.1016/j.jhep.2014.04.043]

494 **Saito T**, Ichimura Y, Taguchi K, Suzuki T, Mizushima T, Takagi K, Hirose Y, Nagahashi M, Iso T, Fukutomi T, Ohishi M, Endo K, Uemura T, Nishito Y, Okuda S, Obata M, Kouno T, Imamura R, Tada Y, Obata R, Yasuda D, Takahashi K, Fujimura T, Pi J, Lee MS, Ueno T, Ohe T, Mashino T, Wakai T, Kojima H, Okabe T, Nagano T, Motohashi H, Waguri S, Soga T, Yamamoto M, Tanaka K, Komatsu M. p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. *Nat Commun* 2016; **7**: 12030 [PMID: 27345495 DOI: 10.1038/ncomms12030]

495 **Mitsuishi Y**, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, Yamamoto M, Motohashi H. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* 2012; **22**: 66-79 [PMID: 22789539 DOI: 10.1016/j.ccr.2012.05.016]

496 **Perra A**, Kowalik MA, Ghiso E, Ledda-Columbano GM, Di Tommaso L, Angioni MM, Raschioni C, Testore E, Roncalli M, Giordano S, Columbano A. YAP activation is an early event and a potential therapeutic target in liver cancer development. *J Hepatol* 2014; **61**: 1088-1096 [PMID: 25010260 DOI: 10.1016/j.jhep.2014.06.033]

497 **Turcios L**, Chacon E, Garcia C, Eman P, Cornea V, Jiang J, Spear B, Liu C, Watt DS, Marti F, Gedaly R. Autophagic flux modulation by Wnt/β-catenin pathway inhibition in hepatocellular carcinoma. *PLoS One* 2019; **14**: e0212538 [PMID: 30794613 DOI: 10.1371/journal.pone.0212538]

498 **Wang W**, Xu L, Liu P, Jairam K, Yin Y, Chen K, Sprengers D, Peppelenbosch MP, Pan Q, Smits R. Blocking Wnt Secretion Reduces Growth of Hepatocellular Carcinoma Cell Lines Mostly Independent of β-Catenin Signaling. *Neoplasia* 2016; **18**: 711-723 [PMID: 27851986 DOI: 10.1016/j.neo.2016.10.004]

499 **Kiyono K**, Suzuki HI, Matsuyama H, Morishita Y, Komuro A, Kano MR, Sugimoto K, Miyazono K. Autophagy is activated by TGF-beta and potentiates TGF-beta-mediated growth inhibition in human hepatocellular carcinoma cells. *Cancer Res* 2009; **69**: 8844-8852 [PMID: 19903843 DOI: 10.1158/0008-5472.CAN-08-4401]

500 **Xie R**, Wang F, McKeehan WL, Liu L. Autophagy enhanced by microtubule- and mitochondrion-associated MAP1S suppresses genome instability and hepatocarcinogenesis. *Cancer Res* 2011; **71**: 7537-7546 [PMID: 22037873 DOI: 10.1158/0008-5472.CAN-11-2170]

501 **Jung KH**, Noh JH, Kim JK, Eun JW, Bae HJ, Chang YG, Kim MG, Park WS, Lee JY, Lee SY, Chu IS, Nam SW. Histone deacetylase 6 functions as a tumor suppressor by activating c-Jun NH2-terminal kinase-mediated beclin 1-dependent autophagic cell death in liver cancer. *Hepatology* 2012; **56**: 644-657 [PMID: 22392728 DOI: 10.1002/hep.25699]

502 **Xie HJ**, Noh JH, Kim JK, Jung KH, Eun JW, Bae HJ, Kim MG, Chang YG, Lee JY, Park H, Nam SW. HDAC1 inactivation induces mitotic defect and caspase-independent autophagic cell death in liver cancer. *PLoS One* 2012; **7**: e34265 [PMID: 22496786 DOI: 10.1371/journal.pone.0034265]

503 **Zou M**, Lu N, Hu C, Liu W, Sun Y, Wang X, You Q, Gu C, Xi T, Guo Q. Beclin 1-mediated autophagy in hepatocellular carcinoma cells: implication in anticancer efficiency of oroxylin A *via* inhibition of mTOR signaling. *Cell Signal* 2012; **24**: 1722-1732 [PMID: 22560876 DOI: 10.1016/j.cellsig.2012.04.009]

504 **Luo Z**, Yu G, Lee HW, Li L, Wang L, Yang D, Pan Y, Ding C, Qian J, Wu L, Chu Y, Yi J, Wang X, Sun Y, Jeong LS, Liu J, Jia L. The Nedd8-activating enzyme inhibitor MLN4924 induces autophagy and apoptosis to suppress liver cancer cell growth. *Cancer Res* 2012; **72**: 3360-3371 [PMID: 22562464 DOI: 10.1158/0008-5472.CAN-12-0388]

505 **Yang D**, Li L, Liu H, Wu L, Luo Z, Li H, Zheng S, Gao H, Chu Y, Sun Y, Liu J, Jia L. Induction of autophagy and senescence by knockdown of ROC1 E3 ubiquitin ligase to suppress the growth of liver cancer cells. *Cell Death Differ* 2013; **20**: 235-247 [PMID: 22935614 DOI: 10.1038/cdd.2012.113]

506 **Wang Y**, Han C, Lu L, Magliato S, Wu T. Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. *Hepatology* 2013; **58**: 995-1010 [PMID: 23504944 DOI: 10.1002/hep.26394]

507 **Chang CP**, Yang MC, Liu HS, Lin YS, Lei HY. Concanavalin A induces autophagy in hepatoma cells and has a therapeutic effect in a murine in situ hepatoma model. *Hepatology* 2007; **45**: 286-296 [PMID: 17256764 DOI: 10.1002/hep.21509]

508 **Lei HY**, Chang CP. Induction of autophagy by concanavalin A and its application in anti-tumor therapy. *Autophagy* 2007; **3**: 402-404 [PMID: 17471013 DOI: 10.4161/auto.4280]

509 **Chang CP**, Yang MC, Lei HY. Concanavalin A/IFN-gamma triggers autophagy-related necrotic hepatocyte death through IRGM1-mediated lysosomal membrane disruption. *PLoS One* 2011; **6**: e28323 [PMID: 22163006 DOI: 10.1371/journal.pone.0028323]

510 **Qian H**, Yang Y. Alterations of cellular organelles in human liver-derived hepatoma G2 cells induced by adriamycin. *Anticancer Drugs* 2009; **20**: 779-786 [PMID: 19617817 DOI: 10.1097/CAD.0b013e32832f4e6f]

511 **Yang F**, Gao YH, Wu KW, Deng R, Li DD, Wei ZX, Jiang S, Wu XQ, Feng GK, Li HJ, Zhu XF. A novel sesquiterpene Hirsutanol A induces autophagical cell death in human hepatocellular carcinoma cells by increasing reactive oxygen species. *Chin J Cancer* 2010; **29**: 655-660 [PMID: 20591217 DOI: 10.5732/cjc.009.10702]

512 **Xie SQ**, Li Q, Zhang YH, Wang JH, Mei ZH, Zhao J, Wang CJ. NPC-16, a novel naphthalimide-polyamine conjugate, induced apoptosis and autophagy in human hepatoma HepG2 cells and Bel-7402 cells. *Apoptosis* 2011; **16**: 27-34 [PMID: 20809291 DOI: 10.1007/s10495-010-0537-1]

513 **Zhang JQ**, Li YM, Liu T, He WT, Chen YT, Chen XH, Li X, Zhou WC, Yi JF, Ren ZJ. Antitumor effect of matrine in human hepatoma G2 cells by inducing apoptosis and autophagy. *World J Gastroenterol* 2010; **16**: 4281-4290 [PMID: 20818811 DOI: 10.3748/wjg.v16.i34.4281]

514 **Wang N**, Feng Y, Zhu M, Tsang CM, Man K, Tong Y, Tsao SW. Berberine induces autophagic cell death and mitochondrial apoptosis in liver cancer cells: the cellular mechanism. *J Cell Biochem* 2010; **111**: 1426-1436 [PMID: 20830746 DOI: 10.1002/jcb.22869]

515 **Wang N**, Pan W, Zhu M, Zhang M, Hao X, Liang G, Feng Y. Fangchinoline induces autophagic cell death *via* p53/sestrin2/AMPK signalling in human hepatocellular carcinoma cells. *Br J Pharmacol* 2011; **164**: 731-742 [PMID: 21418191 DOI: 10.1111/j.1476-5381.2011.01349.x]

516 **Hou Q**, Tang X, Liu H, Tang J, Yang Y, Jing X, Xiao Q, Wang W, Gou X, Wang Z. Berberine induces cell death in human hepatoma cells *in vitro* by downregulating CD147. *Cancer Sci* 2011; **102**: 1287-1292 [PMID: 21443647 DOI: 10.1111/j.1349-7006.2011.01933.x]

517 **Qian H**, Yang Y, Wang X. Curcumin enhanced adriamycin-induced human liver-derived Hepatoma G2 cell death through activation of mitochondria-mediated apoptosis and autophagy. *Eur J Pharm Sci* 2011; **43**: 125-131 [PMID: 21514382 DOI: 10.1016/j.ejps.2011.04.002]

518 **Andrzejak M**, Price M, Kessel DH. Apoptotic and autophagic responses to photodynamic therapy in 1c1c7 murine hepatoma cells. *Autophagy* 2011; **7**: 979-984 [PMID: 21555918 DOI: 10.4161/auto.7.9.15865]

519 **Hu M**, Huang H, Zhao R, Li P, Li M, Miao H, Chen N, Chen M. AZD8055 induces cell death associated with autophagy and activation of AMPK in hepatocellular carcinoma. *Oncol Rep* 2014; **31**: 649-656 [PMID: 24297300 DOI: 10.3892/or.2013.2890]

520 **Song J**, Qu Z, Guo X, Zhao Q, Zhao X, Gao L, Sun K, Shen F, Wu M, Wei L. Hypoxia-induced autophagy contributes to the chemoresistance of hepatocellular carcinoma cells. *Autophagy* 2009; **5**: 1131-1144 [PMID: 19786832 DOI: 10.4161/auto.5.8.9996]

521 **Chen LH**, Loong CC, Su TL, Lee YJ, Chu PM, Tsai ML, Tsai PH, Tu PH, Chi CW, Lee HC, Chiou SH. Autophagy inhibition enhances apoptosis triggered by BO-1051, an N-mustard derivative, and involves the ATM signaling pathway. *Biochem Pharmacol* 2011; **81**: 594-605 [PMID: 21184746 DOI: 10.1016/j.bcp.2010.12.011]

522 **Ding ZB**, Hui B, Shi YH, Zhou J, Peng YF, Gu CY, Yang H, Shi GM, Ke AW, Wang XY, Song K, Dai Z, Shen YH, Fan J. Autophagy activation in hepatocellular carcinoma contributes to the tolerance of oxaliplatin *via* reactive oxygen species modulation. *Clin Cancer Res* 2011; **17**: 6229-6238 [PMID: 21825039 DOI: 10.1158/1078-0432.CCR-11-0816]

523 **Shimizu S**, Takehara T, Hikita H, Kodama T, Tsunematsu H, Miyagi T, Hosui A, Ishida H, Tatsumi T, Kanto T, Hiramatsu N, Fujita N, Yoshimori T, Hayashi N. Inhibition of autophagy potentiates the antitumor effect of the multikinase inhibitor sorafenib in hepatocellular carcinoma. *Int J Cancer* 2012; **131**: 548-557 [PMID: 21858812 DOI: 10.1002/ijc.26374]

524 **Du H**, Yang W, Chen L, Shi M, Seewoo V, Wang J, Lin A, Liu Z, Qiu W. Role of autophagy in resistance to oxaliplatin in hepatocellular carcinoma cells. *Oncol Rep* 2012; **27**: 143-150 [PMID: 21935576 DOI: 10.3892/or.2011.1464]

525 **Xu N**, Zhang J, Shen C, Luo Y, Xia L, Xue F, Xia Q. Cisplatin-induced downregulation of miR-199a-5p increases drug resistance by activating autophagy in HCC cell. *Biochem Biophys Res Commun* 2012; **423**: 826-831 [PMID: 22713463 DOI: 10.1016/j.bbrc.2012.06.048]

526 **Yu HC**, Lin CS, Tai WT, Liu CY, Shiau CW, Chen KF. Nilotinib induces autophagy in hepatocellular carcinoma through AMPK activation. *J Biol Chem* 2013; **288**: 18249-18259 [PMID: 23677989 DOI: 10.1074/jbc.M112.446385]

527 **Wu B**, Cui J, Yang XM, Liu ZY, Song F, Li L, Jiang JL, Chen ZN. Cytoplasmic fragment of CD147 generated by regulated intramembrane proteolysis contributes to HCC by promoting autophagy. *Cell Death Dis* 2017; **8**: e2925 [PMID: 28703811 DOI: 10.1038/cddis.2017.251]

528 **Shi YH**, Ding ZB, Zhou J, Hui B, Shi GM, Ke AW, Wang XY, Dai Z, Peng YF, Gu CY, Qiu SJ, Fan J. Targeting autophagy enhances sorafenib lethality for hepatocellular carcinoma *via* ER stress-related apoptosis. *Autophagy* 2011; **7**: 1159-1172 [PMID: 21691147 DOI: 10.4161/auto.7.10.16818]

529 **Bareford MD**, Park MA, Yacoub A, Hamed HA, Tang Y, Cruickshanks N, Eulitt P, Hubbard N, Tye G, Burow ME, Fisher PB, Moran RG, Nephew KP, Grant S, Dent P. Sorafenib enhances pemetrexed cytotoxicity through an autophagy-dependent mechanism in cancer cells. *Cancer Res* 2011; **71**: 4955-4967 [PMID: 21622715 DOI: 10.1158/0008-5472.CAN-11-0898]

530 **Li X**, Zhou Y, Yang L, Ma Y, Peng X, Yang S, Li H, Liu J. LncRNA NEAT1 promotes autophagy *via* regulating miR-204/ATG3 and enhanced cell resistance to sorafenib in hepatocellular carcinoma. *J Cell Physiol* 2020; **235**: 3402-3413 [PMID: 31549407 DOI: 10.1002/jcp.29230]

531 **Jin F**, Wang Y, Li M, Zhu Y, Liang H, Wang C, Wang F, Zhang CY, Zen K, Li L. MiR-26 enhances chemosensitivity and promotes apoptosis of hepatocellular carcinoma cells through inhibiting autophagy. *Cell Death Dis* 2017; **8**: e2540 [PMID: 28079894 DOI: 10.1038/cddis.2016.461]

532 **Prieto-Domínguez N**, Ordóñez R, Fernández A, Méndez-Blanco C, Baulies A, Garcia-Ruiz C, Fernández-Checa JC, Mauriz JL, González-Gallego J. Melatonin-induced increase in sensitivity of human hepatocellular carcinoma cells to sorafenib is associated with reactive oxygen species production and mitophagy. *J Pineal Res* 2016; **61**: 396-407 [PMID: 27484637 DOI: 10.1111/jpi.12358]

533 **Li W**, Li Y, Siraj S, Jin H, Fan Y, Yang X, Huang X, Wang X, Wang J, Liu L, Du L, Chen Q. FUN14 Domain-Containing 1-Mediated Mitophagy Suppresses Hepatocarcinogenesis by Inhibition of Inflammasome Activation in Mice. *Hepatology* 2019; **69**: 604-621 [PMID: 30053328 DOI: 10.1002/hep.30191]

534 **Huang Q**, Zhan L, Cao H, Li J, Lyu Y, Guo X, Zhang J, Ji L, Ren T, An J, Liu B, Nie Y, Xing J. Increased mitochondrial fission promotes autophagy and hepatocellular carcinoma cell survival through the ROS-modulated coordinated regulation of the NFKB and TP53 pathways. *Autophagy* 2016; **12**: 999-1014 [PMID: 27124102 DOI:

10.1080/15548627.2016.1166318]
535 **Liu K**, Lee J, Kim JY, Wang L, Tian Y, Chan ST, Cho C, Machida K, Chen D, Ou JJ. Mitophagy Controls the Activities of Tumor Suppressor p53 to Regulate Hepatic Cancer Stem Cells. *Mol Cell* 2017; **68**: 281-292. e5 [PMID: 29033320 DOI: 10.1016/j.molcel.2017.09.022]
536 **Gómez de Cedrón M**, Ramírez de Molina A. Microtargeting cancer metabolism: opening new therapeutic windows based on lipid metabolism. *J Lipid Res* 2016; **57**: 193-206 [PMID: 26630911 DOI: 10.1194/jlr.R061812]
537 **Zhao T**, Du H, Ding X, Walls K, Yan C. Activation of mTOR pathway in myeloid-derived suppressor cells stimulates cancer cell proliferation and metastasis in lal(-/-) mice. *Oncogene* 2015; **34**: 1938-1948 [PMID: 24882582 DOI: 10.1038/onc.2014.143]
538 **Mukhopadhyay S**, Schlaepfer IR, Bergman BC, Panda PK, Praharaj PP, Naik PP, Agarwal R, Bhutia SK. ATG14 facilitated lipophagy in cancer cells induce ER stress mediated mitoptosis through a ROS dependent pathway. *Free Radic Biol Med* 2017; **104**: 199-213 [PMID: 28069524 DOI: 10.1016/j.freeradbiomed.2017.01.007]
539 **Lu GD**, Ang YH, Zhou J, Tamilarasi J, Yan B, Lim YC, Srivastava S, Salto-Tellez M, Hui KM, Shen HM, Nguyen LN, Tan BC, Silver DL, Hooi SC. CCAAT/enhancer binding protein α predicts poorer prognosis and prevents energy starvation-induced cell death in hepatocellular carcinoma. *Hepatology* 2015; **61**: 965-978 [PMID: 25363290 DOI: 10.1002/hep.27593]
540 **Kon M**, Kiffin R, Koga H, Chapochnick J, Macian F, Varticovski L, Cuervo AM. Chaperone-mediated autophagy is required for tumor growth. *Sci Transl Med* 2011; **3**: 109ra117 [PMID: 22089453 DOI: 10.1126/scitranslmed.3003182]
541 **Chava S**, Lee C, Aydin Y, Chandra PK, Dash A, Chedid M, Thung SN, Moroz K, Wu T, Nayak NC, Dash S. Chaperone-mediated autophagy compensates for impaired macroautophagy in the cirrhotic liver to promote hepatocellular carcinoma. *Oncotarget* 2017; **8**: 40019-40036 [PMID: 28402954 DOI: 10.18632/oncotarget.16685]
542 **Dash S**, Aydin Y, Moroz K. Chaperone-Mediated Autophagy in the Liver: Good or Bad? *Cells* 2019; **8** [PMID: 31652893 DOI: 10.3390/cells8111308]
543 **Wu WKK**, Zhang L, Chan MTV. Autophagy, NAFLD and NAFLD-Related HCC. *Adv Exp Med Biol* 2018; **1061**: 127-138 [PMID: 29956211 DOI: 10.1007/978-981-10-8684-7_10]
544 **Niture S**, Gyamfi MA, Lin M, Chimeh U, Dong X, Zheng W, Moore J, Kumar D. TNFAIP8 regulates autophagy, cell steatosis, and promotes hepatocellular carcinoma cell proliferation. *Cell Death Dis* 2020; **11**: 178 [PMID: 32152268 DOI: 10.1038/s41419-020-2369-4]
545 **Liu L**, Liao JZ, He XX, Li PY. The role of autophagy in hepatocellular carcinoma: friend or foe. *Oncotarget* 2017; **8**: 57707-57722 [PMID: 28915706 DOI: 10.18632/oncotarget.17202]
546 **Huang F**, Wang BR, Wang YG. Role of autophagy in tumorigenesis, metastasis, targeted therapy and drug resistance of hepatocellular carcinoma. *World J Gastroenterol* 2018; **24**: 4643-4651 [PMID: 30416312 DOI: 10.3748/wjg.v24.i41.4643]
547 **Yang S**, Yang L, Li X, Li B, Li Y, Zhang X, Ma Y, Peng X, Jin H, Li H. New insights into autophagy in hepatocellular carcinoma: mechanisms and therapeutic strategies. *Am J Cancer Res* 2019; **9**: 1329-1353 [PMID: 31392073]
548 **Chen P**, Bonaldo P. Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. *Int Rev Cell Mol Biol* 2013; **301**: 1-35 [PMID: 23317816 DOI: 10.1016/B978-0-12-407704-1.00001-4]
549 **Lin H**, Yan J, Wang Z, Hua F, Yu J, Sun W, Li K, Liu H, Yang H, Lv Q, Xue J, Hu ZW. Loss of immunity-supported senescence enhances susceptibility to hepatocellular carcinogenesis and progression in Toll-like receptor 2-deficient mice. *Hepatology* 2013; **57**: 171-182 [PMID: 22859216 DOI: 10.1002/hep.25991]
550 **Chen W**, Ma T, Shen XN, Xia XF, Xu GD, Bai XL, Liang TB. Macrophage-induced tumor angiogenesis is regulated by the TSC2-mTOR pathway. *Cancer Res* 2012; **72**: 1363-1372 [PMID: 22287548 DOI: 10.1158/0008-5472.CAN-11-2684]
551 **Chen KD**, Lin CC, Tsai MC, Huang KT, Chiu KW. Tumor microenvironment mediated by suppression of autophagic flux drives liver malignancy. *Biomed J* 2018; **41**: 163-168 [PMID: 30080656 DOI: 10.1016/j.bj.2018.03.002]
552 **Hu T**, Li P, Luo Z, Chen X, Zhang J, Wang C, Chen P, Dong Z. Chloroquine inhibits hepatocellular carcinoma cell growth *in vitro* and in vivo. *Oncol Rep* 2016; **35**: 43-49 [PMID: 26530158 DOI: 10.3892/or.2015.4380]
553 **Wang Y**, Zhao H, Wang D, Hao M, Kong C, Zhao X, Gao Y, Li J, Liu B, Yang B, Zhang H, Jiang J. Inhibition of Autophagy Promoted Apoptosis and Suppressed Growth of Hepatocellular Carcinoma Upon Photothermal Exposure. *J Biomed Nanotechnol* 2019; **15**: 813-821 [PMID: 30841973 DOI: 10.1166/jbn.2019.2714]
554 **Amaravadi RK**, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N, Timmer W, DiPaola RS, Lotze MT, White E. Principles and current strategies for targeting autophagy for cancer treatment. *Clin Cancer Res* 2011; **17**: 654-666 [PMID: 21325294 DOI: 10.1158/1078-0432.CCR-10-2634]
555 **Di Fazio P**, Matrood S. Targeting autophagy in liver cancer. *Transl Gastroenterol Hepatol* 2018; **3**: 39 [PMID: 30148224 DOI: 10.21037/tgh.2018.06.09]
556 **Peng YF**, Shi YH, Ding ZB, Ke AW, Gu CY, Hui B, Zhou J, Qiu SJ, Dai Z, Fan J. Autophagy inhibition suppresses pulmonary metastasis of HCC in mice *via* impairing anoikis resistance and colonization of HCC cells. *Autophagy* 2013; **9**: 2056-2068 [PMID: 24157892 DOI: 10.4161/auto.26398]
557 **Gao L**, Song JR, Zhang JW, Zhao X, Zhao QD, Sun K, Deng WJ, Li R, Lv G, Cheng HY, Wei LX. Chloroquine promotes the anticancer effect of TACE in a rabbit VX2 Liver tumor model. *Int J Biol Sci* 2013; **9**: 322-330 [PMID: 23569437 DOI: 10.7150/ijbs.5925]
558 **Wang X**, Deng Q, Feng K, Chen S, Jiang J, Xia F, Ma K, Bie P. Insufficient radiofrequency ablation promotes hepatocellular carcinoma cell progression *via* autophagy and the CD133 feedback loop. *Oncol Rep* 2018; **40**: 241-251 [PMID: 29749472 DOI: 10.3892/or.2018.6403]
559 **Zhao Z**, Wu J, Liu X, Liang M, Zhou X, Ouyang S, Yao J, Wang J, Luo B. Insufficient radiofrequency ablation promotes proliferation of residual hepatocellular carcinoma *via* autophagy. *Cancer Lett* 2018; **421**: 73-81 [PMID: 29458142 DOI: 10.1016/j.canlet.2018.02.024]
560 **Prieto-Domínguez N**, Ordóñez R, Fernández A, García-Palomo A, Muntané J, González-Gallego J, Mauriz JL. Modulation of Autophagy by Sorafenib: Effects on Treatment Response. *Front Pharmacol* 2016; **7**: 151 [PMID:

27375485 DOI: 10.3389/fphar.2016.00151]

561 **Zhao P**, Li M, Wang Y, Chen Y, He C, Zhang X, Yang T, Lu Y, You J, Lee RJ, Xiang G. Enhancing anti-tumor efficiency in hepatocellular carcinoma through the autophagy inhibition by miR-375/sorafenib in lipid-coated calcium carbonate nanoparticles. *Acta Biomater* 2018; **72**: 248-255 [PMID: 29555460 DOI: 10.1016/j.actbio.2018.03.022]

562 **Hou YJ**, Dong LW, Tan YX, Yang GZ, Pan YF, Li Z, Tang L, Wang M, Wang Q, Wang HY. Inhibition of active autophagy induces apoptosis and increases chemosensitivity in cholangiocarcinoma. *Lab Invest* 2011; **91**: 1146-1157 [PMID: 21647092 DOI: 10.1038/labinvest.2011.97]

563 **Sasaki M**, Nitta T, Sato Y, Nakanuma Y. Autophagy may occur at an early stage of cholangiocarcinogenesis *via* biliary intraepithelial neoplasia. *Hum Pathol* 2015; **46**: 202-209 [PMID: 25466963 DOI: 10.1016/j.humpath.2014.09.016]

564 **Dong LW**, Hou YJ, Tan YX, Tang L, Pan YF, Wang M, Wang HY. Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma. *Autophagy* 2011; **7**: 1222-1229 [PMID: 21654208 DOI: 10.4161/auto.7.10.16610]

565 **Wang TT**, Cao QH, Chen MY, Xia Q, Fan XJ, Ma XK, Lin Q, Jia CC, Dong M, Ruan DY, Lin ZX, Wen JY, Wei L, Li X, Chen ZH, Wang L, Wu XY, Wan XB. Beclin 1 deficiency correlated with lymph node metastasis, predicts a distinct outcome in intrahepatic and extrahepatic cholangiocarcinoma. *PLoS One* 2013; **8**: e80317 [PMID: 24303007 DOI: 10.1371/journal.pone.0080317]

566 **Qu X**, Sheng J, Shen L, Su J, Xu Y, Xie Q, Wu Y, Zhang X, Sun L. Autophagy inhibitor chloroquine increases sensitivity to cisplatin in QBC939 cholangiocarcinoma cells by mitochondrial ROS. *PLoS One* 2017; **12**: e0173712 [PMID: 28301876 DOI: 10.1371/journal.pone.0173712]

567 **Jia B**, Xue Y, Yan X, Li J, Wu Y, Guo R, Zhang J, Zhang L, Li Y, Liu Y, Sun L. Autophagy inhibitor chloroquine induces apoptosis of cholangiocarcinoma cells *via* endoplasmic reticulum stress. *Oncol Lett* 2018; **16**: 3509-3516 [PMID: 30127955 DOI: 10.3892/ol.2018.9131]

568 **O'Dell MR**, Huang JL, Whitney-Miller CL, Deshpande V, Rothberg P, Grose V, Rossi RM, Zhu AX, Land H, Bardeesy N, Hezel AF. Kras(G12D) and p53 mutation cause primary intrahepatic cholangiocarcinoma. *Cancer Res* 2012; **72**: 1557-1567 [PMID: 22266220 DOI: 10.1158/0008-5472.CAN-11-3596]

569 **Huang JL**, Hezel AF. Autophagy in intra-hepatic cholangiocarcinoma. *Autophagy* 2012; **8**: 1148-1149 [PMID: 22751196 DOI: 10.4161/auto.20647]

570 **Hong ZF**, Zhao WX, Yin ZY, Xie CR, Xu YP, Chi XQ, Zhang S, Wang XM. Capsaicin Enhances the Drug Sensitivity of Cholangiocarcinoma through the Inhibition of Chemotherapeutic-Induced Autophagy. *PLoS One* 2015; **10**: e0121538 [PMID: 25933112 DOI: 10.1371/journal.pone.0121538]

571 **Ding X**, Chaiteerakij R, Moser CD, Shaleh H, Boakye J, Chen G, Ndzengue A, Li Y, Zhou Y, Huang S, Sinicrope FA, Zou X, Thomas MB, Smith CD, Roberts LR. Antitumor effect of the novel sphingosine kinase 2 inhibitor ABC294640 is enhanced by inhibition of autophagy and by sorafenib in human cholangiocarcinoma cells. *Oncotarget* 2016; **7**: 20080-20092 [PMID: 26956050 DOI: 10.18632/oncotarget.7914]

572 **Lomas DA**, Mahadeva R. Alpha1-antitrypsin polymerization and the serpinopathies: pathobiology and prospects for therapy. *J Clin Invest* 2002; **110**: 1585-1590 [PMID: 12464660 DOI: 10.1172/JCI16782]

573 **Huber R**, Carrell RW. Implications of the three-dimensional structure of alpha 1-antitrypsin for structure and function of serpins. *Biochemistry* 1989; **28**: 8951-8966 [PMID: 2690952 DOI: 10.1021/bi00449a001]

574 **Greene CM**, Marciniak SJ, Teckman J, Ferrarotti I, Brantly ML, Lomas DA, Stoller JK, McElvaney NG. α1-Antitrypsin deficiency. *Nat Rev Dis Primers* 2016; **2**: 16051 [PMID: 27465791 DOI: 10.1038/nrdp.2016.51]

575 **Lomas DA**, Hurst JR, Gooptu B. Update on alpha-1 antitrypsin deficiency: New therapies. *J Hepatol* 2016; **65**: 413-424 [PMID: 27034252 DOI: 10.1016/j.jhep.2016.03.010]

576 **Rudnick DA**, Perlmutter DH. Alpha-1-antitrypsin deficiency: a new paradigm for hepatocellular carcinoma in genetic liver disease. *Hepatology* 2005; **42**: 514-521 [PMID: 16044402 DOI: 10.1002/hep.20815]

577 **Hazari YM**, Bashir A, Habib M, Bashir S, Habib H, Qasim MA, Shah NN, Haq E, Teckman J, Fazili KM. Alpha-1-antitrypsin deficiency: Genetic variations, clinical manifestations and therapeutic interventions. *Mutat Res* 2017; **773**: 14-25 [PMID: 28927525 DOI: 10.1016/j.mrrev.2017.03.001]

578 **Teckman JH**, Perlmutter DH. Retention of mutant alpha(1)-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G961-G974 [PMID: 11052993 DOI: 10.1152/ajpgi.2000.279.5.G961]

579 **Teckman JH**, An JK, Loethen S, Perlmutter DH. Fasting in alpha1-antitrypsin deficient liver: constitutive [correction of consultative] activation of autophagy. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G1156-G1165 [PMID: 12381530 DOI: 10.1152/ajpgi.00041.2002]

580 **Marciniak SJ**, Lomas DA. Alpha1-antitrypsin deficiency and autophagy. *N Engl J Med* 2010; **363**: 1863-1864 [PMID: 21047232 DOI: 10.1056/NEJMcibr1008007]

581 **Teckman JH**, Mangalat N. Alpha-1 antitrypsin and liver disease: mechanisms of injury and novel interventions. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 261-268 [PMID: 25066184 DOI: 10.1586/17474124.2014.943187]

582 **Perlmutter DH**. Liver injury in alpha1-antitrypsin deficiency: an aggregated protein induces mitochondrial injury. *J Clin Invest* 2002; **110**: 1579-1583 [PMID: 12464659 DOI: 10.1172/JCI16787]

583 **Teckman JH**, An JK, Blomenkamp K, Schmidt B, Perlmutter D. Mitochondrial autophagy and injury in the liver in alpha 1-antitrypsin deficiency. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G851-G862 [PMID: 14684378 DOI: 10.1152/ajpgi.00175.2003]

584 **Kamimoto T**, Shoji S, Hidvegi T, Mizushima N, Umebayashi K, Perlmutter DH, Yoshimori T. Intracellular inclusions containing mutant alpha1-antitrypsin Z are propagated in the absence of autophagic activity. *J Biol Chem* 2006; **281**: 4467-4476 [PMID: 16365039 DOI: 10.1074/jbc.M509409200]

585 **Kruse KB**, Brodsky JL, McCracken AA. Characterization of an ERAD gene as VPS30/ATG6 reveals two alternative and functionally distinct protein quality control pathways: one for soluble Z variant of human alpha-1 proteinase inhibitor (A1PiZ) and another for aggregates of A1PiZ. *Mol Biol Cell* 2006; **17**: 203-212 [PMID: 16267277 DOI: 10.1091/mbc.e04-09-0779]

586 **Kaushal S**, Annamali M, Blomenkamp K, Rudnick D, Halloran D, Brunt EM, Teckman JH. Rapamycin reduces

intrahepatic alpha-1-antitrypsin mutant Z protein polymers and liver injury in a mouse model. *Exp Biol Med (Maywood)* 2010; **235**: 700-709 [PMID: 20511674 DOI: 10.1258/ebm.2010.009297]

587 **Perlmutter DH**. The role of autophagy in alpha-1-antitrypsin deficiency: a specific cellular response in genetic diseases associated with aggregation-prone proteins. *Autophagy* 2006; **2**: 258-263 [PMID: 16874089 DOI: 10.4161/auto.2882]

588 **Perlmutter DH**. Autophagic disposal of the aggregation-prone protein that causes liver inflammation and carcinogenesis in alpha-1-antitrypsin deficiency. *Cell Death Differ* 2009; **16**: 39-45 [PMID: 18617899 DOI: 10.1038/cdd.2008.103]

589 **Lindblad D**, Blomenkamp K, Teckman J. Alpha-1-antitrypsin mutant Z protein content in individual hepatocytes correlates with cell death in a mouse model. *Hepatology* 2007; **46**: 1228-1235 [PMID: 17886264 DOI: 10.1002/hep.21822]

590 **Yamamura T**, Ohsaki Y, Suzuki M, Shinohara Y, Tatematsu T, Cheng J, Okada M, Ohmiya N, Hirooka Y, Goto H, Fujimoto T. Inhibition of Niemann-Pick-type C1-like1 by ezetimibe activates autophagy in human hepatocytes and reduces mutant α1-antitrypsin Z deposition. *Hepatology* 2014; **59**: 1591-1599 [PMID: 24214142 DOI: 10.1002/hep.26930]

591 **Hidvegi T**, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, Maurice N, Mukherjee A, Goldbach C, Watkins S, Michalopoulos G, Perlmutter DH. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. *Science* 2010; **329**: 229-232 [PMID: 20522742 DOI: 10.1126/science.1190354]

592 **Pastore N**, Blomenkamp K, Annunziata F, Piccolo P, Mithbaokar P, Maria Sepe R, Vetrini F, Palmer D, Ng P, Polishchuk E, Iacobacci S, Polishchuk R, Teckman J, Ballabio A, Brunetti-Pierri N. Gene transfer of master autophagy regulator TFEB results in clearance of toxic protein and correction of hepatic disease in alpha-1-anti-trypsin deficiency. *EMBO Mol Med* 2013; **5**: 397-412 [PMID: 23381957 DOI: 10.1002/emmm.201202046]

593 **Wang Y**, Cobanoglu MC, Li J, Hidvegi T, Hale P, Ewing M, Chu AS, Gong Z, Muzumdar R, Pak SC, Silverman GA, Bahar I, Perlmutter DH. An analog of glibenclamide selectively enhances autophagic degradation of misfolded α1-antitrypsin Z. *PLoS One* 2019; **14**: e0209748 [PMID: 30673724 DOI: 10.1371/journal.pone.0209748]

594 **Tang Y**, Fickert P, Trauner M, Marcus N, Blomenkamp K, Teckman J. Autophagy induced by exogenous bile acids is therapeutic in a model of α-1-AT deficiency liver disease. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G156-G165 [PMID: 27102560 DOI: 10.1152/ajpgi.00143.2015]

595 **Tang Y**, Blomenkamp KS, Fickert P, Trauner M, Teckman JH. NorUDCA promotes degradation of α1-antitrypsin mutant Z protein by inducing autophagy through AMPK/ULK1 pathway. *PLoS One* 2018; **13**: e0200897 [PMID: 30067827 DOI: 10.1371/journal.pone.0200897]

596 **Puls F**, Goldschmidt I, Bantel H, Agne C, Bröcker V, Dämmrich M, Lehmann U, Berrang J, Pfister ED, Kreipe HH, Baumann U. Autophagy-enhancing drug carbamazepine diminishes hepatocellular death in fibrinogen storage disease. *J Hepatol* 2013; **59**: 626-630 [PMID: 23707368 DOI: 10.1016/j.jhep.2013.05.018]

597 **Członkowska A**, Litwin T, Dusek P, Ferenci P, Lutsenko S, Medici V, Rybakowski JK, Weiss KH, Schilsky ML. Wilson disease. *Nat Rev Dis Primers* 2018; **4**: 21 [PMID: 30190489 DOI: 10.1038/s41572-018-0018-3]

598 **Polishchuk EV**, Merolla A, Lichtmannegger J, Romano A, Indrieri A, Ilyechova EY, Concilli M, De Cegli R, Crispino R, Mariniello M, Petruzzelli R, Ranucci G, Iorio R, Pietrocola F, Einer C, Borchard S, Zibert A, Schmidt HH, Di Schiavi E, Puchkova LV, Franco B, Kroemer G, Zischka H, Polishchuk RS. Activation of Autophagy, Observed in Liver Tissues From Patients With Wilson Disease and From ATP7B-Deficient Animals, Protects Hepatocytes From Copper-Induced Apoptosis. *Gastroenterology* 2019; **156**: 1173-1189. e5 [PMID: 30452922 DOI: 10.1053/j.gastro.2018.11.032]

599 **Farah BL**, Sinha RA, Wu Y, Singh BK, Lim A, Hirayama M, Landau DJ, Bay BH, Koeberl DD, Yen PM. Hepatic mitochondrial dysfunction is a feature of Glycogen Storage Disease Type Ia (GSDIa). *Sci Rep* 2017; **7**: 44408 [PMID: 28317891 DOI: 10.1038/srep44408]

600 **Farah BL**, Landau DJ, Sinha RA, Brooks ED, Wu Y, Fung SYS, Tanaka T, Hirayama M, Bay BH, Koeberl DD, Yen PM. Induction of autophagy improves hepatic lipid metabolism in glucose-6-phosphatase deficiency. *J Hepatol* 2016; **64**: 370-379 [PMID: 26462884 DOI: 10.1016/j.jhep.2015.10.008]

601 **Cho JH**, Kim GY, Pan CJ, Anduaga J, Choi EJ, Mansfield BC, Chou JY. Downregulation of SIRT1 signaling underlies hepatic autophagy impairment in glycogen storage disease type Ia. *PLoS Genet* 2017; **13**: e1006819 [PMID: 28558013 DOI: 10.1371/journal.pgen.1006819]

602 **Aki T**, Unuma K, Uemura K. Emerging roles of mitochondria and autophagy in liver injury during sepsis. *Cell Stress* 2017; **1**: 79-89 [PMID: 31225438 DOI: 10.15698/cst2017.11.110]

603 **Qiu P**, Liu Y, Zhang J. Review: the Role and Mechanisms of Macrophage Autophagy in Sepsis. *Inflammation* 2019; **42**: 6-19 [PMID: 30194660 DOI: 10.1007/s10753-018-0890-8]

604 **Chang FY**, Chen HC, Chen PJ, Ho MS, Hsieh SL, Lin JC, Liu FT, Sytwu HK. Immunologic aspects of characteristics, diagnosis, and treatment of coronavirus disease 2019 (COVID-19). *J Biomed Sci* 2020; **27**: 72 [PMID: 32498686 DOI: 10.1186/s12929-020-00663-w]

605 **Lee JP**, Foote A, Fan H, Peral de Castro C, Lang T, Jones SA, Gavrilescu N, Mills KH, Leech M, Morand EF, Harris J. Loss of autophagy enhances MIF/macrophage migration inhibitory factor release by macrophages. *Autophagy* 2016; **12**: 907-916 [PMID: 27163877 DOI: 10.1080/15548627.2016.1164358]

606 **Thiessen SE**, Derese I, Derde S, Dufour T, Pauwels L, Bekhuis Y, Pintelon I, Martinet W, Van den Berghe G, Vanhorebeek I. The Role of Autophagy in Critical Illness-induced Liver Damage. *Sci Rep* 2017; **7**: 14150 [PMID: 29074879 DOI: 10.1038/s41598-017-14405-w]

607 **Kemp MG**. Crosstalk Between Apoptosis and Autophagy: Environmental Genotoxins, Infection, and Innate Immunity. *J Cell Death* 2017; **9**: 1179670716685085 [PMID: 28469477 DOI: 10.1177/1179670716685085]

608 **Prabakaran T**, Bodda C, Krapp C, Zhang BC, Christensen MH, Sun C, Reinert L, Cai Y, Jensen SB, Skouboe MK, Nyengaard JR, Thompson CB, Lebbink RJ, Sen GC, van Loo G, Nielsen R, Komatsu M, Nejsum LN, Jakobsen MR, Gyrd-Hansen M, Paludan SR. Attenuation of cGAS-STING signaling is mediated by a p62/SQSTM1-dependent autophagy pathway activated by TBK1. *EMBO J* 2018; **37** [PMID: 29496741 DOI: 10.15252/embj.201797858]

609 **Hu Q**, Knight PH, Ren Y, Ren H, Zheng J, Wu X, Ren J, Sawyer RG. The emerging role of stimulator of interferons genes signaling in sepsis: Inflammation, autophagy, and cell death. *Acta Physiol (Oxf)* 2019; **225**: e13194 [PMID:

30269441 DOI: 10.1111/apha.13194]
610 **Lalazar G**, Ilyas G, Malik SA, Liu K, Zhao E, Amir M, Lin Y, Tanaka KE, Czaja MJ. Autophagy confers resistance to lipopolysaccharide-induced mouse hepatocyte injury. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G377-G386 [PMID: 27469366 DOI: 10.1152/ajpgi.00124.2016]
611 **Oami T**, Watanabe E, Hatano M, Teratake Y, Fujimura L, Sakamoto A, Ito C, Toshimori K, Swanson PE, Oda S. Blocking Liver Autophagy Accelerates Apoptosis and Mitochondrial Injury in Hepatocytes and Reduces Time to Mortality in a Murine Sepsis Model. *Shock* 2018; **50**: 427-434 [PMID: 29076973 DOI: 10.1097/SHK.0000000000001040]
612 **Xiong X**, Ren Y, Cui Y, Li R, Wang C, Zhang Y. Obeticholic acid protects mice against lipopolysaccharide-induced liver injury and inflammation. *Biomed Pharmacother* 2017; **96**: 1292-1298 [PMID: 29174575 DOI: 10.1016/j.biopha.2017.11.083]
613 **Xing W**, Yang L, Peng Y, Wang Q, Gao M, Yang M, Xiao X. Ginsenoside Rg3 attenuates sepsis-induced injury and mitochondrial dysfunction in liver *via* AMPK-mediated autophagy flux. *Biosci Rep* 2017; **37** [PMID: 28779013 DOI: 10.1042/BSR20170934]
614 **Inata Y**, Kikuchi S, Samraj RS, Hake PW, O'Connor M, Ledford JR, O'Connor J, Lahni P, Wolfe V, Piraino G, Zingarelli B. Autophagy and mitochondrial biogenesis impairment contribute to age-dependent liver injury in experimental sepsis: dysregulation of AMP-activated protein kinase pathway. *FASEB J* 2018; **32**: 728-741 [PMID: 28974562 DOI: 10.1096/fj.201700576R]
615 **Ying L**, Zhao GJ, Wu Y, Ke HL, Hong GL, Zhang H, Dong N, Wu Y, Yao YM, Lu ZQ. Mitofusin 2 Promotes Apoptosis of $CD4^+$ T Cells by Inhibiting Autophagy in Sepsis. *Mediators Inflamm* 2017; **2017**: 4926205 [PMID: 29358849 DOI: 10.1155/2017/4926205]
616 **Neumann Y**, Bruns SA, Rohde M, Prajsnar TK, Foster SJ, Schmitz I. Intracellular Staphylococcus aureus eludes selective autophagy by activating a host cell kinase. *Autophagy* 2016; **12**: 2069-2084 [PMID: 27629870 DOI: 10.1080/15548627.2016.1226732]
617 **Yin X**, Xin H, Mao S, Wu G, Guo L. The Role of Autophagy in Sepsis: Protection and Injury to Organs. *Front Physiol* 2019; **10**: 1071 [PMID: 31507440 DOI: 10.3389/fphys.2019.01071]
618 **Sydor S**, Manka P, Best J, Jafoui S, Sowa JP, Zoubek ME, Hernandez-Gea V, Cubero FJ, Kälsch J, Vetter D, Fiel MI, Hoshida Y, Bian CB, Nelson LJ, Moshage H, Faber KN, Paul A, Baba HA, Gerken G, Friedman SL, Canbay A, Bechmann LP. Krüppel-like factor 6 is a transcriptional activator of autophagy in acute liver injury. *Sci Rep* 2017; **7**: 8119 [PMID: 28808340 DOI: 10.1038/s41598-017-08680-w]
619 **Baulies A**, Ribas V, Núñez S, Torres S, Alarcón-Vila C, Martínez L, Suda J, Ybanez MD, Kaplowitz N, García-Ruiz C, Fernández-Checa JC. Lysosomal Cholesterol Accumulation Sensitizes To Acetaminophen Hepatotoxicity by Impairing Mitophagy. *Sci Rep* 2015; **5**: 18017 [PMID: 26657973 DOI: 10.1038/srep18017]
620 **James LP**, Alonso EM, Hynan LS, Hinson JA, Davern TJ, Lee WM, Squires RH; Pediatric Acute Liver Failure Study Group. Detection of acetaminophen protein adducts in children with acute liver failure of indeterminate cause. *Pediatrics* 2006; **118**: e676-e681 [PMID: 16950959 DOI: 10.1542/peds.2006-0069]
621 **Xie Y**, McGill MR, Du K, Dorko K, Kumer SC, Schmitt TM, Ding WX, Jaeschke H. Mitochondrial protein adducts formation and mitochondrial dysfunction during N-acetyl-m-aminophenol (AMAP)-induced hepatotoxicity in primary human hepatocytes. *Toxicol Appl Pharmacol* 2015; **289**: 213-222 [PMID: 26431796 DOI: 10.1016/j.taap.2015.09.022]
622 **Qiu Y**, Benet LZ, Burlingame AL. Identification of the hepatic protein targets of reactive metabolites of acetaminophen *in vivo* in mice using two-dimensional gel electrophoresis and mass spectrometry. *J Biol Chem* 1998; **273**: 17940-17953 [PMID: 9651401 DOI: 10.1074/jbc.273.28.17940]
623 **McGill MR**, Lebofsky M, Norris HR, Slawson MH, Bajt ML, Xie Y, Williams CD, Wilkins DG, Rollins DE, Jaeschke H. Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. *Toxicol Appl Pharmacol* 2013; **269**: 240-249 [PMID: 23571099 DOI: 10.1016/j.taap.2013.03.026]
624 **Heard KJ**, Green JL, James LP, Judge BS, Zolot L, Rhyee S, Dart RC. Acetaminophen-cysteine adducts during therapeutic dosing and following overdose. *BMC Gastroenterol* 2011; **11**: 20 [PMID: 21401949 DOI: 10.1186/1471-230X-11-20]
625 **Ni HM**, McGill MR, Chao X, Du K, Williams JA, Xie Y, Jaeschke H, Ding WX. Removal of acetaminophen protein adducts by autophagy protects against acetaminophen-induced liver injury in mice. *J Hepatol* 2016; **65**: 354-362 [PMID: 27151180 DOI: 10.1016/j.jhep.2016.04.025]
626 **Lin Z**, Wu F, Lin S, Pan X, Jin L, Lu T, Shi L, Wang Y, Xu A, Li X. Adiponectin protects against acetaminophen-induced mitochondrial dysfunction and acute liver injury by promoting autophagy in mice. *J Hepatol* 2014; **61**: 825-831 [PMID: 24882054 DOI: 10.1016/j.jhep.2014.05.033]
627 **Igusa Y**, Yamashina S, Izumi K, Inami Y, Fukada H, Komatsu M, Tanaka K, Ikejima K, Watanabe S. Loss of autophagy promotes murine acetaminophen hepatotoxicity. *J Gastroenterol* 2012; **47**: 433-443 [PMID: 22124574 DOI: 10.1007/s00535-011-0500-0]
628 **Sun Y**, Li TY, Song L, Zhang C, Li J, Lin ZZ, Lin SC, Lin SY. Liver-specific deficiency of unc-51 Like kinase 1 and 2 protects mice from acetaminophen-induced liver injury. *Hepatology* 2018; **67**: 2397-2413 [PMID: 29272037 DOI: 10.1002/hep.29759]
629 **Shan S**, Shen Z, Zhang C, Kou R, Xie K, Song F. Mitophagy protects against acetaminophen-induced acute liver injury in mice through inhibiting NLRP3 inflammasome activation. *Biochem Pharmacol* 2019; **169**: 113643 [PMID: 31542387 DOI: 10.1016/j.bcp.2019.113643]
630 **Hua D**, Ju Z, Gan X, Wang Q, Luo C, Gu J, Yu Y. Human amniotic mesenchymal stromal cells alleviate acute liver injury by inhibiting the pro-inflammatory response of liver resident macrophage through autophagy. *Ann Transl Med* 2019; **7**: 392 [PMID: 31555706 DOI: 10.21037/atm.2019.08.83]
631 **Wu Z**, Han M, Chen T, Yan W, Ning Q. Acute liver failure: mechanisms of immune-mediated liver injury. *Liver Int* 2010; **30**: 782-794 [PMID: 20492514 DOI: 10.1111/j.1478-3231.2010.02262.x]
632 **Dechêne A**, Sowa JP, Gieseler RK, Jochum C, Bechmann LP, El Fouly A, Schlattjan M, Saner F, Baba HA, Paul A, Dries

V, Odenthal M, Gerken G, Friedman SL, Canbay A. Acute liver failure is associated with elevated liver stiffness and hepatic stellate cell activation. *Hepatology* 2010; **52**: 1008-1016 [PMID: 20684020 DOI: 10.1002/hep.23754]

633 **Yin C**, Evason KJ, Asahina K, Stainier DY. Hepatic stellate cells in liver development, regeneration, and cancer. *J Clin Invest* 2013; **123**: 1902-1910 [PMID: 23635788 DOI: 10.1172/JCI66369]

634 **Shen K**, Chang W, Gao X, Wang H, Niu W, Song L, Qin X. Depletion of activated hepatic stellate cell correlates with severe liver damage and abnormal liver regeneration in acetaminophen-induced liver injury. *Acta Biochim Biophys Sin (Shanghai)* 2011; **43**: 307-315 [PMID: 21335335 DOI: 10.1093/abbs/gmr005]

635 **He Y**, Jin L, Wang J, Yan Z, Chen T, Zhao Y. Mechanisms of fibrosis in acute liver failure. *Liver Int* 2015; **35**: 1877-1885 [PMID: 25388426 DOI: 10.1111/liv.12731]

636 **Langer DA**, Das A, Semela D, Kang-Decker N, Hendrickson H, Bronk SF, Katusic ZS, Gores GJ, Shah VH. Nitric oxide promotes caspase-independent hepatic stellate cell apoptosis through the generation of reactive oxygen species. *Hepatology* 2008; **47**: 1983-1993 [PMID: 18459124 DOI: 10.1002/hep.22285]

637 **Jin L**, Gao H, Wang J, Yang S, Wang J, Liu J, Yang Y, Yan T, Chen T, Zhao Y, He Y. Role and regulation of autophagy and apoptosis by nitric oxide in hepatic stellate cells during acute liver failure. *Liver Int* 2017; **37**: 1651-1659 [PMID: 28508586 DOI: 10.1111/liv.13476]

638 **Amir M**, Zhao E, Fontana L, Rosenberg H, Tanaka K, Gao G, Czaja MJ. Inhibition of hepatocyte autophagy increases tumor necrosis factor-dependent liver injury by promoting caspase-8 activation. *Cell Death Differ* 2013; **20**: 878-887 [PMID: 23519075 DOI: 10.1038/cdd.2013.21]

639 **Zhong Z**, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, He F, Boassa D, Perkins G, Ali SR, McGeough MD, Ellisman MH, Seki E, Gustafsson AB, Hoffman HM, Diaz-Meco MT, Moscat J, Karin M. NF-κB Restricts Inflammasome Activation *via* Elimination of Damaged Mitochondria. *Cell* 2016; **164**: 896-910 [PMID: 26919428 DOI: 10.1016/j.cell.2015.12.057]

640 **Han J**, Bae J, Choi CY, Choi SP, Kang HS, Jo EK, Park J, Lee YS, Moon HS, Park CG, Lee MS, Chun T. Autophagy induced by AXL receptor tyrosine kinase alleviates acute liver injury *via* inhibition of NLRP3 inflammasome activation in mice. *Autophagy* 2016; **12**: 2326-2343 [PMID: 27780404 DOI: 10.1080/15548627.2016.1235124]

641 **Wang Q**, Wei S, Zhou S, Qiu J, Shi C, Liu R, Zhou H, Lu L. Hyperglycemia aggravates acute liver injury by promoting liver-resident macrophage NLRP3 inflammasome activation *via* the inhibition of AMPK/mTOR-mediated autophagy induction. *Immunol Cell Biol* 2020; **98**: 54-66 [PMID: 31625631 DOI: 10.1111/imcb.12297]

642 **Zhou S**, Gu J, Liu R, Wei S, Wang Q, Shen H, Dai Y, Zhou H, Zhang F, Lu L. Spermine Alleviates Acute Liver Injury by Inhibiting Liver-Resident Macrophage Pro-Inflammatory Response Through ATG5-Dependent Autophagy. *Front Immunol* 2018; **9**: 948 [PMID: 29770139 DOI: 10.3389/fimmu.2018.00948]

643 **Kim JS**, Wang JH, Biel TG, Kim DS, Flores-Toro JA, Vijayvargiya R, Zendejas I, Behrns KE. Carbamazepine suppresses calpain-mediated autophagy impairment after ischemia/reperfusion in mouse livers. *Toxicol Appl Pharmacol* 2013; **273**: 600-610 [PMID: 24126417 DOI: 10.1016/j.taap.2013.10.006]

644 **Biel TG**, Lee S, Flores-Toro JA, Dean JW, Go KL, Lee MH, Law BK, Law ME, Dunn WA Jr, Zendejas I, Behrns KE, Kim JS. Sirtuin 1 suppresses mitochondrial dysfunction of ischemic mouse livers in a mitofusin 2-dependent manner. *Cell Death Differ* 2016; **23**: 279-290 [PMID: 26184910 DOI: 10.1038/cdd.2015.96]

645 **Evankovich J**, Zhang R, Cardinal JS, Zhang L, Chen J, Huang H, Beer-Stolz D, Billiar TR, Rosengart MR, Tsung A. Calcium/calmodulin-dependent protein kinase IV limits organ damage in hepatic ischemia-reperfusion injury through induction of autophagy. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G189-G198 [PMID: 22575222 DOI: 10.1152/ajpgi.00051.2012]

646 **Kim JS**, Nitta T, Mohuczy D, O'Malley KA, Moldawer LL, Dunn WA Jr, Behrns KE. Impaired autophagy: A mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. *Hepatology* 2008; **47**: 1725-1736 [PMID: 18311843 DOI: 10.1002/hep.22187]

647 **Fang H**, Liu A, Dahmen U, Dirsch O. Dual role of chloroquine in liver ischemia reperfusion injury: reduction of liver damage in early phase, but aggravation in late phase. *Cell Death Dis* 2013; **4**: e694 [PMID: 23807223 DOI: 10.1038/cddis.2013.225]

648 **Eng CH**, Yu K, Lucas J, White E, Abraham RT. Ammonia derived from glutaminolysis is a diffusible regulator of autophagy. *Sci Signal* 2010; **3**: ra31 [PMID: 20424262 DOI: 10.1126/scisignal.2000911]

649 **Cheong H**, Lindsten T, Wu J, Lu C, Thompson CB. Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. *Proc Natl Acad Sci USA* 2011; **108**: 11121-11126 [PMID: 21690395 DOI: 10.1073/pnas.1107969108]

650 **Polletta L**, Vernucci E, Carnevale I, Arcangeli T, Rotili D, Palmerio S, Steegborn C, Nowak T, Schutkowski M, Pellegrini L, Sansone L, Villanova L, Runci A, Pucci B, Morgante E, Fini M, Mai A, Russo MA, Tafani M. SIRT5 regulation of ammonia-induced autophagy and mitophagy. *Autophagy* 2015; **11**: 253-270 [PMID: 25700560 DOI: 10.1080/15548627.2015.1009778]

651 **Soria LR**, Allegri G, Melck D, Pastore N, Annunziata P, Paris D, Polishchuk E, Nusco E, Thöny B, Motta A, Häberle J, Ballabio A, Brunetti-Pierri N. Enhancement of hepatic autophagy increases ureagenesis and protects against hyperammonemia. *Proc Natl Acad Sci USA* 2018; **115**: 391-396 [PMID: 29279371 DOI: 10.1073/pnas.1714670115]

652 **Sridhar S**, Botbol Y, Macian F, Cuervo AM. Autophagy and disease: always two sides to a problem. *J Pathol* 2012; **226**: 255-273 [PMID: 21990109 DOI: 10.1002/path.3025]

653 **Golbabapour S**, Bagheri-Lankarani K, Ghavami S, Geramizadeh B. Autoimmune Hepatitis and Stellate Cells: An Insight into the Role of Autophagy. *Curr Med Chem* 2020; **27**: 6073-6095 [PMID: 30947648 DOI: 10.2174/0929867326666190402120231]

654 **Feng Q**, Yao J, Zhou G, Xia W, Lyu J, Li X, Zhao T, Zhang G, Zhao N, Yang J. Quantitative Proteomic Analysis Reveals That Arctigenin Alleviates Concanavalin A-Induced Hepatitis Through Suppressing Immune System and Regulating Autophagy. *Front Immunol* 2018; **9**: 1881 [PMID: 30177931 DOI: 10.3389/fimmu.2018.01881]

655 **Fan X**, Men R, Wang H, Shen M, Wang T, Ye T, Luo X, Yang L. Methylprednisolone Decreases Mitochondria-Mediated Apoptosis and Autophagy Dysfunction in Hepatocytes of Experimental Autoimmune Hepatitis Model *via* the Akt/mTOR Signaling. *Front Pharmacol* 2019; **10**: 1189 [PMID: 31680966 DOI: 10.3389/fphar.2019.01189]

656 **Fan X**, Men R, Huang C, Shen M, Wang T, Ghnewa Y, Ma Y, Ye T, Yang L. Critical roles of conventional dendritic cells in autoimmune hepatitis *via* autophagy regulation. *Cell Death Dis* 2020; **11**: 23 [PMID: 31932577 DOI: 10.1038/s41419-019-2217-6]

657 **Szekerczés T**, Gógl A, Illyés I, Mandl J, Borka K, Kiss A, Schaff Z, Lendvai G, Werling K. Autophagy, Mitophagy and MicroRNA Expression in Chronic Hepatitis C and Autoimmune Hepatitis. *Pathol Oncol Res* 2020; **26**: 2143-2151 [PMID: 32124227 DOI: 10.1007/s12253-020-00799-y]

658 **Kwak BJ**, Choi HJ, Kim OH, Kim KH, You YK, Lee TY, Ahn J, Kim SJ. The Role of Phospho-c-Jun N-Terminal Kinase Expression on hepatocyte Necrosis and Autophagy in the Cholestatic Liver. *J Surg Res* 2019; **241**: 254-263 [PMID: 31035140 DOI: 10.1016/j.jss.2019.03.034]

659 **Huang YH**, Yang YL, Huang FC, Tiao MM, Lin YC, Tsai MH, Wang FS. MicroRNA-29a mitigation of endoplasmic reticulum and autophagy aberrance counteracts in obstructive jaundice-induced fibrosis in mice. *Exp Biol Med (Maywood)* 2018; **243**: 13-21 [PMID: 29105510 DOI: 10.1177/1535370217741500]

660 **Kim S**, Han SY, Yu KS, Han D, Ahn HJ, Jo JE, Kim JH, Shin J, Park HW. Impaired autophagy promotes bile acid-induced hepatic injury and accumulation of ubiquitinated proteins. *Biochem Biophys Res Commun* 2018; **495**: 1541-1547 [PMID: 29198703 DOI: 10.1016/j.bbrc.2017.11.202]

661 **Khambu B**, Li T, Yan S, Yu C, Chen X, Goheen M, Li Y, Lin J, Cummings OW, Lee YA, Friedman S, Dong Z, Feng GS, Wu S, Yin XM. Hepatic Autophagy Deficiency Compromises Farnesoid X Receptor Functionality and Causes Cholestatic Injury. *Hepatology* 2019; **69**: 2196-2213 [PMID: 30520052 DOI: 10.1002/hep.30407]

662 **Gao L**, Lv G, Guo X, Jing Y, Han Z, Zhang S, Sun K, Li R, Yang Y, Wei L. Activation of autophagy protects against cholestasis-induced hepatic injury. *Cell Biosci* 2014; **4**: 47 [PMID: 25922659 DOI: 10.1186/2045-3701-4-47]

663 **Woolbright BL**, Jaeschke H. Novel insight into mechanisms of cholestatic liver injury. *World J Gastroenterol* 2012; **18**: 4985-4993 [PMID: 23049206 DOI: 10.3748/wjg.v18.i36.4985]

664 **Sasaki M**, Miyakoshi M, Sato Y, Nakanuma Y. Modulation of the microenvironment by senescent biliary epithelial cells may be involved in the pathogenesis of primary biliary cirrhosis. *J Hepatol* 2010; **53**: 318-325 [PMID: 20570384 DOI: 10.1016/j.jhep.2010.03.008]

665 **Sasaki M**, Nakanuma Y. Novel approach to bile duct damage in primary biliary cirrhosis: participation of cellular senescence and autophagy. *Int J Hepatol* 2012; **2012**: 452143 [PMID: 21994884 DOI: 10.1155/2012/452143]

666 **Manley S**, Ni HM, Kong B, Apte U, Guo G, Ding WX. Suppression of autophagic flux by bile acids in hepatocytes. *Toxicol Sci* 2014; **137**: 478-490 [PMID: 24189133 DOI: 10.1093/toxsci/kft246]

667 **Sasaki M**, Miyakoshi M, Sato Y, Nakanuma Y. Increased expression of mitochondrial proteins associated with autophagy in biliary epithelial lesions in primary biliary cirrhosis. *Liver Int* 2013; **33**: 312-320 [PMID: 23231002 DOI: 10.1111/liv.12049]

668 **Sasaki M**, Miyakoshi M, Sato Y, Nakanuma Y. Autophagy mediates the process of cellular senescence characterizing bile duct damages in primary biliary cirrhosis. *Lab Invest* 2010; **90**: 835-843 [PMID: 20212459 DOI: 10.1038/labinvest.2010.56]

669 **van de Graaf S**, Beuers U. Autophagy - another piece of the puzzle towards understanding primary biliary cirrhosis? *Liver Int* 2014; **34**: 481-483 [PMID: 24612169 DOI: 10.1111/liv.12357]

670 **Nakanuma Y**, Sasaki M, Harada K. Autophagy and senescence in fibrosing cholangiopathies. *J Hepatol* 2015; **62**: 934-945 [PMID: 25435435 DOI: 10.1016/j.jhep.2014.11.027]

671 **Sasaki M**, Miyakoshi M, Sato Y, Nakanuma Y. Autophagy may precede cellular senescence of bile ductular cells in ductular reaction in primary biliary cirrhosis. *Dig Dis Sci* 2012; **57**: 660-666 [PMID: 21989821 DOI: 10.1007/s10620-011-1929-y]

672 **Sasaki M**, Nakanuma Y. Biliary epithelial apoptosis, autophagy, and senescence in primary biliary cirrhosis. *Hepat Res Treat* 2010; **2010**: 205128 [PMID: 21152179 DOI: 10.1155/2010/205128]

673 **Young AR**, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, Tavaré S, Arakawa S, Shimizu S, Watt FM, Narita M. Autophagy mediates the mitotic senescence transition. *Genes Dev* 2009; **23**: 798-803 [PMID: 19279323 DOI: 10.1101/gad.519709]

674 **Sasaki M**, Ikeda H, Haga H, Manabe T, Nakanuma Y. Frequent cellular senescence in small bile ducts in primary biliary cirrhosis: a possible role in bile duct loss. *J Pathol* 2005; **205**: 451-459 [PMID: 15685690 DOI: 10.1002/path.1729]

675 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *J Hepatol* 2017; **67**: 145-172 [PMID: 28427765 DOI: 10.1016/j.jhep.2017.03.022]

676 **Lindor KD**, Bowlus CL, Boyer J, Levy C, Mayo M. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2019; **69**: 394-419 [PMID: 30070375 DOI: 10.1002/hep.30145]

677 **Gossard AA**, Lindor KD. Current and promising therapy for primary biliary cholangitis. *Expert Opin Pharmacother* 2019; **20**: 1161-1167 [PMID: 30931642 DOI: 10.1080/14656566.2019.1601701]

678 **Sasaki M**, Nakanuma Y. Bile Acids and Deregulated Cholangiocyte Autophagy in Primary Biliary Cholangitis. *Dig Dis* 2017; **35**: 210-216 [PMID: 28249264 DOI: 10.1159/000450913]

679 **Mueller M**, Thorell A, Claudel T, Jha P, Koefeler H, Lackner C, Hoesel B, Fauler G, Stojakovic T, Einarsson C, Marschall HU, Trauner M. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. *J Hepatol* 2015; **62**: 1398-1404 [PMID: 25617503 DOI: 10.1016/j.jhep.2014.12.034]

680 **Panzitt K**, Jungwirth E, Krones E, Lee JM, Pollheimer M, Thallinger GG, Kolb-Lenz D, Xiao R, Thorell A, Trauner M, Fickert P, Marschall HU, Moore DD, Wagner M. FXR-dependent Rubicon induction impairs autophagy in models of human cholestasis. *J Hepatol* 2020; **72**: 1122-1131 [PMID: 32001325 DOI: 10.1016/j.jhep.2020.01.014]

681 **Wagner M**, Zollner G, Trauner M. Nuclear receptors in liver disease. *Hepatology* 2011; **53**: 1023-1034 [PMID: 21319202 DOI: 10.1002/hep.24148]

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MINIREVIEWS

Post-liver transplant biliary complications: Current knowledge and therapeutic advances

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Abstract

Liver transplantation is the current standard of care for end-stage liver disease and an accepted therapeutic option for acute liver failure and primary liver tumors. Despite the remarkable advances in the surgical techniques and immunosuppressive therapy, the postoperative morbidity and mortality still remain high and the leading causes are biliary complications, which affect up to one quarter of recipients. The most common biliary complications are anastomotic and non-anastomotic biliary strictures, leaks, bile duct stones, sludge and casts. Despite the absence of a recommended treatment algorithm many options are available, such as surgery, percutaneous techniques and interventional endoscopy. In the last few years, endoscopic techniques have widely replaced the more aggressive percutaneous and surgical approaches. Endoscopic retrograde cholangiography is the preferred technique when duct-to-duct anastomosis has been performed. Recently, new devices and techniques have been developed and this has led to a remarkable increase in the success rate of minimally invasive procedures. Understanding the mechanisms of biliary complications helps in their early recognition which is the prerequisite for successful treatment. Aggressive endoscopic therapy is essential for the reduction of morbidity and mortality in these cases. This article focuses on the common post-transplant biliary complications and the available interventional treatment modalities.

Key Words: Post-transplant biliary complications; Endoscopic retrograde cholangiopancreatography; Cholangioscopy; Percutaneous biliary interventions; Liver transplantation; Living-donor liver transplantation

Core Tip: Liver transplantation is the current standard of care for end-stage liver disease. Biliary complications are the leading cause of morbidity and mortality among recipients and despite the advances in surgical techniques they are seen in up to 25% of cases. Surgery, interventional endoscopy and percutaneous approaches are the available therapeutic options. Endoscopic retrograde cholangiography when possible is the most recommended therapeutic modality, replacing more aggressive surgical interventions. New techniques such as cholangioscopy overcome many of the limitations of conventional endoscopy. This article discusses the most common post-transplant biliary complications and the advances in treatment modalities.

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INTRODUCTION

Liver transplantation (LT) is the widely endorsed method for treatment of end- stage liver disease, acute liver failure and primary liver cancer. The advances in surgical techniques, postoperative care, immunosuppression, and antiviral therapy have led to remarkable progress in survival of these patients. The currently reported 5-year survival rate is 70%-75%[1,2].

Biliary complications are a significant source of morbidity in the early and long-term period after LT (Table 1). Their overall incidence ranges between 15% and 25%. With associated mortality of 10%, they remain a major problem in post-transplant patients. Timely identification and treatment play a significant role in preserving the graft and improving the overall survival rate of patients[3,4].

The most common current treatment is focused on interventional endoscopic (ERC) and percutaneous (PTC) procedures[4-6].

ERC provides minimal invasion with great long-term results and is a preferred method when surgical reconstruction allows this. ERC has been proven to be safe and highly effective in dealing with most of the early as well as late post-LT biliary complications. Procedural-related adverse events in post-LT cases are comparable with those among the general population[6].

The complication rate in patients after living donor liver transplantation (LDLT) in particular is about 10%, which is 2-fold higher than the standard[7,8].

PTC is an effective alternative in patients with altered anatomy which impedes endoscopy access. There is growing evidence that cholangioscopy could be a beneficial tool in the diagnostics and therapy of selected cases[9].

Surgery is available for cases when endoscopic and PCT methods have failed.

Biliary reconstruction techniques

The two major options for biliary reconstruction are bilio-enteric (hepatico-jejunostomy or choledocho-jejunostomy) and duct-to-duct anastomosis. Duct-to-duct anastomosis is the method of choice for biliary reconstruction in any type of transplantation: Cadaveric liver transplantation (DDLT), split transplantations, LDLT (left lobe or right lobe) transplantations[10,11].

Hepatico-jejunostomy is currently used only for selected cases such as those with primary sclerosing cholangitis, prior bilio-digestive surgery, significant ductal size mismatch, and insufficient length of recipient bile ducts[12].

Many benefits motivate the preference for direct duct-to-duct suturing: Preserved sphincter-of-Oddi function, lower risk of cholangitis, and reduction in the number of anastomoses. Besides, the preserved intestinal continuity ensures an endoscopic access to the biliary tree in case of potential complications [13,14].

T-tube placement has been widely abandoned over the last decades[15]. It has been proven that its usage increases the rate of biliary complications. A single-center retrospective review of 1041 transplantations reported that cholestatic liver disease, Roux-en-Y anastomosis, donor risk index > 2, and T-tubes were independent predictors of post-LT complications[16].

LDLT and DDLT

The rising number of LTs augments the need for liver grafts. This has led to the widespread tendency of LDLT. Multiple factors related to LDLT techniques contribute to the increased incidence of biliary complications[11].

Hepatic resection of the donor liver in LDLT requires dissection of the hilum, which could cause bile duct devascularization or subsequent bile leak from the cut surface of the liver. Excessive use of coagulation diathermy is another risk factor for the occurrence of bile leak. On the other hand, the need

Table 1 Risk factors for the most common biliary complications

	Anastomotic	Non-anastomotic
Strictures	Advanced recipient age; Female donor; Failure to flush the donor duct; Preceding bile leakage; Acute rejection; Chronic rejection; Hepaticojejunostomy reconstruction	HAT; Chronic ductopenic rejection; Blood type ABO incompatibility; PSC, autoimmune hepatitis prolonged warm and cold ischemia times prolonged donor exposure to vasopressors
Leaks	Active bleeding at the bile duct end excessive dissection of periductal tissue tension on ductal anastomosis	T-tube tract, excessive use of electrocautery incorrect suture of the cystic duct stump
Stones and clots	Ischemia, stricture, infection	
Biliary cast syndrome	Acute cellular rejection, bile stasis, ischemia, infection, sepsis, HAT	
Haemobilia	Alcoholic liver disease, high body mass index of recipient; Iatrogenic: PTC, liver biopsy	

PSC: Primary sclerosing cholangitis; PTC: Percutaneous; HAT: Hepatic artery thrombosis.

for dissection of the recipient's left or right hepatic duct could prolong the ischemic time. Bringing the recipient's hepatic duct to the graft's hilum to ensure tension-free anastomosis could cause additional disturbance of the blood supply. In general, the reported biliary complication rate is 2-3-fold higher in LTLD than in DDLT. Furthermore, the treatment is usually more complicated due to the smaller size of the ducts or the presence of multiple anastomoses. Therefore, the success rate of treatment for complications is lower in LDLT[14,17-19].

Classification

The most common complications are strictures, leaks, and biliary stones. According to the timeframe of their occurrence, post-LT complications can be divided into early (occurring within the first 4 wk after transplantation) and late. Biliary leaks are the most common complication in the early postoperative period, while biliary strictures are the predominant complication as a whole. According to the lesion location, strictures and leaks are divided into anastomotic and non-anastomotic[20-22].

It is appropriate to make a distinction between biliary stricture and biliary obstruction. While the obstruction can be caused by external compression (biloma, haematoma), luminal cast, stones or tube remnants, the stricture is narrowing of the duct lumen, causing bile outflow disturbance.

Multiple factors can play a role in the occurrence of biliary complications. Anastomotic lesions are mostly due to technical issues, while non-anastomotic lesions are the result of ischemia or immune reactions[23].

With respect to the etiology, some authors divide the complications into five groups[21]: (1) Hepatic artery thrombosis-related; (2) Technical biliary complications; (3) Ischemic-type biliary lesions; (4) Infectious biliary complications; and (5) Uncommon: Sphincter of Oddi dysfunction (SOD), bile cast syndrome, haemobilia, lymphoproliferative disease, and other neoplasms.

Biliary strictures

Up to 50% of post-LT biliary complications consist of biliary strictures[24]. They are divided into two major morphological types: Anastomotic (AS) and non-anastomotic (NAS).

Most frequently, the strictures are anastomotic. AS appear more often in LDLT than in DDLT. They are short, single narrowings, located at the anastomotic site. The incidence ranges between 5%-15% in DDLT and 13%-36% in LDLT[21-26]. They occur mostly during the first year after transplantation within a mean time of 5-8 wk[23,27].

The most common factors associated with AS are surgical issues over the first months and ischemia leading to fibrous healing at the later stages. Additionally, ABO incompatibility, advanced recipient age, small bile duct caliber, prolonged warm and cold ischemia time, and cytomegalovirus infection are reported to be significant risk factors[25,28-30].

Endoscopic retrograde cholangiography (ERCP) is the standard of care for AS treatment, whenever anatomy allows it. The overall reported success rate ranges between 70%-100%[31-33].

For patients with hepatico-jejunostomy, different scopes such as single or double balloon enteroscope, spiral enteroscope or pediatric colonoscope are used. These techniques are time-consuming and complex; they require additional expertise and are related to higher risk and higher cost[32,34-37].

For all these reasons, PTC is a widely accepted approach in cases of altered anatomy[38]. Surgical therapy is now used as salvage therapy and is required in about 1% of cases[39].

AS treatment aims to normalize bile outflow through the anastomosis. The endpoint of ERC is lack of narrowing during occlusive cholangiography or free contrast outflow during fluoroscopy (Figure 1). Clinical and laboratory resolution of cholestasis are the most reliable measures of successful treatment.

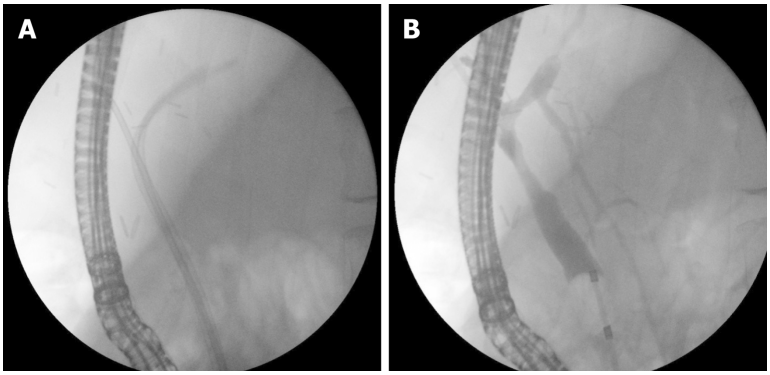

Figure 1 Endoscopic treatment of anastomotic stricture after living donor liver transplantation. A: Two plastic stents; and B: Occlusive cholangiogram after treatment.

The standard treatment includes guidewire insertion across the stricture, followed by balloon dilation and stent insertion. Most commonly 10Fr or 7Fr plastic stents are used. These stents can be easily removed or replaced. Balloon dilation in itself is effective as a non-invasive technique, which has shown less than promising long-term results with a 30%-40% success rate[34,40] (Figure 2).

Numerous large studies have proven that the combination of balloon dilation plus stent placement is more effective than dilation or stenting alone[41].

Several endoscopic strategies are applied in the management of anastomotic strictures. The most frequently used technique is balloon dilation with placement of a maximum number of 10Fr plastic stents with subsequent stent exchange until full resolution of the stricture on fluoroscopy (Table 2).

The initial dilation requires 4-10 mm balloons. In rare cases of tight strictures a Soehendra catheter can be used to overcome the stricture. The progressive increase in the number of stents with every subsequent procedure has ensured more sustained resolution of the stricture[42,43].

Different time intervals between stent exchanges were investigated. In a study from 2008, a short-term stent exchange of every 2 wk was investigated. The reported resolution rate was 87%, achieved for a mean period of 107 d and a mean number of stents inserted of 2.5. More often stents are replaced every three months to prevent occlusion and cholangitis. The reported success rate in many large studies is 80%-95%[39,41-43]. In a review of 440 post-LT patients with AS, the success rate of stent therapy was 84%. The resolution rate was established to be dependent on therapy duration and was highest (94%-100%) when therapy lasted 12 mo or more[44].

The time it takes for the structure to evolve has also been proved to be a predictive factor for healing. Strictures manifested within the first 6 mo after LT have better prognosis for sustained resolution[25,31,45].

Due to elevated rupture risk, it is preferable for ERC to be postponed at least one month after the transplantation. When necessary, a 7-8.5 Fr stent is applied without balloon dilation. In tight strictures, a 4 mm angioplasty balloon may be considered[46].

Some new dilation balloons have been tested in order to improve bile stricture resolution. There are few published data on the usage of peripheral cutting balloons[47].

Paclitaxel-eluting balloons have been investigated, due to the fact that paclitaxel can suppress fibrotic proliferation[48]. The latter two are not in common use.

An available alternative to the standard multiple-plastic-stent therapy is the placement of fully covered self-expanding metal stents (fSEMS). Their major benefits are a reduction in the number of procedures and cost-effectiveness[49-52].

In a substantive study with 200 cases, the reported success was 80%-95%[51]. Eight and 10 mm SEMS are available according to the stricture size. FSEMS are not considered suitable for AS smaller than 5 mm[24].

Stent migration is the major limitation of this technique. The main strategies to prevent migration include skipping dilation of the stricture, using stents with flaps, and leaving the stent in the duodenum for a long period[49-51].

A large systematic review, published in 2013, reported a migration rate of fSEMS of 16%; the authors also mentioned a low risk of stent ingrowth and stent impaction. The comparison analysis in that study showed that neither technique was superior[49,53].

Management in LDLT is more challenging due to the frequent presence of multiple anastomoses with a smaller size (Figure 3). According to Coté *et al*[24], significant risk factors for treatment failure in LDLT are higher LT recipient age, longer operation duration, and a pouched morphology of the AS.

Non-anastomotic strictures consist of one or more duct narrowings proximal to the anastomosis. They are longer, complex, and usually multiple, and can affect intra- and extrahepatic ducts. NAS are more rarely observed: 5%-10% of biliary complications[54]. Ischemia and immunological reactions are the main aetiological mechanisms. The most common risk factors reported in the literature are hepatic

Table 2 Studies on the effectiveness of maximal stent therapy in post-operative biliary strictures

Ref.	Patients	Treatment duration	Mean number of stents	Number of ERCPs	Success rate
Costamagna *et al*[41], 2001	45	12.1 mo (range 2-24 mo)	3.2 (range 1-6)	4.1 (range 2-8)	89% (40/45)
Hsieh *et at*[23], 2012	41	5.3 (range 3.8-8.9)	7.0 (range 4-10)	4.0 (range 3.0-5.3)	100% (41/41)
Morelli *et al*[43], 2008	38	107 d (range 20-198 d)	2.5 (range 1-6)	3.4 (range 2-6)	87% (33/38)
Pasha *et al*[90], 2004	25	3.3 mo (range, 2.2-7 mo)	2.0 (range 1-4)	3.5 (range 1-9)	88% (22 of 25)
Tabibian *et al*[42], 2010	69	15 mo (range 12-60 mo)	3.0 (range 2-7)	2.5 (range 2-5)	94% (65/69)

ERCP: Endoscopic retrograde cholangiography.

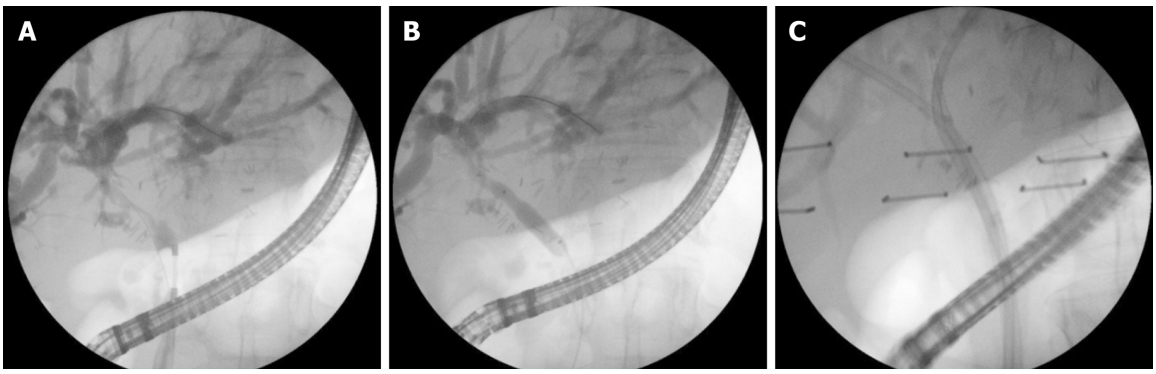

Figure 2 Anastomotic stricture. A: Cholangiogram; B: Balloon dilation; and C: Multiple stent treatment.

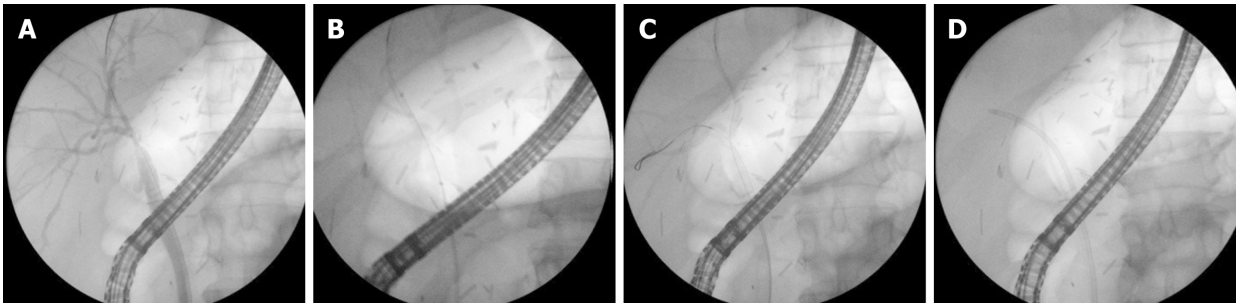

Figure 3 Anastomotic stricture after living donor liver transplantation (right lobe). A: Guidewire insertion; B: Balloon dilation; C: Second guidewire insertion; and D: Stent placement (7Fr + 5Fr).

artery thrombosis, prolonged cold and warm ischemia, prolonged exposure to vasopressors of the donor, ABO incompatibility, chronic ductopenic rejection, PSC or autoimmune hepatitis in the recipient [55,56]. In the case of acute hepatic artery thrombosis, early revascularization therapy is required to prevent multiple stricture formation.

Cases with NAS could benefit from mini-invasive (endoscopic and percutaneous) treatment, but the estimated results are significantly worse than in cases with AS. In cases with dominant strictures and extrahepatic localization ERC is the first treatment option. Endoscopic access to NAS is much more challenging due to the small caliber and relatively proximal location[53]. Cases with angulated, complex strictures, not suitable for ERC passage benefit from percutaneous approaches, followed by hybrid procedures such as the rendezvous technique. When intrahepatic strictures are present, PTC with direct radiology-guided percutaneous stent insertion could be in order[57,58].

Stricture recurrence and continued stricture formation are possible even after successful endoscopic therapy. Long-term observation (MRCP and laboratory) of these patients is required to evaluate the disease course and the response to treatment. Cases resistant to stent treatment, or those with diffuse bile duct injury, must be listed for re-transplantation. Percutaneous drainage could be a bridging therapy to the operation[54,58].

The reported success rate of stenting therapy in the literature is 50% to 75% for DDLT and 33%-50% for LDLT[26,50]. In most NAS cases, the treatment process also takes longer than with AS[11,59].

Passing a guidewire through the stricture is considered the most critical moment. Occlusion balloons and swing-tip catheters for selective cannulation are used for this purpose[54]. The rendezvous technique could also be used to deal with this issue[59,60].

After successful cannulation, the standard technique of balloon dilation followed by plastic stent insertion is performed. For this type of stricture, 4-6 mm balloons with a subsequent increase in caliber are used. Even when cannulated, the width, angulation and proximal location of the strictures often limit the stent insertion. The stents used are usually 7 or 8.5Fr and carry a high migration risk due to rigidity of the plastic[58,59].

A working group from Minnesota reported their treatment for NAS with long (12-20 cm), 10Fr flexible stents with side fenestration. They provide better bile drainage through the stent and through the side holes and could be inserted higher due to their flexibility[61].

Cholangioscopy provides direct visualisation of the biliary tree. This allows visual assessment of the biliary epithelium at the stricture and tissue sampling if needed. In cases of strictures, not suitable for standard cannulation, cholangioscopy enables guidewire insertion under visual control (Figure 4). This facilitates guidewire placement in tight, angulated strictures. Cholangioscopy has been proven to increase the stricture cannulation rate and the success rate of endoscopy treatment as a whole (Figure 5). The implementation of cholangioscopy in stricture therapy could spare the need for percutaneous drainage and surgical interventions[62,63].

Bile leaks

Bile leaks are the second most frequent biliary complication after LT. Bile leaks are also divided into anastomotic and non-anastomotic. Most of them are anastomotic and occur early - within the first 4 wk after LT[8,10,64].

The reported incidence in the literature ranges from 2% to 25%[13,22].

Their occurrence is slightly higher in patients with bilio-enteric reconstruction than with cases of duct-to-duct anastomosis. A systematic review, including data from 61 studies, reported the incidence of bile leaks to be 9.5% in LDLT and 7.8% in DDLT[64]. The presence of a bile leak is an independent risk factor for further development of a stricture[65].

Early bile leaks are usually caused by technical issues related to surgery, such as tension of the anastomosis, incomplete cystic stump suture, excessive use of diathermy, bleeding from the cut ends of ducts, premature T-tube extraction, and the cut surface of the graft. Ischemic injury is the other major cause of bile leaks[8,18,19,52]. Large studies have shown double and triple hepatico-jejunostomy and warm ischemia time as independent risk factors for the occurrence of bile leaks[17,18].

Bile diversion is the key to bile leak healing. Therapeutic options include ERC followed by stenting or nasobiliary drainage, percutaneous drainage, and surgical revision. Sphincterotomy with endoscopic stenting leads to reduction in the transpapillary pressure, usually followed by fast lesion closure. Stent placement leads to successful treatment in over 90% of cases with early leaks (Figure 6)[13,66]. Simple defects like T-tube exit, cystic duct remnant or small anastomotic leaks usually close in 2-5 wk. The biliary stent is usually extracted after no less than 3 mo due to potentially delayed tissue healing on account of immunosuppression. Some centers prefer the placement of nasobiliary drainage for early small defects. This allows close fluoroscopic follow-up of the defect closure and avoids the need for a second stent extraction procedure. Given the low patient tolerance, displacement risk, and prolonged hospital stay, this practice is currently of limited use[19,66].

In cases with defects, refractory to plastic stent treatment, fcSEMS usage could be considered. Small studies have reported good closure success rate[67-69].

According to a study including 35 cases treated with 8 mm and 10 mm fcSEMS, the achieved leak resolution was 94%[68].

In some studies, a high incidence of stricture was observed after stent removal[70].

In cases with bilio-enteric anastomosis, percutaneous access to the biliary tree is used for bile diversion. An internal-external drainage placement for 3-6 mo is an effective alternative to the endoscopic approach. A technique with EUS-guided gastrostomy, used for ERCP access, is also reported in a small study from 2011[33].

In cases with a T-tube, drainage unclamping is sufficient. When bile juice is diverted outside the body (nasobiliary, percutaneous, T-tube drainage), the level of immunosuppression medication, in particular cyclosporine, should be closely monitored. If a significant collection is formed, the latter must be drained to prevent infection, sepsis, and late adhesion. Large or complex leaks often require surgical revision due to a high probability of intra-abdominal abscess formation[54,55].

Bile stones and sludge

Formation of sludge, clots, casts and stones can cause bile obstruction. The reported incidence after LT ranges widely between 4%-10%[71,72].

Cyclosporine therapy, mucosal damage due to ischemia or infection and cholesterol supersaturation (often seen post-LT) could predispose to lithogenesis. In many cases, there is an underlying stricture. Usually, an ERC and sludge/stone extraction procedure is sufficient for definitive treatment with a success rate over 90%[72-76].

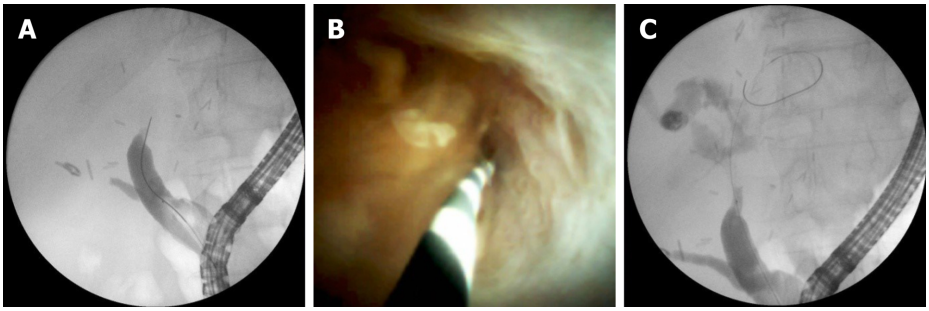

Figure 4 Complex anastomotic stricture. A: Impossible insertion of guidewire through a stricture; B: Guidewire insertion under direct visual control; and C: Guidewire inserted above anastomosis.

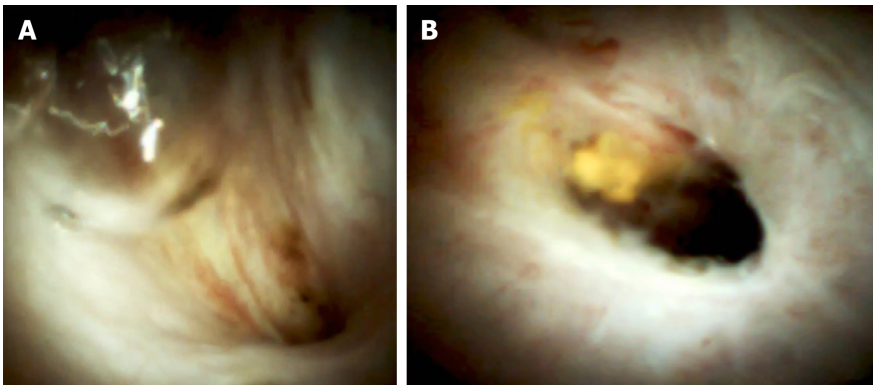

Figure 5 Digital cholangioscopy image of an anastomotic stricture.

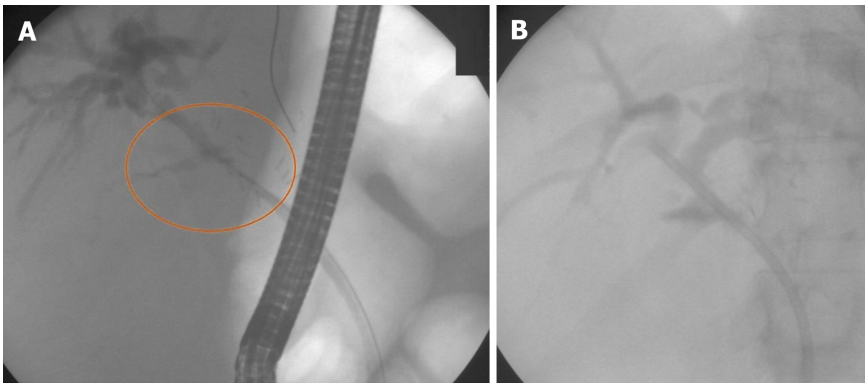

Figure 6 Anastomotic leak. A: Guidewire insertion; and B: Stent placement (10Fr).

According to Alazmi *et al*[45], there is a 17% incidence rate of recurrence within the first 6 mo after the procedure.

Well-known techniques, such as large balloon dilation and mechanical lithotripsy, are used in cases of large stones. In cases of difficult lithiasis such as multiple, large or intrahepatic stones, as well as stones over the stricture, extracorporeal lithotripsy could be applied. A study in 2015 reported six cases of difficult lithiasis that could not be treated with standard ERCP. Five of the six cases were managed with ECSL with no reported adverse events[77].

The limitations of endoscopic therapy can be overcome by means of digital cholangioscopy. Cholangioscopy provides an opportunity for visually controlled fragmentation of large biliary stones with little risk of biliary injury. Advanced intraductal techniques such as Holmium laser or Electrohydraulic lithotripsy achieve outstanding results in difficult cases, not suitable for ERC treatment (Figure 7)[62,78,79].

A research team from South Korea (Nam *et al*[79]) reported a case series of 15 patients (intrahepatic lithiasis n = 10, biliary cast syndrome n = 3, stones over the stricture n = 2) treated with percutaneous intrahepatic cholangioscopy. Eleven patients were successfully managed and no procedure-related adverse events were observed[79].

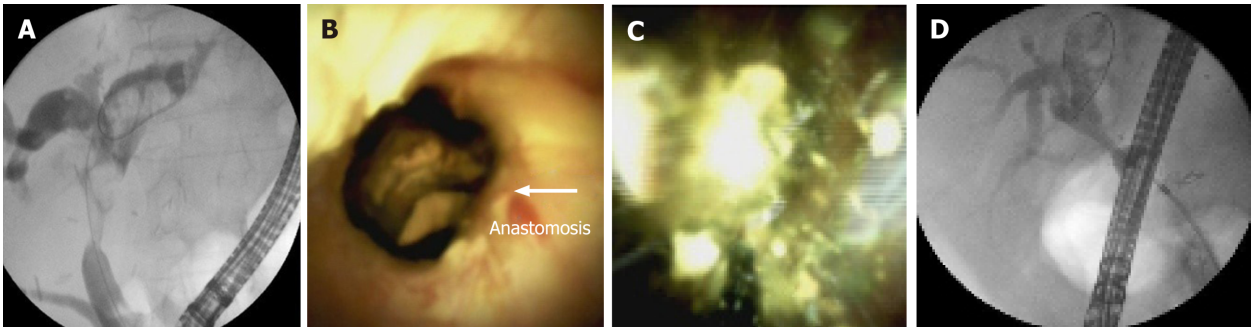

Figure 7 Multiple intrahepatic stones above anastomotic stricture. A: Fluoroscopic image; B: Digital cholangioscopic image; C: Electrohydraulic lithotripsy performance; and D: Fluoroscopic image after treatment.

Biliary cast syndrome

This disorder represents multiple filling defects in intra- and extrahepatic bile ducts, caused by casts adherent to the biliary epithelium. The reported incidence varies between 2.5% and 18%[80,81].

The pathogenetic mechanism is considered to be cell injury as a result of ischemia, acute cellular rejection, chronic rejection, infection, or bile stasis. The desquamated epithelial cells combined with bile components may form hard casts[82].

ERC with bile tree flushing and cast extraction will suffice in many cases. Balloon extractors and Dormia baskets are used for this purpose[81]. In cases of extended intrahepatic involvement or altered anatomy, a percutaneous procedure could be needed. In a study of 10 patients with biliary cast syndrome, mini-invasive (endoscopic/percutaneous) treatment was successful in 60% of cases[83].

Several studies noted good outcomes following cholangioscopy-guided therapy of bile cast syndrome (Figure 8). Nam *et al*[79] reported three cases, treated by percutaneous cholangioscopy.

Ursodeoxycholic acid is considered to have a role in the prophylaxis of bile cast syndrome. In cases refractory to mini-invasive therapy, surgery is required.

Sphincter of Oddi dysfunction

Chronic injury, denervation of the recipient's common bile duct (CBD), or fibrotic tissue formation could cause impaired ampullary relaxation and hypertension of the papilla of Vater. The role of biliary manometry in the diagnosis of SOD after transplantation is uncertain. Sphincterotomy is usually sufficient to resolve the obstruction[27,29]. In cases of firm fibrosis, stent placement could be in order[8,21].

Mucocele

In rare cases, the donor's cystic duct could be incorporated in the suture line of the anastomosis. As a consequence, a blind mucosa-lined sac is formed. Due to accumulation of mucin, this sac can increase in size and cause bile obstruction due to external compression. Endoscopy could not provide sustainable resolution in such cases. Percutaneous drainage or surgical resection are effective treatment options. The differential diagnosis of mucocele is made with any type of fluid collection such as biloma, abscess, hemorrhage, and aneurysm[8,84,85].

Redundant CBD

The excessive length of the donor's common hepatic duct could lead to a sigmoid-shaped deflection of the CBD. This could entail bile outflow deterioration. The reported incidence is 1.6% in all LT. ERC with long plastic stent placement usually resolves cholestasis. In very rare cases, surgery with a new biliodigestive anastomosis is needed[86].

Haemobilia

Spontaneous haemorrhage in the biliary tree after LT occurs rarely with a reported frequency of 1.2%. There are reported cases of haemobilia associated with large biliary stones over the stricture. More often, haemobilia is iatrogenic, *i.e.* subsequent to percutaneous biliary drainage or liver biopsy. Rupture of a hepatic artery pseudoaneurysm can cause severe biliary haemorrhage. Recipient high BMI and alcoholic liver disease were significant risk factors for spontaneous haemobilia reported in a study including 2701 post-LT patients[87].

ERC with clot extraction and nasobiliary drainage placement is the first-choice therapy. Nasobiliary drainage ensures an opportunity for biliary lavage, which prevents the development of cholangitis and indicates the presence of recurrent bleeding. In most cases, the combination of endoscopic desobstruction therapy, coagulation correction, and supportive medication yields good results. In cases of severe haemorrhage, selective embolization techniques are reported to be successful. Plastic biliary stents or fSEMS were reported to be effective haemostatic tools in studies of non-transplant patients

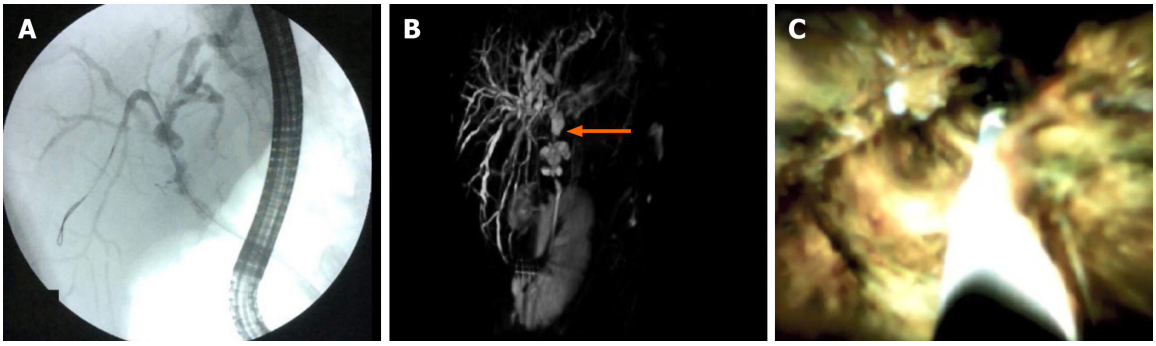

Figure 8 Biliary cast syndrome. A: Fluoroscopic image; B: Magnetic resonance cholangiopancreatography; and C: Digital cholangioscopic image.

with significant haemobilia[88-90].

Due to low incidence, there are not enough data regarding post-transplant patients with severe haemobilia.

Foreign bodies

Suture materials or T-tube remnants could form a nidus for bile sediment and stones. ERC and PTC are effective methods for detection and clearance of bile duct remnants[59].

CONCLUSION

Known as the Achilles' heel of liver transplantation, biliary complications are observed in one quarter of all patients. Their prevalence has increased due to the worldwide increase in liver transplantation. Living donor liver transplantations have a higher complication rate and presuppose more complicated treatment scenarios with lower success rates. Endoscopic stent insertion is the key treatment for most biliary complications. Percutaneous or EUS-guided puncture and cholangioscopy are feasible options for biliary access when standard fluoroscopic cannulation fails. A wide variety of accessories have been developed to overcome the complexity of living donor liver transplantation complications, but the treatment success rate remains unsatisfactory. Early recognition and aggressive management are essential for the reduction of morbidity and mortality in patients with biliary complications.

FOOTNOTES

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REFERENCES

1 **Yoshida EM**, Haque M, Scudamore CH. Adult live donor liver transplantation: routine, commonplace, standard care for

end stage liver disease (we hope). *Ann Hepatol* 2010; **9**: 89-90 [PMID: 20308727]
2 **Charlton MR**. Roadmap for improving patient and graft survival in the next 10 years. *Liver Transpl* 2016; **22**: 71-78 [PMID: 27514705 DOI: 10.1002/lt.24602]
3 **Girotra M**, Soota K, Klair JS, Dang SM, Aduli F. Endoscopic management of post-liver transplant biliary complications. *World J Gastrointest Endosc* 2015; **7**: 446-459 [PMID: 25992185 DOI: 10.4253/wjge.v7.i5.446]
4 **Moy BT**, Birk JW. A Review on the Management of Biliary Complications after Orthotopic Liver Transplantation. *J Clin Transl Hepatol* 2019; **7**: 61-71 [PMID: 30944822 DOI: 10.14218/JCTH.2018.00028]
5 **Patel P**, DiNorcia J. The devil is in the detail: current management of perioperative surgical complications after liver transplantation. *Curr Opin Organ Transplant* 2019; **24**: 138-147 [PMID: 30694994 DOI: 10.1097/MOT.0000000000000616]
6 **Stratta RJ**, Wood RP, Langnas AN, Hollins RR, Bruder KJ, Donovan JP, Burnett DA, Lieberman RP, Lund GB, Pillen TJ. Diagnosis and treatment of biliary tract complications after orthotopic liver transplantation. *Surgery* 1989; **106**: 675-683; discussion 683-684 [PMID: 2799642]
7 **Balderramo D**, Bordas JM, Sendino O, Abraldes JG, Navasa M, Llach J, Cardenas A. Complications after ERCP in liver transplant recipients. *Gastrointest Endosc* 2011; **74**: 285-294 [PMID: 21704993 DOI: 10.1016/j.gie.2011.04.025]
8 **Thuluvath PJ**, Pfau PR, Kimmey MB, Ginsberg GG. Biliary complications after liver transplantation: the role of endoscopy. *Endoscopy* 2005; **37**: 857-863 [PMID: 16116539 DOI: 10.1055/s-2005-870192]
9 **Elmunzer BJ**, Debenedet AT, Volk ML, Sonnenday CJ, Waljee AK, Fontana RJ, Oza AB, Singal A, Englesbe MJ, Scheiman JM. Clinical yield of diagnostic endoscopic retrograde cholangiopancreatography in orthotopic liver transplant recipients with suspected biliary complications. *Liver Transpl* 2012; **18**: 1479-1484 [PMID: 22888069 DOI: 10.1002/lt.23535]
10 **Greif F**, Bronsther OL, Van Thiel DH, Casavilla A, Iwatsuki S, Tzakis A, Todo S, Fung JJ, Starzl TE. The incidence, timing, and management of biliary tract complications after orthotopic liver transplantation. *Ann Surg* 1994; **219**: 40-45 [PMID: 8297175]
11 **Graziadei IW**, Schwaighofer H, Koch R, Nachbaur K, Koenigsrainer A, Margreiter R, Vogel W. Long-term outcome of endoscopic treatment of biliary strictures after liver transplantation. *Liver Transpl* 2006; **12**: 718-725 [PMID: 16482553 DOI: 10.1002/Lt.20644]
12 **Graziadei IW**. Recurrence of primary sclerosing cholangitis after liver transplantation. *Liver Transpl* 2002; **8**: 575-581 [PMID: 12089708 DOI: 10.1053/jlts.2002.33952]
13 **Akamatsu N**, Sugawara Y, Hashimoto D. Biliary reconstruction, its complications and management of biliary complications after adult liver transplantation: a systematic review of the incidence, risk factors and outcome. *Transpl Int* 2011; **24**: 379-392 [PMID: 21143651 DOI: 10.1111/j.1432-2277.2010.01202.x]
14 **Welling TH**, Heidt DG, Englesbe MJ, Magee JC, Sung RS, Campbell DA, Punch JD, Pelletier SJ. Biliary complications following liver transplantation in the model for end-stage liver disease era: effect of donor, recipient, and technical factors. *Liver Transpl* 2008; **14**: 73-80 [PMID: 18161843 DOI: 10.1002/Lt.21354]
15 **Rizk RS**, McVicar JP, Emond MJ, Rohrmann CA Jr, Kowdley KV, Perkins J, Carithers RL Jr, Kimmey MB. Endoscopic management of biliary strictures in liver transplant recipients: effect on patient and graft survival. *Gastrointest Endosc* 1998; **47**: 128-135 [PMID: 9512276 DOI: 10.1016/s0016-5107(98)70344-x]
16 **Senter-Zapata M**, Khan AS, Subramanian T, Vachharajani N, Dageforde LA, Wellen JR, Shenoy S, Majella Doyle MB, Chapman WC. Patient and Graft Survival: Biliary Complications after Liver Transplantation. *J Am Coll Surg* 2018; **226**: 484-494 [PMID: 29360615 DOI: 10.1016/j.jamcollsurg.2017.12.039]
17 **Kasahara M**, Egawa H, Takada Y, Oike F, Sakamoto S, Kiuchi T, Yazumi S, Shibata T, Tanaka K. Biliary reconstruction in right lobe living-donor liver transplantation: Comparison of different techniques in 321 recipients. *Ann Surg* 2006; **243**: 559-566 [PMID: 16552210 DOI: 10.1097/01.sla.0000206419.65678.2e]
18 **Johnston TD**, Gates R, Reddy KS, Nickl NJ, Ranjan D. Nonoperative management of bile leaks following liver transplantation. *Clin Transplant* 2000; **14**: 365-369 [PMID: 10946772 DOI: 10.1034/j.1399-0012.2000.14040102.x]
19 **Oh DW**, Lee SK, Song TJ, Park DH, Lee SS, Seo DW, Kim MH. Endoscopic management of bile leakage after liver transplantation. *Gut Liver* 2015; **9**: 417-423 [PMID: 25717048 DOI: 10.5009/gnl14117]
20 **El Deen FZ**, Lee CS, Lee WC. Endoscopic Management of Biliary Complications after Liver Transplantation. *Ther Gastrointest Endosc* **2011**: 93-114 [DOI: 10.5772/23525]
21 **Wojcicki M**, Milkiewicz P, Silva M. Biliary tract complications after liver transplantation: a review. *Dig Surg* 2008; **25**: 245-257 [PMID: 18628624 DOI: 10.1159/000144653]
22 **Williams ED**, Draganov PV. Endoscopic management of biliary strictures after liver transplantation. *World J Gastroenterol* 2009; **15**: 3725-3733 [PMID: 19673012 DOI: 10.3748/wjg.15.3725]
23 **Tashiro H**, Itamoto T, Sasaki T, Ohdan H, Fudaba Y, Amano H, Fukuda S, Nakahara H, Ishiyama K, Ohshita A, Kohashi T, Mitsuta H, Chayama K, Asahara T. Biliary complications after duct-to-duct biliary reconstruction in living-donor liver transplantation: causes and treatment. *World J Surg* 2007; **31**: 2222-2229 [PMID: 17885788 DOI: 10.1007/s00268-007-9217-x]
24 **Coté GA**, Slivka A, Tarnasky P, Mullady DK, Elmunzer BJ, Elta G, Fogel E, Lehman G, McHenry L, Romagnuolo J, Menon S, Siddiqui UD, Watkins J, Lynch S, Denski C, Xu H, Sherman S. Effect of Covered Metallic Stents Compared With Plastic Stents on Benign Biliary Stricture Resolution: A Randomized Clinical Trial. *JAMA* 2016; **315**: 1250-1257 [PMID: 27002446 DOI: 10.1001/jama.2016.2619]
25 **Verdonk RC**, Buis CI, Porte RJ, van der Jagt EJ, Limburg AJ, van den Berg AP, Slooff MJ, Peeters PM, de Jong KP, Kleibeuker JH, Haagsma EB. Anastomotic biliary strictures after liver transplantation: causes and consequences. *Liver Transpl* 2006; **12**: 726-735 [PMID: 16628689 DOI: 10.1002/Lt.20714]
26 **Hsieh TH**, Mekeel KL, Crowell MD, Nguyen CC, Das A, Aqel BA, Carey EJ, Byrne TJ, Vargas HE, Douglas DD, Mulligan DC, Harrison ME. Endoscopic treatment of anastomotic biliary strictures after living donor liver transplantation: outcomes after maximal stent therapy. *Gastrointest Endosc* 2013; **77**: 47-54 [PMID: 23062758 DOI: 10.1016/j.gie.2012.08.034]

27 **Sun N**, Zhang J, Li X, Zhang C, Zhou X, Zhang C. Biliary tract reconstruction with or without T-tube in orthotopic liver transplantation: a systematic review and meta-analysis. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 529-538 [PMID: 25583036 DOI: 10.1586/17474124.2015.1002084]

28 **Shibuya ML**, Buddenbaum WE, Don AL, Utsumi H, Suciu D, Kosaka T, Elkind MM. Amsacrine-induced lesions in DNA and their modulation by novobiocin and 2,4-dinitrophenol. *Cancer Res* 1991; **51**: 573-580 [PMID: 1985775 DOI: 10.1016/j.transproceed.2009.09.026]

29 **Sawyer RG**, Punch JD. Incidence and management of biliary complications after 291 liver transplants following the introduction of transcystic stenting. *Transplantation* 1998; **66**: 1201-1207 [PMID: 9825818 DOI: 10.1097/00007890-199811150-00015]

30 **O'Connor TP**, Lewis WD, Jenkins RL. Biliary tract complications after liver transplantation. *Arch Surg* 1995; **130**: 312-317 [PMID: 7887800 DOI: 10.1001/archsurg.1995.01430030082017]

31 **Leidy K**. Enjoyable learning experiences--an aid to retention? *J Contin Educ Nurs* 1992; **23**: 206-208 [PMID: 1452821 DOI: 10.1067/s0016-5107(03)00011-7]

32 **Arain MA**, Attam R, Freeman ML. Advances in endoscopic management of biliary tract complications after liver transplantation. *Liver Transpl* 2013; **19**: 482-498 [PMID: 23417867 DOI: 10.1002/lt.23624]

33 **Balderramo D**, Navasa M, Cardenas A. Current management of biliary complications after liver transplantation: emphasis on endoscopic therapy. *Gastroenterol Hepatol* 2011; **34**: 107-115 [PMID: 20692731 DOI: 10.1016/j.gastrohep.2010.05.008]

34 **Saleem A**, Baron TH. Successful endoscopic treatment of biliary cast syndrome in an orthotopic liver transplant patient with a Roux-en-Y anastomosis *via* balloon enteroscopy. *Liver Transpl* 2010; **16**: 527-529 [PMID: 20213831 DOI: 10.1002/lt.22007]

35 **Di Pisa M**, Miraglia R, Volpes R, Gruttadauria S, Traina M. Single balloon enteroscopy for endoscopic retrograde cholangiography in a patient with hepaticojejunostomy after liver transplant. *Gastroenterol Res Pract* 2010; **2010**: 701696 [PMID: 20454574 DOI: 10.1155/2010/701696]

36 **Sanada Y**, Mizuta K, Yano T, Hatanaka W, Okada N, Wakiya T, Umehara M, Egami S, Urahashi T, Hishikawa S, Fujiwara T, Sakuma Y, Hyodo M, Yamamoto H, Yasuda Y, Kawarasaki H. Double-balloon enteroscopy for bilioenteric anastomotic stricture after pediatric living donor liver transplantation. *Transpl Int* 2011; **24**: 85-90 [PMID: 20738835 DOI: 10.1111/j.1432-2277.2010.01156.x]

37 **Haruta H**, Yamamoto H, Mizuta K, Kita Y, Uno T, Egami S, Hishikawa S, Sugano K, Kawarasaki H. A case of successful enteroscopic balloon dilation for late anastomotic stricture of choledochojejunostomy after living donor liver transplantation. *Liver Transpl* 2005; **11**: 1608-1610 [PMID: 16315301 DOI: 10.1002/Lt.20623]

38 **Attam R**, Leslie D, Freeman M, Ikramuddin S, Andrade R. EUS-assisted, fluoroscopically guided gastrostomy tube placement in patients with Roux-en-Y gastric bypass: a novel technique for access to the gastric remnant. *Gastrointest Endosc* 2011; **74**: 677-682 [PMID: 21872717 DOI: 10.1016/j.gie.2011.05.018]

39 **Zoepf T**, Maldonado-Lopez EJ, Hilgard P, Malago M, Broelsch CE, Treichel U, Gerken G. Balloon dilatation vs. balloon dilatation plus bile duct endoprostheses for treatment of anastomotic biliary strictures after liver transplantation. *Liver Transpl* 2006; **12**: 88-94 [PMID: 16382450 DOI: 10.1002/Lt.20548]

40 **Kim TH**, Lee SK, Han JH, Park DH, Lee SS, Seo DW, Kim MH, Song GW, Ha TY, Kim KH, Hwang S, Lee SG. The role of endoscopic retrograde cholangiography for biliary stricture after adult living donor liver transplantation: technical aspect and outcome. *Scand J Gastroenterol* 2011; **46**: 188-196 [PMID: 20955089 DOI: 10.3109/00365521.2010.522722]

41 **Holt AP**, Thorburn D, Mirza D, Gunson B, Wong T, Haydon G. A prospective study of standardized nonsurgical therapy in the management of biliary anastomotic strictures complicating liver transplantation. *Transplantation* 2007; **84**: 857-863 [PMID: 17984838 DOI: 10.1097/01.tp.0000282805.33658.ce]

42 **Costamagna G**, Pandolfi M, Mutignani M, Spada C, Perri V. Long-term results of endoscopic management of postoperative bile duct strictures with increasing numbers of stents. *Gastrointest Endosc* 2001; **54**: 162-168 [PMID: 11474384 DOI: 10.1067/mge.2001.116876]

43 **Tabibian JH**, Asham EH, Han S, Saab S, Tong MJ, Goldstein L, Busuttil RW, Durazo FA. Endoscopic treatment of postorthotopic liver transplantation anastomotic biliary strictures with maximal stent therapy (with video). *Gastrointest Endosc* 2010; **71**: 505-512 [PMID: 20189508 DOI: 10.1016/j.gie.2009.10.023]

44 **Morelli G**, Fazel A, Judah J, Pan JJ, Forsmark C, Draganov P. Rapid-sequence endoscopic management of posttransplant anastomotic biliary strictures. *Gastrointest Endosc* 2008; **67**: 879-885 [PMID: 18178206 DOI: 10.1016/j.gie.2007.08.046]

45 **Alazmi WM**, Fogel EL, Watkins JL, McHenry L, Tector JA, Fridell J, Mosler P, Sherman S, Lehman GA. Recurrence rate of anastomotic biliary strictures in patients who have had previous successful endoscopic therapy for anastomotic narrowing after orthotopic liver transplantation. *Endoscopy* 2006; **38**: 571-574 [PMID: 16802268 DOI: 10.1055/s-2006-925027]

46 **Freeman ML**, Cass OW, Dailey J. Dilation of high-grade pancreatic and biliary ductal strictures with small-caliber angioplasty balloons. *Gastrointest Endosc* 2001; **54**: 89-92 [PMID: 11427852 DOI: 10.1067/mge.2001.116176]

47 **Hüsing A**, Reinecke H, Cicinnati VR, Beckebaum S, Wilms C, Schmidt HH, Kabar I. Paclitaxel-eluting balloon dilation of biliary anastomotic stricture after liver transplantation. *World J Gastroenterol* 2015; **21**: 977-981 [PMID: 25624733 DOI: 10.3748/wjg.v21.i3.977]

48 **Hansen T**, Hollemann D, Pitton MB, Heise M, Hoppe-Lotichius M, Schuchmann M, Kirkpatrick CJ, Otto G. Histological examination and evaluation of donor bile ducts received during orthotopic liver transplantation--a morphological clue to ischemic-type biliary lesion? *Virchows Arch* 2012; **461**: 41-48 [PMID: 22588496 DOI: 10.1007/s00428-012-1245-8]

49 **Kao D**, Zepeda-Gomez S, Tandon P, Bain VG. Managing the post-liver transplantation anastomotic biliary stricture: multiple plastic versus metal stents: a systematic review. *Gastrointest Endosc* 2013; **77**: 679-691 [PMID: 23473000 DOI: 10.1016/j.gie.2013.01.015]

50 **Kurita A**, Kodama Y, Minami R, Sakuma Y, Kuriyama K, Tanabe W, Ohta Y, Maruno T, Shiokawa M, Sawai Y, Uza N, Yazumi S, Yoshizawa A, Uemoto S, Chiba T. Endoscopic stent placement above the intact sphincter of Oddi for biliary strictures after living donor liver transplantation. *J Gastroenterol* 2013; **48**: 1097-1104 [PMID: 23325164 DOI:

10.1007/s00535-012-0705-x]
51 **Villa NA**, Harrison ME. Management of Biliary Strictures After Liver Transplantation. *Gastroenterol Hepatol (NY)* 2015; **11**: 316-328 [PMID: 27482175]
52 **Weiss S**, Schmidt SC, Ulrich F, Pascher A, Schumacher G, Stockmann M, Puhl G, Guckelberger O, Neumann UP, Pratschke J, Neuhaus P. Biliary reconstruction using a side-to-side choledochocholedochostomy with or without T-tube in deceased donor liver transplantation: a prospective randomized trial. *Ann Surg* 2009; **250**: 766-771 [PMID: 19809299 DOI: 10.1097/SLA.0b013e3181bd920a]
53 **Kaffes A**, Griffin S, Vaughan R, James M, Chua T, Tee H, Dinesen L, Corte C, Gill R. A randomized trial of a fully covered self-expandable metallic stent versus plastic stents in anastomotic biliary strictures after liver transplantation. *Therap Adv Gastroenterol* 2014; **7**: 64-71 [PMID: 24587819 DOI: 10.1177/1756283X13503614]
54 **Lee HW**, Shah NH, Lee SK. An Update on Endoscopic Management of Post-Liver Transplant Biliary Complications. *Clin Endosc* 2017; **50**: 451-463 [PMID: 28415168 DOI: 10.5946/ce.2016.139]
55 **Macías-Gómez C**, Dumonceau JM. Endoscopic management of biliary complications after liver transplantation: An evidence-based review. *World J Gastrointest Endosc* 2015; **7**: 606-616 [PMID: 26078829 DOI: 10.4253/wjge.v7.i6.606]
56 **Yazumi S**, Chiba T. Endoscopic management of biliary stricture after right-lobe living-donor liver transplantation with biliary anastomosis. *Clin Gastroenterol Hepatol* 2006; **4**: 1296; author reply 1296 [PMID: 17045213]
57 **Park JS**, Kim MH, Lee SK, Seo DW, Lee SS, Han J, Min YI, Hwang S, Park KM, Lee YJ, Lee SG, Sung KB. Efficacy of endoscopic and percutaneous treatments for biliary complications after cadaveric and living donor liver transplantation. *Gastrointest Endosc* 2003; **57**: 78-85 [PMID: 12518136 DOI: 10.1067/mge.2003.11]
58 **Verdonk RC**, Buis CI, van der Jagt EJ, Gouw AS, Limburg AJ, Slooff MJ, Kleibeuker JH, Porte RJ, Haagsma EB. Nonanastomotic biliary strictures after liver transplantation, part 2: Management, outcome, and risk factors for disease progression. *Liver Transpl* 2007; **13**: 725-732 [PMID: 17457935 DOI: 10.1002/Lt.21165]
59 **Choi JH**, Lee SK. Percutaneous transhepatic cholangioscopy: does its role still exist? *Clin Endosc* 2013; **46**: 529-536 [PMID: 24143316 DOI: 10.5946/ce.2013.46.5.529]
60 **Chang JH**, Lee IS, Chun HJ, Choi JY, Yoon SK, Kim DG, You YK, Choi MG, Choi KY, Chung IS. Usefulness of the rendezvous technique for biliary stricture after adult right-lobe living-donor liver transplantation with duct-to-duct anastomosis. *Gut Liver* 2010; **4**: 68-75 [PMID: 20479915 DOI: 10.5009/gnl.2010.4.1.68]
61 **Shin M**, Joh JW. Advances in endoscopic management of biliary complications after living donor liver transplantation: Comprehensive review of the literature. *World J Gastroenterol* 2016; **22**: 6173-6191 [PMID: 27468208 DOI: 10.3748/wjg.v22.i27.6173]
62 **Karagyozov P**, Boeva I Tishkov I. The benefits of adding digital cholangioscopy in the evaluation and treatment of post-transplant biliary complication. *Gastrointest Endosc* 2020; **5S**: AB304 [DOI: 10.1016/j.gie.2020.03.3874]
63 **Wright H**, Sharma S, Gurakar A, Sebastian A, Kohli V, Jabbour N. Management of biliary stricture guided by the Spyglass Direct Visualization System in a liver transplant recipient: an innovative approach. *Gastrointest Endosc* 2008; **67**: 1201-1203 [PMID: 18308314 DOI: 10.1016/j.gie.2007.10.055]
64 **Kochhar G**, Parungao JM, Hanouneh IA, Parsi MA. Biliary complications following liver transplantation. *World J Gastroenterol* 2013; **19**: 2841-2846 [PMID: 23704818 DOI: 10.3748/wjg.v19.i19.2841]
65 **Shah SA**, Grant DR, McGilvray ID, Greig PD, Selzner M, Lilly LB, Girgrah N, Levy GA, Cattral MS. Biliary strictures in 130 consecutive right lobe living donor liver transplant recipients: results of a Western center. *Am J Transplant* 2007; **7**: 161-167 [PMID: 17227565 DOI: 10.1111/j.1600-6143.2006.01601.x]
66 **Dumonceau JM**, Tringali A, Blero D, Devière J, Laugiers R, Heresbach D, Costamagna G; European Society of Gastrointestinal Endoscopy. Biliary stenting: indications, choice of stents and results: European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. *Endoscopy* 2012; **44**: 277-298 [PMID: 22297801 DOI: 10.1055/s-0031-1291633]
67 **Irani S**, Baron TH, Law R, Akbar A, Ross AS, Gluck M, Gan I, Kozarek RA. Endoscopic treatment of nonstricture-related benign biliary diseases using covered self-expandable metal stents. *Endoscopy* 2015; **47**: 315-321 [PMID: 25521570 DOI: 10.1055/s-0034-1391093]
68 **Luigiano C**, Bassi M, Ferrara F, Fabbri C, Ghersi S, Morace C, Consolo P, Maimone A, Galluccio G, D'Imperio N, Cennamo V. Placement of a new fully covered self-expanding metal stent for postoperative biliary strictures and leaks not responding to plastic stenting. *Surg Laparosc Endosc Percutan Tech* 2013; **23**: 159-162 [PMID: 23579510 DOI: 10.1097/SLE.0b013e318278c201]
69 **Ayoub WS**, Esquivel CO, Martin P. Biliary complications following liver transplantation. *Dig Dis Sci* 2010; **55**: 1540-1546 [PMID: 20411422 DOI: 10.1007/s10620-010-1217-2]
70 **Phillips MS**, Bonatti H, Sauer BG, Smith L, Javaid M, Kahaleh M, Schmitt T. Elevated stricture rate following the use of fully covered self-expandable metal biliary stents for biliary leaks following liver transplantation. *Endoscopy* 2011; **43**: 512-517 [PMID: 21618151 DOI: 10.1055/s-0030-1256389]
71 **Spier BJ**, Pfau PR, Lorenze KR, Knechtle SJ, Said A. Risk factors and outcomes in post-liver transplantation bile duct stones and casts: A case-control study. *Liver Transpl* 2008; **14**: 1461-1465 [PMID: 18825682 DOI: 10.1002/lt.21511]
72 **Sheng R**, Ramirez CB, Zajko AB, Campbell WL. Biliary stones and sludge in liver transplant patients: a 13-year experience. *Radiology* 1996; **198**: 243-247 [PMID: 8539387 DOI: 10.1148/radiology.198.1.8539387]
73 **Farouk M**, Branum GD, Watters CR, Cucchiaro G, Helms M, McCann R, Bollinger R, Meyers WC. Bile compositional changes and cholesterol stone formation following orthotopic liver transplantation. *Transplantation* 1991; **52**: 727-730 [PMID: 1926353]
74 **Waldram R**, Williams R, Calne RY. Bile composition and bile cast formation after transplantation of the liver in man. *Transplantation* 1975; **19**: 382-387 [PMID: 168674 DOI: 10.1097/00007890-197505000-00004]
75 **Maheshwari A**, Maley W, Li Z, Thuluvath PJ. Biliary complications and outcomes of liver transplantation from donors after cardiac death. *Liver Transpl* 2007; **13**: 1645-1653 [PMID: 18044778 DOI: 10.1002/Lt.21212]
76 **Rerknimitr R**, Sherman S, Fogel EL, Kalayci C, Lumeng L, Chalasani N, Kwo P, Lehman GA. Biliary tract complications after orthotopic liver transplantation with choledochocholedochostomy anastomosis: endoscopic findings and results of therapy. *Gastrointest Endosc* 2002; **55**: 224-231 [PMID: 11818927 DOI: 10.1067/mge.2002.120813]

77 **Lisotti A**, Caponi A, Gibiino G, Muratori R. Safety and efficacy of extracorporeal shock-wave lithotripsy in the management of biliary stones after orthotopic liver transplantation. *Dig Liver Dis* 2015; **47**: 817-818 [PMID: 26143107 DOI: 10.1016/j.dld.2015.06.001]

78 **Gürakar A**, Wright H, Camci C, Jaboour N. The application of SpyScope® technology in evaluation of pre and post liver transplant biliary problems. *Turk J Gastroenterol* 2010; **21**: 428-432 [PMID: 21331998 DOI: 10.4318/tjg.2010.0131]

79 **Nam K**, Lee SK, Song TJ, Park DH, Lee SS, Seo DW, Kim MH. Percutaneous transhepatic cholangioscopy for biliary complications after liver transplantation: a single center experience. *J Hepatobiliary Pancreat Sci* 2016; **23**: 650-657 [PMID: 27474863 DOI: 10.1002/jhbp.388]

80 **Gor NV**, Levy RM, Ahn J, Kogan D, Dodson SF, Cohen SM. Biliary cast syndrome following liver transplantation: Predictive factors and clinical outcomes. *Liver Transpl* 2008; **14**: 1466-1472 [PMID: 18825683 DOI: 10.1002/lt.21492]

81 **Starzl TE**, Putnam CW, Hansbrough JF, Porter KA, Reid HA. Biliary complications after liver transplantation: with special reference to the biliary cast syndrome and techniques of secondary duct repair. *Surgery* 1977; **81**: 212-221 [PMID: 319551]

82 **Voigtländer T**, Negm AA, Strassburg CP, Lehner F, Manns MP, Lankisch TO. Biliary cast syndrome post-liver transplantation: risk factors and outcome. *Liver Int* 2013; **33**: 1287-1292 [PMID: 23601581 DOI: 10.1111/liv.12181]

83 **Navaneethan U**, Venkatesh PG, Al Mohajer M, Gelrud A. Successful diagnosis and management of biliary cast syndrome in a liver transplant patient using single operator cholangioscopy. *JOP* 2011; **12**: 461-463 [PMID: 21904071]

84 **Zajko AB**, Bennett MJ, Campbell WL, Koneru B. Mucocele of the cystic duct remnant in eight liver transplant recipients: findings at cholangiography, CT, and US. *Radiology* 1990; **177**: 691-693 [PMID: 2243970 DOI: 10.1148/radiology.177.3.2243970]

85 **Chatterjee S**, Das D, Hudson M, Bassendine MF, Scott J, Oppong KE, Sen G, French JJ. Mucocele of the cystic duct remnant after orthotopic liver transplant: a problem revisited. *Exp Clin Transplant* 2011; **9**: 214-216 [PMID: 21649573]

86 **Torres V**, Martinez N, Lee G, Almeda J, Gross G, Patel S, Rosenkranz L. How do we manage post-OLT redundant bile duct? *World J Gastroenterol* 2013; **19**: 2501-2506 [PMID: 23674851 DOI: 10.3748/wjg.v19.i16.2501]

87 **Park TY**, Lee SK, Nam K, Oh D, Song TJ, Park DH, Lee SS, Seo DW, Kim MH. Spontaneous hemobilia after liver transplantation: Frequency, risk factors, and outcome of endoscopic management. *J Gastroenterol Hepatol* 2017; **32**: 583-588 [PMID: 27449807 DOI: 10.1111/jgh.13497]

88 **Shinjo K**, Matsubayashi H, Matsui T, Kawata N, Uemura S, Yamamoto Y, Ono H. Biliary hemostasis using an endoscopic plastic stent placement for uncontrolled hemobilia caused by transpapillary forceps biopsy (with video). *Clin J Gastroenterol* 2016; **9**: 86-88 [PMID: 26960930 DOI: 10.1007/s12328-016-0637-8]

89 **Goenka MK**, Harwani Y, Rai V, Goenka U. Fully covered self-expandable metal biliary stent for hemobilia caused by portal biliopathy. *Gastrointest Endosc* 2014; **80**: 1175 [PMID: 24818548 DOI: 10.1016/j.gie.2014.03.029]

90 **Pasha SF**, Harrison ME, Das A, Nguyen CC, Vargas HE, Balan V, Byrne TJ, Douglas DD, Mulligan DC. Endoscopic treatment of anastomotic biliary strictures after deceased donor liver transplantation: outcomes after maximal stent therapy. *Gastrointest Endosc* 2007; **66**: 44-51 [PMID: 17591473 DOI: 10.1016/j.gie.2007.02.017]

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MINIREVIEWS

Shifting perspectives – interplay between non-alcoholic fatty liver disease and insulin resistance in lean individuals

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has become a significant public health burden affecting not only obese individuals but also people with normal weight. As opposed to previous beliefs, this particular subset of patients has an increased risk of all-cause mortality and worse outcomes than their obese counterparts. The development of NAFLD in lean subjects seems to be interconnected with metabolic phenotype, precisely visceral fat tissue, sarcopenia, and insulin resistance. Here, we summarize available data focusing on the co-dependent relationship between metabolic phenotype, insulin resistance, and development of NAFLD in lean individuals, suggesting more appropriate tools for measuring body fat distribution for the screening of patients at risk.

Key Words: Non-alcoholic fatty liver disease; Metabolic phenotype; Lean individuals; Insulin resistance; Visceral fat tissue; Sarcopenia

Core Tip: The prevalence of non-alcoholic fatty liver disease among non-obese (overweight or lean) individuals seems to be much higher than previously reported, affecting almost 20% of the non-obese population. Non-alcoholic fatty liver disease is no longer considered solely an obesity-related disorder since non-obese individuals participate significantly in this entity. The metabolic phenotype is the key role-player in the development of non-alcoholic fatty liver disease in lean individuals. The detection of lean patients with non-alcoholic fatty liver disease is particularly challenging since the body-mass index is not a good indicator of metabolic health.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), recently known as metabolic-associated fatty liver disease [1], is one of the most common causes of chronic liver disease. NAFLD was traditionally associated with metabolic syndrome encompassing obesity, insulin resistance, hypertension, and atherogenic dyslipidemia[2]. Recently, a new clinical entity, including NAFLD in non-obese/lean individuals has emerged. It soon became apparent that the existence of NAFLD in non-obese subjects should not be neglected since its prevalence has significantly increased. According to a recently published meta-analysis, up to 40% of NAFLD patients are non-obese, with the highest prevalence in western countries as opposed to previous findings dominantly allocating this entity in Asian regions[3]. The clinical consequences of NAFLD can be detrimental; for instance, progression to significant fibrosis remains uncertain as well as long-term cardiometabolic complications and mortality[4-7]. However, prevalence data and terminology are quite variable since definitions used to determine lean and obese patients differ among various studies, depending on Asian or Caucasian cutoff values. In addition, a body mass index (BMI) cutoff value of 25 kg/m^2 is frequently used to differ between lean and obese individuals, thus excluding the overweight population (Table 1). Here, we decided to use terms "non-obese" or "lean NAFLD" depending on the study in question and definitions used.

The recognition of NAFLD in lean individuals is associated with a concept known as the metabolic phenotype. There are separate subgroups of individuals divided according to their phenotype and metabolic profile to metabolically unhealthy normal weight (MUHNW) and metabolically healthy obese (MHO), the latter being disputable due to higher incidence of cardiovascular disease (CVD) in long-term studies[8]. Distinguishing between those phenotypes is based on BMI, an inadequate surrogate marker for determining the quantity of skeletal muscle mass and adipose tissue, especially in the visceral area[9]. As a consequence, a MUHNW individual could be a person with sarcopenia and a high proportion of fat tissue, with a high probability of developing insulin resistance and/or metabolic syndrome (MetS), subsequently leading to the development of NAFLD[10]. In addition, other factors could be involved in the pathogenesis of NAFLD in lean subjects such as genetics [*e.g.*, patatin-like phospholipase domain-containing 3 (*PNPLA3*) variant (rs738409 C/G)][11], environmental factors including dietary habits[12,13] and physical activity[14], changes in gut microbiota[15], and secondary causes such as hypothyroidism or polycystic ovary syndrome.

Lean NAFLD patients were traditionally considered to have milder metabolic disturbances, thus carrying a lower risk for the development of CVD and progression to non-alcoholic steatohepatitis (NASH) and fibrosis[6,16,17]. However, recent data suggest that progression to diabetes as well as NASH and fibrosis is higher in lean NAFLD individuals, undoubtedly linking visceral fat tissue with undesirable consequences of MUHNW phenotype[5,10,18,19]. Still, a contribution of specific components of MetS to fibrosis remains unclear, although insulin resistance seems the most probable culprit[20-22], Table 1.

In this critical review, we summarized available data and addressed practical issues of whether it is time to shift perspectives away from the scale and how to screen for non-obese patients with a metabolically unhealthy profile.

METABOLIC PHENOTYPE – THE KEY ROLE PLAYER IN THE DEVELOPMENT OF NAFLD IN LEAN INDIVIDUALS

Obesity is generally associated with severe health consequences, mainly related to increased

Table 1 Prevalence, characteristics, and outcomes in lean/non-obese individuals with non-alcoholic fatty liver disease

Author, year	Population, study design, sample size	Prevalence of NAFLD in lean subjects	Main findings
Zou *et al*[4], 2020	Mixed population, 1999-2016 NHANES databases	32.3% overall NAFLD prevalence; 22.7% obese and 9.6% non-obese; Amongst NAFLD patients, 29.7% were non-obese (Caucasian BMI 25-30 kg/m^2, Asian BMI 23-27 kg/m^2), of which 13.6% had lean NAFLD (Caucasian BMI < 25 kg/m^2, Asian BMI < 23 kg/m^2)	Non-obese NAFLD individuals had higher 15-year cumulative all-cause mortality (51.7%) than obese NAFLD (27.2%) and non-NAFLD (20.7%)
Huang *et al* [20], 2020	2483 Asian participants, community based study	44.5% NAFLD and 15.8%, MetS prevalence; Among NAFLD subjects, 48.8%were obese (BMI ≥ 24 kg/m^2)	IR is predictive of NAFLD irrespective of BMI; CV risk calculated by Framingham Risk Score may exist in lean NAFLD subjects
Tobari *et al* [18], 2020	Asian, biopsy-proven 762 NAFLD patients, cross sectional study	Over 25% men and almost 40% women were non-obese, but most of them had visceral fat obesity and/or IR; BMI cutoff 25 kg/m^2	NAFLD was not milder in non-obese patients; Histological steatosis was associated with BMI; Advanced fibrosis was not associated with BMI and showed a significant sex difference
Kim *et al*[10], 2020	664 Asian subjects with biopsy-proven NAFLD and controls, cross sectional study	542 subjects with biopsy-proven NAFLD132 non-obese NAFLD (BMI < 25 kg/m^2) ; 410 obese NAFLD (BMI > 25 kg/m^2) ; 122 controls	Non-obese subjects with NAFLD displayed a similar severity of histological liver damage; Sagittal abdominal diameter was independently associated with significant fibrosis among subjects with non-obese NAFLD
Alferink *et al* [71], 2019	4609 elderly European, population based study	1623 had NAFLD (n = 161 normal-weight and n = 1462 overweight, BMI cutoff 25 kg/m^2)	Both high fat mass and low SMI were associated with normal-weight NAFLD; Fat distribution (assessed by AGR) could best predict NAFLD prevalence
Denkmayr *et al*[19], 2018	European, 466 patients diagnosed with NAFLD, cross sectional study	Lean (BMI ≤ 25.0 kg/m², n = 74) ; Overweight (BMI > 25.0 ≤ 30.0 kg/m², n = 242) ; Obese (BMI > 30.0 kg/m², n = 150)	Lean NAFLD patients had a histological picture similar to obese patients but more severe compared to overweight patients.
Gonzalez-Cantero *et al* [21], 2018	European, cross-sectional study 113 non-obese, non-diabetic individuals	55 patients diagnosed with NAFLD; NAFLD defined as hepatic triglyceride content > 5.56% (quantified by 3T H1-MRS) ; BMI cutoff 25 kg/m^2	Lean-with-NAFLD group had significantly higher HOMA-IR and lower serum adiponectin than the overweight-without-NAFLD group; IR was independently associated with NAFLD but not with waist circumference or BMI
Hagström *et al*[5], 2017	European, prospective cohort study of 646 patients with biopsy-proven NAFLD	19% lean NAFLD; 52% overweight NAFLD; 29% obese NAFLD; BMI cutoff 25 and 30 kg/m^2	Lean NAFLD had lower stages of fibrosis and higher risk for severe liver disease development compared to patients with NAFLD and a higher BMI, independent of available confounders (follow-up 19.9 years)
Leung *et al* [6], 2017	Asian, prospective, 307 NAFLD patients	23.5% were non-obese; BMI cutoff 25 kg/m^2	Non-obese NAFLD patients have less-severe disease and may have a better prognosis than obese patients; Hypertriglyceridemia and higher creatinine are the key factors associated with advanced liver disease in non-obese patients
Fracanzani *et al*[11], 2017	European, retrospective cohort study of 669 patients with biopsy-proven NAFLD	143 patients had BMI < 25 kg/m^2 and NAFLD	20% of patients with lean NAFLD have NASH, fibrosis scores of 2 or higher, and carotid athero-sclerosis
Feldman *et al* [22], 2017	Caucasian, cross sectional, 187 subjects with hepatic steatosis on ultrasound	Lean healthy (BMI ≤ 25 kg/m^2, no steatosis, n = 71) ; Lean NAFLD (BMI ≤ 25 kg/m^2, steatosis, n = 55) ; obese NAFLD (BMI ≥ 30 kg/m^2, steatosis; n = 61)	Lean NAFLD have impaired glucose tolerance, low adiponectin concentrations and an increased rate of PNPLA3 risk allele carriage
Feng *et al*[7], 2014	Asian, population based, 1779 participants	The prevalence of NAFLD was 18.33% in the lean group and 72.90% in the overweight-obese groupBMI cutoff 24 kg/m^2	Lean-NAFLD was more strongly associated with diabetes, hypertension, and MetS than overweight-obese-NAFLD; NAFLD patients were more likely to have central obesity especially in lean groups
Younossi *et al* [17], 2012	Mixed population, 1988-1994 NHANES databases	2185 (18.77% ± 0.76%) of subjects had NAFLD; 7.39% ± 0.65% had lean NAFLD; 27.75% ± 1.00% had overweight/obese NAFLDBMI cutoff 25 kg/m^2	Lean NAFLD was independently associated with younger age, female sex, and a decreased likelihood of having IR and hypercholesterolemia
Margariti *et al* [16], 2012	European, cross sectional, 162 NAFLD patients	Normal BMI was present in 12% of patients; BMI cutoff 25 kg/m^2	Lean NAFLD patients do not have IR-associated metabolic disorders, but they have higher levels of ALT/AST than the overweight or obese NAFLD patients

3T H1-MRS: 3Tesla H1-magnetic resonance spectroscopy; ALT: Alanine aminotransferase; AGR: Android gynoid ratio; AST: Aspartate aminotransferase; BMI: Body mass index; CV: Cardiovascular; IR: Insulin resistance; MetS: Metabolic syndrome; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-

alcoholic steatohepatitis; SMI: Skeletal muscle index.

cardiovascular risk[8]. However, a subset of obese patients will never develop cardiovascular disease and is therefore considered an MHO. Conversely, metabolically unhealthy patients exist even in the group of normal-weight people, the category known as the MUHNW. People with this phenotype seem to have 1.5 to 3-times higher risk for cardiometabolic complications than metabolically healthy normal-weight people and even higher risk than MHO[23,24], but unfortunately often go under the radar for cardiovascular screening and primary outcome prevention.

Generally, the assessment of cardiovascular risk, regardless of the patient's BMI, was historically mainly based on the presence of the MetS. However, according to data from prospective studies, only a smaller proportion of individuals in the normal-weight category with cardiovascular events have MetS compared to patients with cardiovascular events who were overweight or obese (20% compared to 52% and 76%, respectively)[25]. Although MetS as such might not be an accurate predictor of CV risk in normal-weight individuals, its components, especially, lipids and glucose level, as well as waist circumference and waist-to-hip ratio might be useful for risk stratification[9,26,27]. On the other hand, up to 30% of normal-weight individuals can be classified as metabolically obese normal weight having an increased cardiometabolic risk.

It seems that the distribution and health of fatty tissue, rather than its amount, is likely the major determinant of disease risk. For example, higher amounts of visceral fat compared to peripheral and subcutaneous fat comprise a higher metabolic risk and are directly linked to both liver inflammation and fibrosis, independently of insulin resistance and hepatic steatosis[24,28-30].

Some previously published studies have failed to show an association of insulin resistance and NAFLD in lean individuals[16,17]. However, more recently published studies have demonstrated the opposite, linking insulin resistance with the development of NAFLD, irrespective of BMI[10,20-22].

In a study published by Kim *et al*[10] comparing non-obese with Mets and obese without MetS, the ratio of visceral adipose tissue area-to-subcutaneous adipose tissue area (VAT/SAT) was independently linked with NASH or fibrosis in a dose-dependent manner, confirming that metabolic phenotype is crucial in the progression of liver disease, irrespective of the presence of obesity. Lean with MetS were non-obese, had insulin resistance, and an increased VAT area[10]. Another community-based study in the Asian population demonstrated that insulin resistance was a significant predictive factor for NAFLD in both obese and lean subjects[20].

Obviously, metabolic disturbances are responsible for disease progression, with insulin resistance being a key role player (Figure 1). The mechanisms involved seem to be similar as in obese individuals [22]. Higher levels of free fatty acids, enhanced adipose tissue lipolysis, and decreased fat storage capacity of subcutaneous fat tissue overcome fatty acid oxidation and triglyceride secretion leading to the accumulation of triglycerides in hepatocytes[23,31]. An increase in lipotoxicity causes pronounced oxidative stress[32], whereas chronic inflammation is continuously being fueled by changed adipokine secretion from visceral adipocytes, primarily decreased adiponectin secretion together with mitochondrial dysfunction leading to further liver injury[23,31].

Some of the major game-changers determining the nature of metabolic profiles are dietary intake and physical activity. To date, published data indicate a correlation between weight gain in non-obese individuals with the development of NAFLD[12,13], suggesting that calorie intake and modest weight gain in non-obese individuals have deleterious effects on metabolic disturbances primarily through an increase in visceral adipose tissue. Conversely, waist circumference and body weight reduction achieved through lifestyle intervention were independent predictors of NAFLD resolution in lean subjects[33]. Furthermore, sarcopenia is positively correlated to insulin resistance in obese patients and is considered one of the major factors responsible for the obesity paradox[14]. The potential mechanisms involved are the accumulation of intramyocellular lipid and intermuscular adipocytes, chronic inflammation, and loss of insulin sensitivity to protein synthesis preceding insulin resistance to glucose metabolism[34]. Thus, we could hypothesize that the unfavorable ratio of skeletal muscle mass and visceral adipose tissue in non-obese individuals is one of the main determinants of insulin resistance. Indeed, it has been shown that physical activity increases skeletal muscle mass, thus improving sarcopenia and lean/fat tissue mass ratio advancing metabolic health in non-obese individuals through the reduction of insulin resistance[18,35].

OTHER RISK FACTORS INVOLVED IN THE DEVELOPMENT OF NAFLD IN LEAN INDIVIDUALS

Compared to obese and overweight NAFLD patients, some clinical, biochemical, and histological distinctions have been observed in lean NAFLD subjects, going far beyond the simple differences in the BMI. Specifically, low adiponectin levels and high concentrations of proinflammatory cytokines suggest a pronounced degree of adipose tissue dysfunction and distinct metabolic and gut microbiota profiles

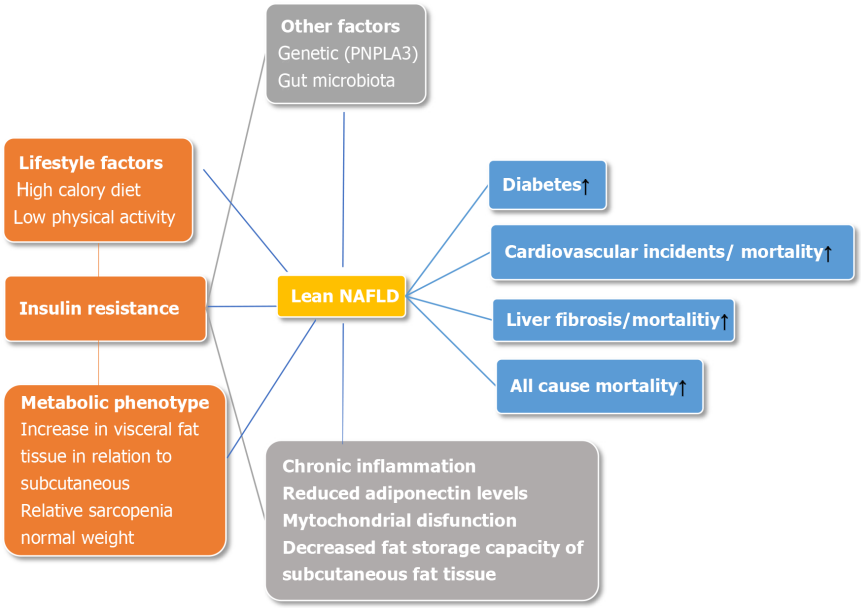

Figure 1 Pathophysiological mechanisms and outcomes of non-alcoholic fatty liver disease in non-obese individuals. NAFLD: Non-alcoholic fatty liver disease.

[11,19,36-38]. Additionally, impaired glucose metabolism and carriage of the *PNPLA3* minor allele was seen in lean Caucasian NAFLD patients[22].

Genetic factors

Several genes and single-nucleotide polymorphisms (SNPs) associated with NAFLD have been identified, of which transmembrane 6 superfamily member 2 (*TM6SF2*)[39-41] and the patatin like *PNPLA3*[42-44] are the most investigated ones.

The rs58542926 genetic variant of *TM6SF2* gene, which encodes the E167K aminoacidic substitution and determines neutral fat accumulation in the liver, has been implicated in NAFLD development. Previous studies suggested a significant association between the *TM6SF2* polymorphism and disease severity and/or progression[39,41].

The rs738409 genetic variant of the *PNPLA3* gene, which takes part in lipid transformation, is now recognized as the major genetic determinant of NAFLD. A meta-analysis based on 23 case-control studies involving 6071 NAFLD patients and 10366 controls showed that *PNPLA3* rs738409 polymorphism is associated with disease severity and progression and that these changes were not influenced by the ethnicities or age of subjects[45]. In addition, Shen and al. demonstrated that the G allele in *PNPLA3* rs738409 increases the risk of NAFLD, especially in subjects without MetS, independent of dietary pattern and metabolic factors[46].

Genetic background for developing NAFLD in the absence of obesity has also been investigated in different populations. Initial reports on NAFLD in lean individuals originated mostly from an Asian background[7,47,48], and implicated Asian ethnic preponderance. However, "non-obese" NAFLD makes just over 40% of the NAFLD population and is common in both eastern and western countries[3].

Earlier studies in Asian populations found that the G allele at the *PNPLA3* rs738409 mutation has been more common in lean than obese NAFLD patients (78.4% *vs* 59.8%; P = 0.001)[49]. However, a study investigating the prevalence of metabolic co-morbidities and *PNPLA3* risk alleles (GG) in the Japanese population did not confirm the difference among the non-obese, obese, and severely obese groups of both sexes[18]. Similarly, a recently published study in the Chinese population found no difference in the SNPs of several genes (*SIRT1*, *APOC3*, *PNPLA3*, *AGTR1*, and *PPARGC1A*) between lean subjects with and without NAFLD[50].

In the Caucasian population, Feldman *et al*[11] showed a high rate of *PNPLA3* risk alleles (CG/GG) in the lean NAFLD group compared with lean controls (odds ratio [OR] 2.676, P = 0.007), but at a comparable rate to obese NAFLD subjects (OR 0.759, P = 0.464)[22]. Another study investigating gene polymorphisms in the Caucasian population demonstrated that in lean NAFLD subjects, the only independent variable associated with NASH and significant fibrosis (≥ 2) was the GG *PNPLA3* polymorphism[11]. In addition, in lean NAFLD patients, a significantly higher prevalence of *TM6SF2* E167K variant carriers was associated with more severe steatosis, inflammation, and NASH.

Gut microbiota

The human gut microbiota (GM) forms a complex ecosystem involving different microorganisms

(bacteria; dominated by four bacterial phyla: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria[51], viruses, uni/pluricellular eukaryotes) that have been implicated in various physiological processes[52]. The impact of diet on GM composition and function is well established, and alterations in the microbiome composition have been associated with the development of obesity, diabetes, MetS and NAFLD[15,53,54]. Previous studies have identified that NAFLD patients have altered microbiome with fewer proportions of Bacteroidetes and higher proportions of Porphyromas and Prevotella than healthy individuals[55,56]. Moreover, an increase in *Lactobacillus, Escherichia, Streptococcus* abundance, decrease in *Ruminococcaceae*, and *Faecalibacterium prausnitzii,* have also been identified in NAFLD patients[57-59].

In addition, substantial differences in fecal and blood microbiota profiles between obese and lean individuals with NAFLD have been identified in the Asian population[18]. Similarly, a Brazilian study confirmed a specific gut microbiota composition in lean NASH patients, showing a lower abundance of *Faecalibacterium* and *Ruminococcus*, and a deficiency in Lactobacillus compared with overweight and obese NASH patients[60]. These differences in microbiota composition between lean and obese NAFLD patients may serve as biomarkers for identifying the specific metabolic NAFLD phenotype.

AVOIDING PITFALLS IN THE DIAGNOSIS OF LEAN NAFLD

After publishing a meta-analysis on metabolic health, which suggested the highest CV risk among individuals of normal weight who are metabolically unhealthy (response rate [RR] 3.14, 95% confidence interval [CI] 2.36-3.93)[61], Kramer *et al*[61] raised the need to phenotype metabolically unhealthy individuals.

Currently, definitions of metabolic health are not unique (Table 2). Sometimes they include either the absence of insulin resistance[62,63], or the absence of insulin resistance and low C-reactive protein (CRP) levels as a surrogate marker for inflammation, in combination with up to any two parameters of MetS[64,65]. In clinical practice, only the latter are used[66,67].

The study by Stefan *et al*[23] (2017) was the first head-to-head comparison of cardiometabolic risk phenotypes suggesting that metabolically unhealthy lean people mainly have insulin secretion failure, insulin resistance, and increased carotid intima-media thickness. Among the aforementioned, insulin resistance is the most widely used cardiovascular risk marker. Metabolically unhealthy normal-weight subjects (defined by a BMI < 25 kg/m^2 and presence of insulin resistance), compared to their healthy counterparts, in addition to elevated CV risk, have an elevated risk of colorectal cancer (OR = 1.59, 95%CI: 1.10-2.28)[68].

As already mentioned, BMI is an inadequate surrogate marker of metabolic health, especially in determining the ratio of visceral and subcutaneous fat tissue, the most important risk factors of NAFLD's insulin resistance and progression in lean individuals[10]. In addition, data on muscle mass are missing, thus providing no information on sarcopenia[69], which is clinically relevant in the development of NAFLD in lean patients. Thus waist circumference and/or waist-to-hip ratio might be a better tool. However, waist circumference is mostly dependent on BMI, meaning that normal-weight patients could have waist circumference in the normal range, but still have higher visceral fat tissue and increased cardiometabolic risk[9]. This issue could be avoided by using waist circumference adjusted for BMI, which has shown a strong linear increase in risk for cardiovascular mortality[70], but no data are available on the association of adjusted waist circumference and NAFLD in lean individuals.

Additionally, in an elderly population-based study, both high-fat mass and low skeletal muscle index were associated with normal-weight NAFLD, although fat distribution assessed by the android gynoid ratio was the best predictor of NAFLD prevalence[71].

CLINICAL AND THERAPEUTIC IMPLICATIONS OF NAFLD IN LEAN INDIVIDUALS ASSOCIATED WITH INSULIN RESISTANCE

The liver-related and general outcomes of patients with NAFLD depend on a number of factors including the presence of metabolic risk factors, especially type 2 diabetes mellitus and hypertension, severity of fibrosis, genetic predisposition, age, diet and other environmental factors.

Metabolic consequences

Regarding metabolic health and clinical outcomes, cardiometabolic complications take the most prominent place in driving the mortality. It seems that metabolically unhealthy, regardless of BMI, including individuals within the normal range of BMI category, have the highest risk of cardiometabolic consequences[72]. Moreover, in a recently published study, normal-weight patients with central adiposity and coronary artery disease had a worse survival rate than normal, overweight, or obese subjects without central obesity[73]. However, long term studies in lean NAFLD patients and cardiovascular health are lacking. In a retrospective study of lean Caucasian patients with biopsy-

Table 2 Definitions of metabolic health in non-obese

Definitions of metabolic health in non-obese individuals:	
Absence of insulin resistance	Meigs *et al*[62]; Stefan *et al*[63]
Absence of insulin resistance and low CRP levels as a surrogate marker for inflammation, in combination with up to any two parameters of metabolic syndrome	Wildman *et al*[64]; Karelis *et al*[65]
Combination with up to any two parameters of metabolic syndrome	Stefan *et al*[66]; Phillips[67]
Definition of metabolically unhealthy non-obese individuals:	
BMI < 25 kg/m^2 and presence of insulin resistance	Stefan *et al*[23]
Waist circumference adjusted for BMI and/or android gynoid ratio and presence of insulin resistance	Suggested by authors

BMI: Body mass index; CRP: C-reactive protein.

proven NAFLD *vs* obese or overweight individuals, 20% of patients who were lean developed NASH, significant fibrosis, and carotid atherosclerosis[11].

A study by Feng and coauthors addressed the question of metabolic consequences and laboratory discrepancies in lean subjects with NAFLD. Compared to obese and overweight NAFLD counterparts, lean Chinese NAFLD individuals had a higher risk of developing diabetes (OR = 2.47, 95%CI: 1.14-5.35), hypertension (OR = 1.72, 95%CI: 1.00-2.96) and MetS (OR = 3.19, 95%CI: 1.17-4.05), making them prone to the development of cardiovascular disease[7].

In terms of mortality, the higher fat mass could be associated with better nutritional state associated with higher survival rates (also known as obesity paradox); thus, lean individuals with the more severe and advanced liver disease could have a poor prognosis, especially if sarcopenia is present[74]. This was confirmed in a recently published meta-analysis, encompassing 93 studies including lean NAFLD individuals, demonstrating that all-cause mortality, liver-related mortality, and cardiovascular-related mortality in non-obese individuals with NAFLD was higher than that of obese individuals with NAFLD (12.1 *vs* 7.5 per 1000 person-years; 4.1 *vs* 2.4 per 1000 person-years; 4.0 *vs* 2.4 per 1000 person-years respectively)[3].

In addition, NHANES based study demonstrated that non-obese NAFLD individuals had increased 15-year cumulative all-cause mortality (51.7%) compared to obese NAFLD (27.2%) and non-NAFLD (20.7%) patients[4].

Therefore it seems that NAFLD in lean individuals has serious cardiometabolic complications leading to an increase in mortality, even higher than in their obese counterparts.

Liver consequences - fibrosis, cirrhosis and cancer

Non-alcoholic fatty liver disease encompasses a spectrum of histological changes with different evolution and outcomes, ranging from simple steatosis to NASH with varying degree of fibrosis. The later entity is characterized by lobular inflammation and hepatocyte ballooning degeneration accompanied by various stages of fibrosis that more often progresses to cirrhosis. However, fibrosis can be found in liver biopsy specimens in the absence of significant inflammation; in a recent multicenter study from Italy and Finland, 34% of patients with significant fibrosis did not have NASH and 10.0% had no inflammation[75].

Currently there are no published data on the specific inflammatory pathways or hepatic stellate cells activation pathways that would be unique to the development of NASH in lean patients as opposed to obese NASH patients. It is therefore believed that progression of NASH in lean individuals follows pathways similar to those demonstrated in obese patients with NASH, and that rate of progression probably depends on the similar risk factors as in their obese counterparts[76].

In general, NAFLD is a slowly progressive disease, but more rapid progression occurs in 20% of patients[77]. In a meta-analysis of over 400 patients with paired liver biopsy, 34% of NAFLD patients had fibrosis progression, 43% had stable fibrosis, and 22% showed an improvement in the fibrosis stage during follow-up[77]. The rate of progression was doubled in the presence of arterial hypertension[77]. The data on the natural history and prognosis of lean patients with NAFLD remains conflicting. Although better or similar metabolic and histological profiles than in obese NAFLD patients are mainly suggested, long term liver related outcomes remain an open question[5,6,19].

In a retrospective cohort study from Italy, significantly lower proportions of lean NAFLD patients had NASH (17% *vs* 40% of obese or overweight patients), and significant fibrosis of F2 or more (17% *vs* 42% for obese/overweight NAFLD patients)[11]. However, lean patients with high waist circumference had increased risk of significant fibrosis of F2 or more, compared to overweight/obese subjects with the same waist circumference[11]. A study from two university centers from Sweden with a median follow-up of 20 years reported that 50% of lean patients had NASH compared to 65% and 80% of overweight

and obese subjects[5]. Yet, lean patients with NAFLD had slightly more events of severe liver disease (defined as decompensated liver disease, liver failure, hepatocellular carcinoma, or cirrhosis) compared to overweight patients (16% *vs* 9%), but similar to obese patients (14%)[5]. The main finding of the study was that although lean patients had a better prognostic profile at baseline with less advanced fibrosis and NASH, an increased risk for the development of severe liver disease was found compared to patients with a higher BMI[5].

In a study from Hong Kong, non-obese patients had lower NAFLD activity score and lower fibrosis stages compared to obese patients[6]. In a recently published meta-analysis, 39% of non-obese or lean NAFLD patients had NASH (compared to 53% of obese individuals), 25% had significant lobular inflammation (compared to 36% of obese), 29% had significant fibrosis of F2 or more (compared to 38% of obese individuals), and 3% had cirrhosis in one study[3]. However liver related mortality was higher in non-obese NAFLD subjects compared to obese equivalents (4·1 per 1000 person-years *vs* 2·4 per 1000 person-years)[3].

Additionally, in a study published by Kim *et al*[10] progression to NASH and fibrosis was equally present in non-obese patients with MetS and obese patients without MetS (55%-60%) linking metabolic phenotype with the liver disease progression.

Cirrhosis of any etiology is a well-known risk factor for the development of hepatocellular carcinoma (HCC); the same is true for NAFLD-induced cirrhosis. The reported incidence of HCC development in patients with NAFLD varies significantly depending on the study population, ranging from 0.25% to 11% after 5 years[78,79]. Furthermore, in a significant proportion of patients, ranging from 23% to 46%, HCC has been reported to develop in the earlier stages of the disease, before the development of cirrhosis[80,81]. Except for the study of Hagström *et al*[5] where the incidence of hepatocellular carcinoma was collectively reported with other liver-related outcomes, no data on the incidence and risk of HCC development in the subgroup of lean patients with NAFLD has been published. Until new data becomes available, no conclusions can be drawn on the risk for HCC development in lean individuals with NAFLD.

MANAGEMENT

As 3%-25% of lean/non-obese and non-diabetic individuals are diagnosed with NAFLD, with potential for progression to NASH and subsequently liver fibrosis with metabolic dysfunction, it is of interest to find pharmacological modalities and lifestyle interventions to treat this specific phenotype[82-84]. Animal studies on obese rats and mice showed significant reductions in hepatic steatosis and oxidative stress when glucagon-like peptide-1 receptor agonists (GLP-1RAs) were used to treat liver steatosis with no or mild fibrosis[85,86]. Moreover, randomized control trial investigating the role of liraglutide (daily GLP-1RA) reported on histological resolution of NASH after 48 wk of treating obese and overweight NASH patients[87]. Data on lean NAFLD/NASH counterparts are lacking, but recently published animal study gave promising results. Ipsen and colleagues reported on liraglutide effects in reducing both inflammation and hepatocyte ballooning in advanced NAFLD in an animal model. The treatment was more effective than dietary intervention, and when the two were combined, they led to rapid weight loss[88].

Still, available data on the treatment and management of lean subjects with NAFLD are practically non-existent, and further studies are needed to evaluate the effects of lifestyle changes and pharmacotherapy in this vulnerable population.

CONCLUSION

NAFLD in lean individuals presents a severe global burden with detrimental clinical consequences. Determining metabolic phenotype is crucial for detecting normal-weight patients at risk of developing NAFLD and preventing possible long-term complications, such as the cardiometabolic, liver, and all-cause mortality, which may be even more pronounced than in the obese individuals. The main characteristic of MUHNW seems to be insulin resistance associated with visceral adiposity; thus, waist circumference or the android gynoid ratio along with HOMA IR could be better predictors of NAFLD in lean subjects than traditionally used BMI and other components of metabolic syndrome. Insulin resistance is undoubtedly associated with the development of NAFLD in lean individuals irrespective of BMI and the presence of MetS; however, is it causality or correlation remains an open question.

FOOTNOTES

Author contributions: Bilic-Curcic I made contributions to the conception and design of the study, involved in drafting and revising the manuscript critically; Cigrovski Berkovic M, Virovic-Jukic L and Mrzljak A were involved

in collecting the data, and drafting and writing the manuscript; All authors read and approved the final manuscript.

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REFERENCES

1 **Kanaskie ML**, Noerr B. Implementing the use of nursing diagnosis in a university hospital setting. *J Nurs Staff Dev* 1988; **4**: 179-185 [PMID: 3204431 DOI: 10.1053/j.gastro.2019.11.312]
2 **Brar G**, Tsukamoto H. Alcoholic and non-alcoholic steatohepatitis: global perspective and emerging science. *J Gastroenterol* 2019; **54**: 218-225 [PMID: 30643981 DOI: 10.1007/s00535-018-01542-w]
3 **Ye Q**, Zou B, Yeo YH, Li J, Huang DQ, Wu Y, Yang H, Liu C, Kam LY, Tan XXE, Chien N, Trinh S, Henry L, Stave CD, Hosaka T, Cheung RC, Nguyen MH. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 739-752 [PMID: 32413340 DOI: 10.1016/S2468-1253(20)30077-7]
4 **Zou B**, Yeo YH, Nguyen VH, Cheung R, Ingelsson E, Nguyen MH. Prevalence, characteristics and mortality outcomes of obese, nonobese and lean NAFLD in the United States, 1999-2016. *J Intern Med* 2020; **288**: 139-151 [PMID: 32319718 DOI: 10.1111/joim.13069]
5 **Hagström H**, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, Kechagias S. Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: A long-term follow-up study. *Hepatol Commun* 2018; **2**: 48-57 [PMID: 29404512 DOI: 10.1002/hep4.1124]
6 **Leung JC**, Loong TC, Wei JL, Wong GL, Chan AW, Choi PC, Shu SS, Chim AM, Chan HL, Wong VW. Histological severity and clinical outcomes of nonalcoholic fatty liver disease in nonobese patients. *Hepatology* 2017; **65**: 54-64 [PMID: 27339817 DOI: 10.1002/hep.28697]
7 **Feng RN**, Du SS, Wang C, Li YC, Liu LY, Guo FC, Sun CH. Lean-non-alcoholic fatty liver disease increases risk for metabolic disorders in a normal weight Chinese population. *World J Gastroenterol* 2014; **20**: 17932-17940 [PMID: 25548491 DOI: 10.3748/wjg.v20.i47.17932]
8 **Schulze MB**. Metabolic health in normal-weight and obese individuals. *Diabetologia* 2019; **62**: 558-566 [PMID: 30569272 DOI: 10.1007/s00125-018-4787-8]
9 **Emerging Risk Factors Collaboration**, Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, Sarwar N, Kizer JR, Lawlor DA, Nordestgaard BG, Ridker P, Salomaa V, Stevens J, Woodward M, Sattar N, Collins R, Thompson SG, Whitlock G, Danesh J. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet* 2011; **377**: 1085-1095 [PMID: 21397319 DOI: 10.1016/S0140-6736(11)60105-0]
10 **Kim D**, Kim W, Joo SK, Han J, Kim JH, Harrison SA, Younossi ZM, Ahmed A. Association between body size-metabolic phenotype and nonalcoholic steatohepatitis and significant fibrosis. *J Gastroenterol* 2020; **55**: 330-341 [PMID: 31535207 DOI: 10.1007/s00535-019-01628-z]
11 **Fracanzani AL**, Petta S, Lombardi R, Pisano G, Russello M, Consonni D, Di Marco V, Cammà C, Mensi L, Dongiovanni P, Valenti L, Craxì A, Fargion S. Liver and Cardiovascular Damage in Patients With Lean Nonalcoholic Fatty Liver Disease, and Association With Visceral Obesity. *Clin Gastroenterol Hepatol* 2017; **15**: 1604-1611. e1 [PMID: 28554682 DOI: 10.1016/j.cgh.2017.04.045]
12 **Zelber-Sagi S**, Lotan R, Shlomai A, Webb M, Harrari G, Buch A, Nitzan Kaluski D, Halpern Z, Oren R. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *J Hepatol* 2012; **56**: 1145-1151 [PMID: 22245895 DOI: 10.1016/j.jhep.2011.12.011]
13 **Chang Y**, Ryu S, Sung E, Woo HY, Cho SI, Yoo SH, Ahn HY, Choi NK. Weight gain within the normal weight range predicts ultrasonographically detected fatty liver in healthy Korean men. *Gut* 2009; **58**: 1419-1425 [PMID: 19505882 DOI: 10.1136/gut.2008.161885]
14 **Srikanthan P**, Hevener AL, Karlamangla AS. Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: findings from the National Health and Nutrition Examination Survey III. *PLoS One* 2010; **5**: e10805 [PMID: 22421977 DOI: 10.1371/journal.pone.0010805]
15 **Yun Y**, Kim HN, Lee EJ, Ryu S, Chang Y, Shin H, Kim HL, Kim TH, Yoo K, Kim HY. Fecal and blood microbiota profiles and presence of nonalcoholic fatty liver disease in obese *vs* lean subjects. *PLoS One* 2019; **14**: e0213692 [PMID:

30870486 DOI: 10.1371/journal.pone.0213692]
16 **Margariti E**, Deutsch M, Manolakopoulos S, Papatheodoridis GV. Non-alcoholic fatty liver disease may develop in individuals with normal body mass index. *Ann Gastroenterol* 2012; **25**: 45-51 [PMID: 24713801]
17 **Younossi ZM**, Stepanova M, Negro F, Hallaji S, Younossi Y, Lam B, Srishord M. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine (Baltimore)* 2012; **91**: 319-327 [PMID: 23117851 DOI: 10.1097/MD.0b013e3182779d49]
18 **Tobari M**, Hashimoto E, Taniai M, Ikarashi Y, Kodama K, Kogiso T, Tokushige K, Takayoshi N, Hashimoto N. Characteristics of non-alcoholic steatohepatitis among lean patients in Japan: Not uncommon and not always benign. *J Gastroenterol Hepatol* 2019; **34**: 1404-1410 [PMID: 30590868 DOI: 10.1111/jgh.14585]
19 **Denkmayr L**, Feldman A, Stechemesser L, Eder SK, Zandanell S, Schranz M, Strasser M, Huber-Schönauer U, Buch S, Hampe J, Paulweber B, Lackner C, Haufe H, Sotlar K, Datz C, Aigner E. Lean Patients with Non-Alcoholic Fatty Liver Disease Have a Severe Histological Phenotype Similar to Obese Patients. *J Clin Med* 2018; **7**: 562 [PMID: 30562976 DOI: 10.3390/jcm7120562]
20 **Huang JF**, Tsai PC, Yeh ML, Huang CF, Huang CI, Hsieh MH, Dai CY, Yang JF, Chen SC, Yu ML, Chuang WL, Chang WY. Risk stratification of non-alcoholic fatty liver disease across body mass index in a community basis. *J Formos Med Assoc* 2020; **119**: 89-96 [PMID: 30952479 DOI: 10.1016/j.jfma.2019.03.014]
21 **Gonzalez-Cantero J**, Martin-Rodriguez JL, Gonzalez-Cantero A, Arrebola JP, Gonzalez-Calvin JL. Insulin resistance in lean and overweight non-diabetic Caucasian adults: Study of its relationship with liver triglyceride content, waist circumference and BMI. *PLoS One* 2018; **13**: e0192663 [PMID: 29425212 DOI: 10.1371/journal.pone.0192663]
22 **Feldman A**, Eder SK, Felder TK, Kedenko L, Paulweber B, Stadlmayr A, Huber-Schönauer U, Niederseer D, Stickel F, Auer S, Haschke-Becher E, Patsch W, Datz C, Aigner E. Clinical and Metabolic Characterization of Lean Caucasian Subjects With Non-alcoholic Fatty Liver. *Am J Gastroenterol* 2017; **112**: 102-110 [PMID: 27527746 DOI: 10.1038/ajg.2016.318]
23 **Stefan N**, Schick F, Häring HU. Causes, Characteristics, and Consequences of Metabolically Unhealthy Normal Weight in Humans. *Cell Metab* 2017; **26**: 292-300 [PMID: 28768170 DOI: 10.1016/j.cmet.2017.07.008]
24 **Eckel N**, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition from metabolic healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI categories in 90 257 women (the Nurses' Health Study): 30 year follow-up from a prospective cohort study. *Lancet Diabetes Endocrinol* 2018; **6**: 714-724 [PMID: 29859908 DOI: 10.1016/S2213-8587(18)30137-2]
25 **Lassale C**, Tzoulaki I, Moons KGM, Sweeting M, Boer J, Johnson L, Huerta JM, Agnoli C, Freisling H, Weiderpass E, Wennberg P, van der A DL, Arriola L, Benetou V, Boeing H, Bonnet F, Colorado-Yohar SM, Engström G, Eriksen AK, Ferrari P, Grioni S, Johansson M, Kaaks R, Katsoulis M, Katzke V, Key TJ, Matullo G, Melander O, Molina-Portillo E, Moreno-Iribas C, Norberg M, Overvad K, Panico S, Quirós JR, Saieva C, Skeie G, Steffen A, Stepien M, Tjønneland A, Trichopoulou A, Tumino R, van der Schouw YT, Verschuren WMM, Langenberg C, Di Angelantonio E, Riboli E, Wareham NJ, Danesh J, Butterworth AS. Separate and combined associations of obesity and metabolic health with coronary heart disease: a pan-European case-cohort analysis. *Eur Heart J* 2018; **39**: 397-406 [PMID: 29020414 DOI: 10.1093/eurheartj/ehx448]
26 **Emerging Risk Factors Collaboration**, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009; **302**: 1993-2000 [PMID: 19903920 DOI: 10.1001/jama.2009.1619]
27 **Emerging Risk Factors Collaboration**. , Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; **375**: 2215-2222 [PMID: 20609967 DOI: 10.1016/S0140-6736(10)60484-9]
28 **Winkler TW**, Günther F, Höllerer S, Zimmermann M, Loos RJ, Kutalik Z, Heid IM. A joint view on genetic variants for adiposity differentiates subtypes with distinct metabolic implications. *Nat Commun* 2018; **9**: 1946 [PMID: 29769528 DOI: 10.1038/s41467-018-04124-9]
29 **Perseghin G**. Lipids in the wrong place: visceral fat and nonalcoholic steatohepatitis. *Diabetes Care* 2011; **34** Suppl 2: S367-S370 [PMID: 21525484 DOI: 10.2337/dc11-s249]
30 **Yaghootkar H**, Lotta LA, Tyrrell J, Smit RA, Jones SE, Donnelly L, Beaumont R, Campbell A, Tuke MA, Hayward C, Ruth KS, Padmanabhan S, Jukema JW, Palmer CC, Hattersley A, Freathy RM, Langenberg C, Wareham NJ, Wood AR, Murray A, Weedon MN, Sattar N, Pearson E, Scott RA, Frayling TM. Genetic Evidence for a Link Between Favorable Adiposity and Lower Risk of Type 2 Diabetes, Hypertension, and Heart Disease. *Diabetes* 2016; **65**: 2448-2460 [PMID: 27207519 DOI: 10.2337/db15-1671]
31 **Conus F**, Rabasa-Lhoret R, Péronnet F. Characteristics of metabolically obese normal-weight (MONW) subjects. *Appl Physiol Nutr Metab* 2007; **32**: 4-12 [PMID: 17332780 DOI: 10.1139/h06-092]
32 **Bedossa P**. Diagnosis of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Why liver biopsy is essential. *Liver Int* 2018; **38** Suppl 1: 64-66 [PMID: 29427497 DOI: 10.1111/Liv.13653]
33 **Wong VW**, Wong GL, Chan RS, Shu SS, Cheung BH, Li LS, Chim AM, Chan CK, Leung JK, Chu WC, Woo J, Chan HL. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. *J Hepatol* 2018; **69**: 1349-1356 [PMID: 30142427 DOI: 10.1016/j.jhep.2018.08.011]
34 **Cleasby ME**, Jamieson PM, Atherton PJ. Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. *J Endocrinol* 2016; **229**: R67-R81 [PMID: 26931135 DOI: 10.1530/JOE-15-0533]
35 **Kwak JH**, Jun DW, Lee SM, Cho YK, Lee KN, Lee HL, Lee OY, Choi HS, Yoon BC. Lifestyle predictors of obese and non-obese patients with nonalcoholic fatty liver disease: A cross-sectional study. *Clin Nutr* 2018; **37**: 1550-1557 [PMID: 28918170 DOI: 10.1016/j.clnu.2017.08.018]
36 **Chitturi S**, Wong VW, Farrell G. Nonalcoholic fatty liver in Asia: Firmly entrenched and rapidly gaining ground. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 163-172 [PMID: 21199528 DOI: 10.1111/j.1440-1746.2010.06548.x]

37 **Akyuz U**, Yesil A, Yilmaz Y. Characterization of lean patients with nonalcoholic fatty liver disease: potential role of high hemoglobin levels. *Scand J Gastroenterol* 2015; **50**: 341-346 [PMID: 25540973 DOI: 10.3109/00365521.2014.983160]

38 **Chen F**, Esmaili S, Rogers GB, Bugianesi E, Petta S, Marchesini G, Bayoumi A, Metwally M, Azardaryany MK, Coulter S, Choo JM, Younes R, Rosso C, Liddle C, Adams LA, Craxì A, George J, Eslam M. Lean NAFLD: A Distinct Entity Shaped by Differential Metabolic Adaptation. *Hepatology* 2020; **71**: 1213-1227 [PMID: 31442319 DOI: 10.1002/hep.30908]

39 **Liu YL**, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, Allison ME, Alexander GJ, Piguet AC, Anty R, Donaldson P, Aithal GP, Francque S, Van Gaal L, Clement K, Ratziu V, Dufour JF, Day CP, Daly AK, Anstee QM. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014; **5**: 4309 [PMID: 24978903 DOI: 10.1038/ncomms5309]

40 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]

41 **Sookoian S**, Castaño GO, Scian R, Mallardi P, Fernández Gianotti T, Burgueño AL, San Martino J, Pirola CJ. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. *Hepatology* 2015; **61**: 515-525 [PMID: 25302781 DOI: 10.1002/hep.27556]

42 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

43 **Valenti L**, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, Nobili V, Mozzi E, Roviaro G, Vanni E, Bugianesi E, Maggioni M, Fracanzani AL, Fargion S, Day CP. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1209-1217 [PMID: 20373368 DOI: 10.1002/hep.23622]

44 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

45 **Xu R**, Tao A, Zhang S, Deng Y, Chen G. Association between patatin-like phospholipase domain containing 3 gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. *Sci Rep* 2015; **5**: 9284 [PMID: 25791171 DOI: 10.1038/srep09284]

46 **Shen J**, Wong GL, Chan HL, Chan HY, Yeung DK, Chan RS, Chim AM, Chan AW, Choi PC, Woo J, Chu WC, Wong VW. PNPLA3 gene polymorphism accounts for fatty liver in community subjects without metabolic syndrome. *Aliment Pharmacol Ther* 2014; **39**: 532-539 [PMID: 24417250 DOI: 10.1111/apt.12609]

47 **Kim HJ**, Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, Lim SK, Kim KR, Lee HC, Huh KB, Cha BS. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch Intern Med* 2004; **164**: 2169-2175 [PMID: 15505132 DOI: 10.1001/archinte.164.19.2169]

48 **Liu CJ**. Prevalence and risk factors for non-alcoholic fatty liver disease in Asian people who are not obese. *J Gastroenterol Hepatol* 2012; **27**: 1555-1560 [PMID: 22741595 DOI: 10.1111/j.1440-1746.2012.07222.x]

49 **Wei JL**, Leung JC, Loong TC, Wong GL, Yeung DK, Chan RS, Chan HL, Chim AM, Woo J, Chu WC, Wong VW. Prevalence and Severity of Nonalcoholic Fatty Liver Disease in Non-Obese Patients: A Population Study Using Proton-Magnetic Resonance Spectroscopy. *Am J Gastroenterol* 2015; **110**: 1306-14; quiz 1315 [PMID: 26215532 DOI: 10.1038/ajg.2015.235]

50 **Zeng J**, Yang RX, Sun C, Pan Q, Zhang RN, Chen GY, Hu Y, Fan JG. Prevalence, clinical characteristics, risk factors, and indicators for lean Chinese adults with nonalcoholic fatty liver disease. *World J Gastroenterol* 2020; **26**: 1792-1804 [PMID: 32351294 DOI: 10.3748/wjg.v26.i15.1792]

51 **Clemente JC**, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell* 2012; **148**: 1258-1270 [PMID: 22424233 DOI: 10.1016/j.cell.2012.01.035]

52 **Gilbert JA**, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med* 2018; **24**: 392-400 [PMID: 29634682 DOI: 10.1038/nm.4517]

53 **Vrieze A**, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913-6. e7 [PMID: 22728514 DOI: 10.1053/j.gastro.2012.06.031]

54 **Qin J**, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**: 55-60 [PMID: 23023125 DOI: 10.1038/nature11450]

55 **Zhu L**, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013; **57**: 601-609 [PMID: 23055155 DOI: 10.1002/hep.26093]

56 **Betrapally NS**, Gillevet PM, Bajaj JS. Changes in the Intestinal Microbiome and Alcoholic and Nonalcoholic Liver Diseases: Causes or Effects? *Gastroenterology* 2016; **150**: 1745-1755.e3 [PMID: 26948887 DOI: 10.1053/j.gastro.2016.02.073]

57 **Machado MV**, Cortez-Pinto H. Diet, Microbiota, Obesity, and NAFLD: A Dangerous Quartet. *Int J Mol Sci* 2016; **17**: 481 [PMID: 27043550 DOI: 10.3390/ijms17040481]

58 **Wong VW**, Tse CH, Lam TT, Wong GL, Chim AM, Chu WC, Yeung DK, Law PT, Kwan HS, Yu J, Sung JJ, Chan HL. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis--a longitudinal study. *PLoS*

One 2013; **8**: e62885 [PMID: 23638162 DOI: 10.1371/journal.pone.0062885]
59 **Jiang W**, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J, Liu Y. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep* 2015; **5**: 8096 [PMID: 25644696 DOI: 10.1038/srep08096]
60 **Duarte SMB**, Stefano JT, Miele L, Ponziani FR, Souza-Basqueira M, Okada LSRR, de Barros Costa FG, Toda K, Mazo DFC, Sabino EC, Carrilho FJ, Gasbarrini A, Oliveira CP. Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: A prospective pilot study. *Nutr Metab Cardiovasc Dis* 2018; **28**: 369-384 [PMID: 29482963 DOI: 10.1016/j.numecd.2017.10.014]
61 **Kramer CK**, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions? *Ann Intern Med* 2013; **159**: 758-769 [PMID: 24297192 DOI: 10.7326/0003-4819-159-11-201312030-00008]
62 **Meigs JB**, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, D'Agostino RB. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. *J Clin Endocrinol Metab* 2006; **91**: 2906-2912 [PMID: 16735483 DOI: 10.1210/jc.2006-0594]
63 **Stefan N**, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Häring HU. Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 2008; **168**: 1609-1616 [PMID: 18695074 DOI: 10.1001/archinte.168.15.1609]
64 **Wildman RP**, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, Sowers MR. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med* 2008; **168**: 1617-1624 [PMID: 18695075 DOI: 10.1001/archinte.168.15.1617]
65 **Karelis AD**, Rabasa-Lhoret R. Inclusion of C-reactive protein in the identification of metabolically healthy but obese (MHO) individuals. *Diabetes Metab* 2008; **34**: 183-184 [PMID: 18329310 DOI: 10.1016/j.diabet.2007.11.004]
66 **Stefan N**, Häring HU, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. *Lancet Diabetes Endocrinol* 2013; **1**: 152-162 [PMID: 24622321 DOI: 10.1016/S2213-8587(13)70062-7]
67 **Phillips CM**. Metabolically healthy obesity across the life course: epidemiology, determinants, and implications. *Ann N Y Acad Sci* 2017; **1391**: 85-100 [PMID: 27723940 DOI: 10.1111/nyas.13230]
68 **Murphy N**, Cross AJ, Abubakar M, Jenab M, Aleksandrova K, Boutron-Ruault MC, Dossus L, Racine A, Kühn T, Katzke VA, Tjønneland A, Petersen KE, Overvad K, Quirós JR, Jakszyn P, Molina-Montes E, Dorronsoro M, Huerta JM, Barricarte A, Khaw KT, Wareham N, Travis RC, Trichopoulou A, Lagiou P, Trichopoulos D, Masala G, Krogh V, Tumino R, Vineis P, Panico S, Bueno-de-Mesquita HB, Siersema PD, Peeters PH, Ohlsson B, Ericson U, Palmqvist R, Nyström H, Weiderpass E, Skeie G, Freisling H, Kong SY, Tsilidis K, Muller DC, Riboli E, Gunter MJ. A Nested Case-Control Study of Metabolically Defined Body Size Phenotypes and Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLoS Med* 2016; **13**: e1001988 [PMID: 27046222 DOI: 10.1371/journal.pmed.1001988]
69 **Bosy-Westphal A**, Müller MJ. Identification of skeletal muscle mass depletion across age and BMI groups in health and disease--there is need for a unified definition. *Int J Obes (Lond)* 2015; **39**: 379-386 [PMID: 25174451 DOI: 10.1038/ijo.2014.161]
70 **Pischon T**, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, van der Schouw YT, Spencer E, Moons KG, Tjønneland A, Halkjaer J, Jensen MK, Stegger J, Clavel-Chapelon F, Boutron-Ruault MC, Chajes V, Linseisen J, Kaaks R, Trichopoulou A, Trichopoulos D, Bamia C, Sieri S, Palli D, Tumino R, Vineis P, Panico S, Peeters PH, May AM, Bueno-de-Mesquita HB, van Duijnhoven FJ, Hallmans G, Weinehall L, Manjer J, Hedblad B, Lund E, Agudo A, Arriola L, Barricarte A, Navarro C, Martinez C, Quirós JR, Key T, Bingham S, Khaw KT, Boffetta P, Jenab M, Ferrari P, Riboli E. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* 2008; **359**: 2105-2120 [PMID: 19005195 DOI: 10.1056/NEJMoa0801891]
71 **Alferink LJM**, Trajanoska K, Erler NS, Schoufour JD, de Knegt RJ, Ikram MA, Janssen HLA, Franco OH, Metselaar HJ, Rivadeneira F, Darwish Murad S. Nonalcoholic Fatty Liver Disease in The Rotterdam Study: About Muscle Mass, Sarcopenia, Fat Mass, and Fat Distribution. *J Bone Miner Res* 2019; **34**: 1254-1263 [PMID: 31074909 DOI: 10.1002/jbmr.3713]
72 **Ruderman N**, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. *Diabetes* 1998; **47**: 699-713 [PMID: 9588440 DOI: 10.2337/diabetes.47.5.699]
73 **Coutinho T**, Goel K, Corrêa de Sá D, Carter RE, Hodge DO, Kragelund C, Kanaya AM, Zeller M, Park JS, Kober L, Torp-Pedersen C, Cottin Y, Lorgis L, Lee SH, Kim YJ, Thomas R, Roger VL, Somers VK, Lopez-Jimenez F. Combining body mass index with measures of central obesity in the assessment of mortality in subjects with coronary disease: role of "normal weight central obesity". *J Am Coll Cardiol* 2013; **61**: 553-560 [PMID: 23369419 DOI: 10.1016/j.jacc.2012.10.035]
74 **Curcic IB**, Berkovic MC, Kuna L, Roguljic H, Smolic R, Varzic SC, Jukic LV, Smolic M. Obesity Paradox in Chronic Liver Diseases: Product of Bias or a Real Thing? *J Clin Transl Hepatol* 2019; **7**: 275-279 [PMID: 31608220 DOI: 10.14218/JCTH.2019.00029]
75 **Pelusi S**, Cespiati A, Rametta R, Pennisi G, Mannisto V, Rosso C, Baselli G, Dongiovanni P, Fracanzani AL, Badiali S, Maggioni M, Craxi A, Fargion S, Prati D, Nobili V, Bugianesi E, Romeo S, Pihlajamaki J, Petta S, Valenti L. Prevalence and Risk Factors of Significant Fibrosis in Patients With Nonalcoholic Fatty Liver Without Steatohepatitis. *Clin Gastroenterol Hepatol* 2019; **17**: 2310-2319. e6 [PMID: 30708111 DOI: 10.1016/j.cgh.2019.01.027]
76 **Albhaisi S**, Chowdhury A, Sanyal AJ. Non-alcoholic fatty liver disease in lean individuals. *JHEP Rep* 2019; **1**: 329-341 [PMID: 32039383 DOI: 10.1016/j.jhepr.2019.08.002]
77 **Singh S**, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver *vs* nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015; **13**: 643-54. quiz e39-40 [PMID: 24768810 DOI: 10.1016/j.cgh.2014.04.014]
78 **European Association for the Study of the Liver (EASL)**. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]
79 **Degasperi E**, Colombo M. Distinctive features of hepatocellular carcinoma in non-alcoholic fatty liver disease. *Lancet*

Gastroenterol Hepatol 2016; **1**: 156-164 [PMID: 28404072 DOI: 10.1016/S2468-1253(16)30018-8]

80 **Dyson J**, Jaques B, Chattopadyhay D, Lochan R, Graham J, Das D, Aslam T, Patanwala I, Gaggar S, Cole M, Sumpter K, Stewart S, Rose J, Hudson M, Manas D, Reeves HL. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014; **60**: 110-117 [PMID: 23978719 DOI: 10.1016/j.jhep.2013.08.011]

81 **Piscaglia F**, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, Tiribelli C, Bellentani S; HCC-NAFLD Italian Study Group. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: A multicenter prospective study. *Hepatology* 2016; **63**: 827-838 [PMID: 26599351 DOI: 10.1002/hep.28368]

82 **Wattacheril J**, Sanyal AJ. Lean NAFLD: An Underrecognized Outlier. *Curr Hepatol Rep* 2016; **15**: 134-139 [PMID: 27668144 DOI: 10.1007/s11901-016-0302-1]

83 **Ratziu V**, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. *J Hepatol* 2015; **62**: S65-S75 [PMID: 25920092 DOI: 10.1016/j.jhep.2015.02.041]

84 **Kumar R**, Mohan S. Non-alcoholic Fatty Liver Disease in Lean Subjects: Characteristics and Implications. *J Clin Transl Hepatol* 2017; **5**: 216-223 [PMID: 28936403 DOI: 10.14218/JCTH.2016.00068]

85 **Mells JE**, Fu PP, Sharma S, Olson D, Cheng L, Handy JA, Saxena NK, Sorescu D, Anania FA. Glp-1 analog, liraglutide, ameliorates hepatic steatosis and cardiac hypertrophy in C57BL/6J mice fed a Western diet. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G225-G235 [PMID: 22038829 DOI: 10.1152/ajpgi.00274.2011]

86 **Gao H**, Zeng Z, Zhang H, Zhou X, Guan L, Deng W, Xu L. The Glucagon-Like Peptide-1 Analogue Liraglutide Inhibits Oxidative Stress and Inflammatory Response in the Liver of Rats with Diet-Induced Non-alcoholic Fatty Liver Disease. *Biol Pharm Bull* 2015; **38**: 694-702 [PMID: 25947915 DOI: 10.1248/bpb.b14-00505]

87 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team; Abouda G; Aldersley MA; Stocken D; Gough SC; Tomlinson JW; Brown RM; Hübscher SG; Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]

88 **Ipsen DH**, Rolin B, Rakipovski G, Skovsted GF, Madsen A, Kolstrup S, Schou-Pedersen AM, Skat-Rørdam J, Lykkesfeldt J, Tveden-Nyborg P. Liraglutide Decreases Hepatic Inflammation and Injury in Advanced Lean Non-Alcoholic Steatohepatitis. *Basic Clin Pharmacol Toxicol* 2018; **123**: 704-713 [PMID: 29953740 DOI: 10.1111/bcpt.13082]

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Basic Study

Integrative analysis of layers of data in hepatocellular carcinoma reveals pathway dependencies

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Abstract

BACKGROUND

The broader use of high-throughput technologies has led to improved molecular characterization of hepatocellular carcinoma (HCC).

AIM

To comprehensively analyze and characterize all publicly available genomic, gene expression, methylation, miRNA and proteomic data in HCC, covering 85 studies and 3355 patient sample profiles, to identify the key dysregulated genes and pathways they affect.

METHODS

We collected and curated all well-annotated and publicly available high-throughput datasets from PubMed and Gene Expression Omnibus derived from human HCC tissue. Comprehensive pathway enrichment analysis was performed using pathDIP for each data type (genomic, gene expression, methylation, miRNA and proteomic), and the overlap of pathways was assessed to elucidate pathway dependencies in HCC.

RESULTS

We identified a total of 8733 abstracts retrieved by the search on PubMed on HCC

for the different layers of data on human HCC samples, published until December 2016. The common key dysregulated pathways in HCC tissue across different layers of data included epidermal growth factor (EGFR) and β1-integrin pathways. Genes along these pathways were significantly and consistently dysregulated across the different types of high-throughput data and had prognostic value with respect to overall survival. Using CTD database, estradiol would best modulate and revert these genes appropriately.

CONCLUSION

By analyzing and integrating all available high-throughput genomic, transcriptomic, miRNA, methylation and proteomic data from human HCC tissue, we identified EGFR, β1-integrin and axon guidance as pathway dependencies in HCC. These are master regulators of key pathways in HCC, such as the mTOR, Ras/Raf/MAPK and p53 pathways. The genes implicated in these pathways had prognostic value in HCC, with Netrin and Slit3 being novel proteins of prognostic importance to HCC. Based on this integrative analysis, EGFR, and β1-integrin are master regulators that could serve as potential therapeutic targets in HCC.

Key Words: Hepatocellular carcinoma; Gene expression; miRNA; Methylation; Proteomics; High throughput data

Core Tip: Analyzing all available high-throughput genomic, transcriptomic, miRNA, methylation and proteomic data from human hepatocellular carcinoma tissue, we identified master regulators of key pathways in hepatocellular carcinoma, such as the mTOR, Ras/Raf/MAPK and p53 pathways.

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INTRODUCTION

The molecular basis of hepatocellular carcinoma (HCC) has been elusive, given the significant heterogeneity of this tumor that arises in the context of various chronic liver diseases[1]. HCC remains a high-fatality cancer, despite large-scale efforts to better characterize and therapeutically target this malignancy. Since prevalence of cirrhosis due to hepatitis C and fatty liver disease is increasing in North America, HCC continues to rise[2]. Five-year survival remains poor at 18% due to late diagnosis and inability to tolerate chemotherapy in patients with cirrhosis[2]. Consequently, there is an urgent need to better understand the molecular basis of this highly fatal cancer.

Clinical management of HCC is optimized based on disease stage[3]. Curative treatment with resection, radiofrequency ablation or transplantation is possible in early stage disease[4]. When HCC is diagnosed at a later stage, sorafenib is the first-line chemotherapy, which is directed against the Ras/Raf/MAPK pathway[4]. This is associated with a very modest improvement in overall survival of 3 additional months as compared to placebo (10.7 mo *vs* 7.9 mo)[5].

The cancer genome atlas (TCGA) is a large-scale project that has enabled improved characterization of cancers with several layers of data. The TCGA multi-platform analysis of 196 HCC tumors described this cancer as highly heterogeneous and difficult to characterize, although certain key pathways did emerge including the Ras/Raf/MAPK, mTOR, Wnt/B-catenin, and Sonic Hedgehog pathways[1,6]. Integration of various types of data has previously been performed to map interaction networks. By integrating genomic, transcriptomic and proteomic data, one can understand potential interactions that contribute to a disease condition or process[7,8]. These interactions may otherwise not be uncovered, on the basis of a single type of data. This systems biology approach has been especially important in cancer, given that alterations in one gene can have a ripple effect on proteins in the rest of a protein-protein interaction network. Therefore, elucidating the layers of data in a disease can provide additional insights into the pathways that drive cancer[9].

In the current study, we aim to characterize the landscape of high-throughput data profiling in HCC and determine the patterns in key dysregulated genes and pathways across these different layers of data. The patterns that emerge could help in better understanding the pathways that drive HCC and

could be considered as therapeutic targets.

MATERIALS AND METHODS

Data collection, analysis and database compiling

We downloaded all available high-throughput genomic, transcriptomic, microRNA, methylation, and proteomic datasets related to human HCC samples from published datasets (PubMed, http://www.ncbi.nlm.nih.gov/PubMed and Gene Expression Omnibus (GEO), https://www.ncbi.nlm.nih.gov/geo).

Using PubMed, the following search was performed for whole exome sequencing data on HCC: ("carcinoma, hepatocellular" [MeSH Terms] OR ("carcinoma" [All Fields] AND "hepatocellular" [All Fields]) OR "hepatocellular carcinoma" [All Fields] OR ("hepatocellular" [All Fields] AND "carcinoma" [All Fields])) AND (whole [All Fields] AND ("exome" [MeSH Terms] OR "exome" [All Fields]) AND sequencing [All Fields]). The following MeSH terms were used to identify gene expression papers: ("carcinoma, hepatocellular" [MeSH Terms] OR ("carcinoma" [All Fields] AND "hepatocellular" [All Fields]) OR "hepatocellular carcinoma" [All Fields] OR ("hepatocellular" [All Fields] AND "carcinoma" [All Fields])) AND ("gene expression" [MeSH Terms] OR ("gene" [All Fields] AND "expression" [All Fields]) OR "gene expression" [All Fields]) AND ("humans" [MeSH Terms] OR "humans" [All Fields]) AND English [All Fields] NOT ("review" [Publication Type] OR "review literature as topic" [MeSH Terms] OR "reviews" [All Fields]). To identify suitable papers regarding methylation in HCC, we used the following terms: ("methylation" [MeSH Terms] OR "methylation"[All Fields]) AND ("carcinoma, hepatocellular" [MeSH Terms] OR ("carcinoma" [All Fields] AND "hepatocellular" [All Fields]) OR "hepatocellular carcinoma" [All Fields] OR ("hepatocellular" [All Fields] AND "carcinoma" [All Fields]) AND ("humans" [MeSH Terms] AND English [lang]). Proteomics papers were retrieved using the following search: [("proteomics" [MeSH Terms] OR "proteomics" [All Fields]) AND high [All Fields] AND throughput [All Fields]] AND ("carcinoma, hepatocellular" [MeSH Terms]) OR ("carcinoma" [All Fields] AND "hepatocellular" [All Fields]) OR "hepatocellular carcinoma" [All Fields] OR ("hepatocellular"[All Fields] AND "carcinoma"[All Fields]). MicroRNAs reported in HCC were identified using these MeSH terms: ("micrornas" [MeSH Terms] OR "micrornas"[All Fields] OR "mirna" [All Fields]) AND profile [All Fields] AND ("carcinoma, hepatocellular" [MeSH Terms] OR ("carcinoma" [All Fields] AND "hepatocellular" [All Fields]) OR "hepatocellular carcinoma" [All Fields] OR ("hepatocellular" [All Fields] AND "carcinoma" [All Fields]).

We considered for inclusion all datasets available in PubMed.

The datasets publicly available on the GEO, a public functional genomics data repository of high-throughput array data (https://www.ncbi.nlm.nih.gov/geo) were retrieved and analyzed using GEO2R (https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html), a web tool available on the portal, identifying genes differentially expressed between samples of HCC and the non-tumoral liver portion. GEO2R compares original submitter-supplied processed data tables using the GEOquery and limma R packages from the Bioconductor project. Following instructions available online at (https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html), we retrieved all dysregulated genes. Only those with an adjusted P value < 0.05, and expression fold change value below ≤ 0.5 or above ≥ 1.5 were considered for further analysis (Table 1, Supplementary Table 1). The genes included in our list from WES papers were reported as affected by nonsynonymous mutations, and synonymous mutations were not considered. Putative microRNA gene targets were identified using an online database, mirDIP 4.1 [10], (http://ophid.utoronto.ca/mirDIP). The most stringent predictive search option (top 1%) was used to obtain the list of putative targets of all differentially expressed miRNAs.

From the selected 11 methylation datasets, raw data from eight studies were available on the GEO website (https://www.ncbi.nlm.nih.gov/geo/). We selected the CpG sites or genes reported to be hyper-or hypo- methylated in these publications. The genomic region was considered differentially methylated between HCC tissue and the adjacent non-tumoral sample, if the FDR corrected P value < 0.01. Furthermore, we filtered out everything that did not satisfy the criteria: $\Delta\beta \geq 0.20$ or $\Delta\beta \leq -0.20$, where $\Delta\beta = \beta HCC - \beta adjacent$ was the difference in methylation between above specified groups. When the CpG sites were considered, the Illumina HumanMethylation450K and 27K platforms were used for mapping to the genes. When multiple sites or genes were found to have the same sense of differential methylation, the mean value of Δβ was calculated. Only the CpGs in the 5′UTR, 1st Exon, TSS200, TSS1500 or in CpG islands were considered in our analysis. Proteomic results were retrieved and included only if protein abundance was reported as different in HCC liver samples compared to control samples.

Figure 1 outlines our study workflow. Papers were excluded from each specific search for the following reasons: Data from cell lines, or animal models, studying efficacy or drugs, or the presence of long non-coding RNA, mechanistic studies not performing high-throughput or evaluating the role of one molecule, papers focused on liver diseases but not HCC or liver tissue, not original data such as review articles, or those studies using already selected datasets, not reporting the modulation of the molecules, and papers without data available.

Table 1 List of the final 85 selected publications for each layer of data. For each publication the number of hepatocellular carcinoma samples and controls and the platform used for the analysis are reported

Gene expression					
No.	**year**	**PMID**	**HCC (*n*)**	**Controls (*n*)**	**GEO dataset**
1	2004	17393520	35	13	GSE6764
2	2008	18504433	11	2	GSE6222
3	2008	18923165	80	82	GSE10143
4	2009	19098997	47	58	GSE14323
5	2009	19861515	16	47	GSE17967
6	2011	21320499	34	34	GSE20140 (GSE10141, GSE10140)
7	2011	21712445	40	40	GSE28248
8	2013	23691139	15	15	GSE17548
9	2013	23800896	GSE36376_276; GSE25097_211	GSE36376_247; GSE25097_283	GSE36376, GSE25097
10	2014	24498002	46	46	GSE47595
11	2014	24564407	45	45	GSE45114
12	2014	25093504	39	40	GSE57958
13	2014	25141867	11	11	GSE55092
14	2014	25376302	18	18	GSE60502
15	2014	25536056	72	72	GSE39791
16	2015	25666192	132	132	GSE54236
17	2015	25645722	228	168	GSE63898
18	2016	27499918	60	60	GSE64041
19	2016	25964079	26	20	GSE54238
Proteomics					
No.	year	PMID	HCC (*n*)	Controls (*n*)	
1	2004	14726492	8	8	
2	2008	19003864	12	12	
3	2005	15759316	10	10	
4	2005	16097030	14	14	
5	2007	17627933	12	12	
6	2014	23621634	3	3	
7	2009	19562805	3	3	
8	2016	26709725	24	12	
9	2013	23589362	20	20	
10	2012	22813877	10	10	
11	2012	22082227	11	11	
12	2011	21631109	69	123	
13	2010	20230046	5	5	
14	2010	19956837	20	20	
15	2009	19715608	18	18	
16	2009	19535095	3	3	
17	2009	19161326	80	80	

18	2004	15221772	20	20	
19	2003	14673798	21	21	
20	2003	14654528	21	21	
21	2002	12481271	11	11	
22	2013	23462207	7	7	
23	2005	16335951	8	8	
24	2006	16342242	10	10	
25	2011	22034872	3	3	
26	2005	15852300	7	7	
27	2011	21913717	3	3	
28	2007	17203974	25	28	
29	2007	17586277	10	10	
Whole exome sequencing					
No.	year	PMID	HCC (*n*)	Controls (*n*)	GEO dataset
1	2013	23912677	3	3	N/A
2	2014	24055508	4	7	N/A
3	2017	28323123	5	5	N/A
4	2014	24798001	231	231	GSE54504
5	2012	22561517	24	24	N/A
Epigenetic_miRNAs					
No.	year	PMID	HCC (*n*)	Controls (*n*)	GEO dataset
1	2015	26190160	9	7	N/A
2	2014	24789420	10	9	GSE31383
3	2014	24564407	45	45	GSE10694
4	2011	21298008	73	73	GSE21362
5	2008	18649363	78	10	N/A
6	2012	22135159	20	20	N/A
7	2011	21319996	94	94	N/A
8	2009	19473441	20	20	N/A
9	2009	19173277	35		N/A
10	2007	18171346	10	10	N/A
11	2006	16331254	25	25	N/A
12	2015	26062888	30	30	N/A
13	2015	26046780	327	43	N/A
14	2015	25861255	66	66	GSE54751
15	2015	25500075	6	6	GSE54537
16	2014	24875649	24	24	
17	2013	23812667	166	166	GSE31384
18	2013	23390000	9	17	GSE40744
19	2012	23082062	18	18	N/A
20	2014	24586785	29	29	N/A
21	2013	24417970	78	78	N/A
Epigenetic methylation					

No.	year	PMID	HCC (*n*)	Controls (*n*)	GEO dataset
1	2011	21500188	13	12	N/A
2	2014	24306662	45	45	N/A
3	2014	25376292	22	22	N/A
4	2015	25945129	8	8	GSE59260
5	2011	21747116	12	12	GSE29720
6	2010	20165882	20	20	GSE18081
7	2012	22234943	62	62	GSE37988
8	2013	24012984	20	8	GSE44970
9	2013	23208076	66	66	GSE54503
10	2014	25093504	59	59	GSE57956
11	2014	25294808	27	27	GSE60753

HCC: Hepatocellular carcinoma; GEO: Gene Expression Omnibus; N/A: Not applicable.

Available patient data, including etiology of liver disease (hepatitis C, hepatitis B, alcohol, fatty liver disease) on the basis of which the HCC tumors developed, presence of cirrhosis, the Model for End-stage Liver Disease score (MELD score, an assessment of the severity of liver dysfunction), tumor histology, stage of cancer, alpha-fetoprotein level, overall and recurrence-free survival following treatment were also documented (Supplementary Table 2).

Pathway enrichment analysis

The key dysregulated genes from each type of data (genomic, miRNA, methylation, transcriptomic, and proteomic) were fed into the Integrated Interactions Database[11] (IID, http://ophid.utoronto.ca/iid), to obtain a list of the protein-protein interactions. For the miRNA dataset, we determined the target genes of the differentially expressed miRNAs in tumors using the miRNA Data Integration Portal mirDIP v4.1 [10]. The individual lists derived from each type of data were then fed into the pathway Data Integration Portal, pathDIP v3.0 (http://ophid.utoronto.ca/pathDIP)[12], in order to determine the significantly dysregulated pathways in HCC. pathDIP integrates data from 20 major pathway databases, and computationally predicts gene association to curated pathways using protein-protein interactions from IID significance of their connectivity[12]. We used this comprehensive pathway enrichment analysis portal to obtain a list of significantly enriched pathways using literature curated (core) pathway memberships *P* value (FDR: BH-method) less than 0.05.

The lists of pathways from each type of data were then assessed for overlap using Venny 2.1, an online tool for Venn diagram design (http://bioinfogp.cnb. csic.es/tools/venny/index.html).

Retrospective validation on independent dataset

In order to determine whether key differentially expressed genes along the overlapping pathways had prognostic value, we used KMplotter, a web-based tool that enables survival analysis across multiple cancers and datasets[13]. Patient samples were split into two groups per autoselection of the best cutoff for each gene, in order to assess its prognostic value. We ran multivariate overall survival analysis based on the high *vs* low expression of each gene in HCC tumors. The two groups were compared by a Kaplan-Meier survival plot, and the hazard ratio with 95% confidence intervals and log-rank *P* value were calculated.

Drug identification by CTD

The identification of putative therapeutic agents able to revert the modulation of genes of interest based on their modulation associated with a worse prognosis was obtained using the online Comparative Toxicogenomics Database http://ctdbase.org[14]. This database provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships.

RESULTS

We identified a total of 8733 abstracts retrieved by the search on PubMed on HCC for the different layers of data on human HCC samples, published until December 2016. The flow chart outlining the selection process is detailed in Figure 1.

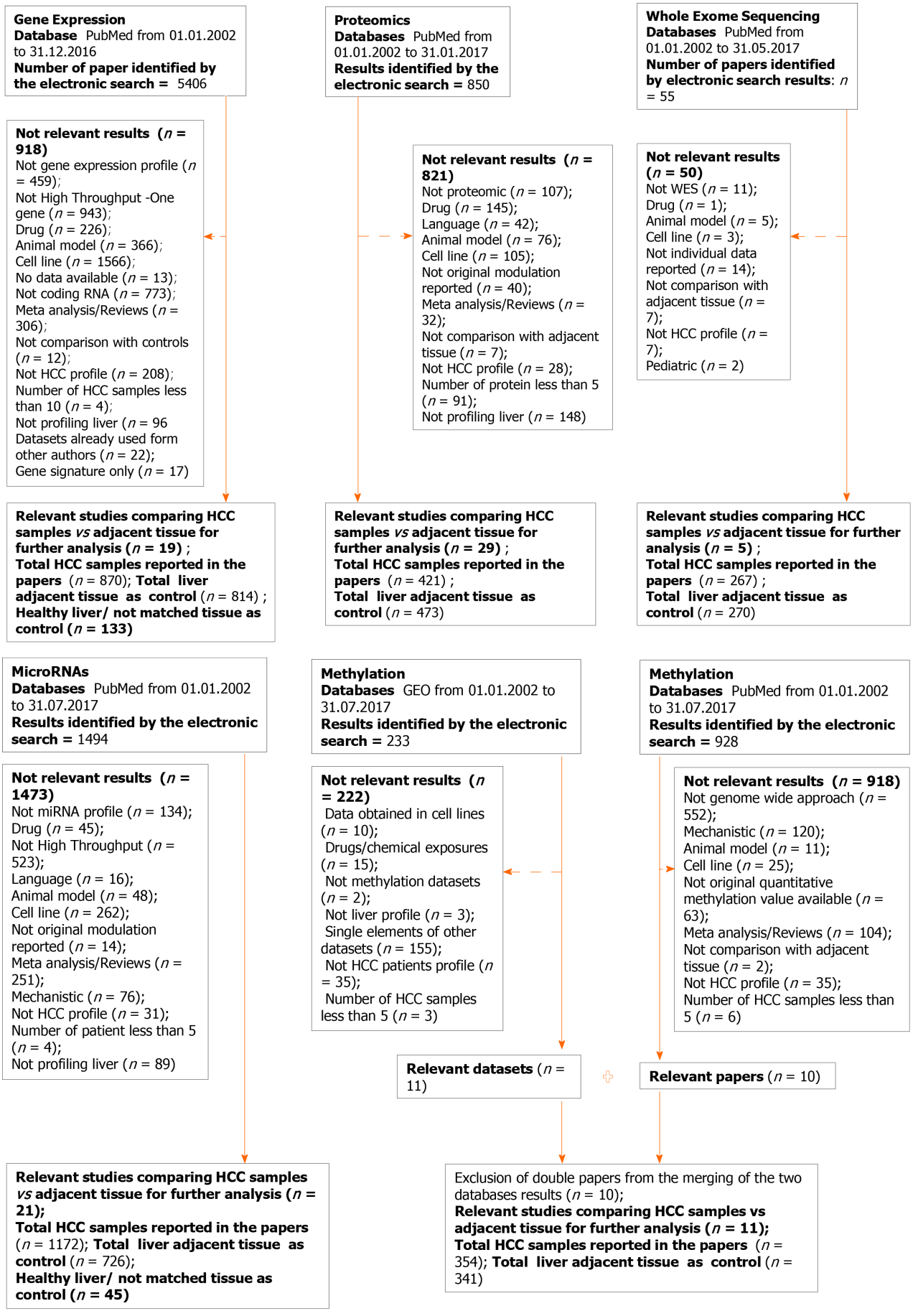

Figure 1 Flow chart showing the paper selection process and exclusion criteria for each data type: Gene expression, proteomics, whole

exome sequencing, microRNAs and methylation.

The number of samples included in our analysis are as follows: (1) Whole exome sequencing: 267 HCC and 270 control samples; (2) Gene expression: 870 HCC and 814 control samples; (3) miRNA: 1172 HCC and 771 control samples; (4) Methylation: 354 HCC and 341 control samples; and (5) Proteomics: 421 HCC and 473 control samples. The methodologies and platforms used to obtain these high-throughput data are reported by type of data (genomic, transcriptomic, miRNA, methylation and proteomic) in Table 1. Clinical data, regarding etiology of liver disease (hepatitis C, hepatitis B, alcohol, fatty liver disease) were frequently reported, on the other side serum levels of liver enzymes, AST and ALT, frequently used to assess liver functions were not available. Pathological details relative to differentiation or stage were frequently absent as well as other crucial variables in the clinic setting, such as Child Pugh/MELD score (Supplementary Table 2).

Integrative analysis reveals most important pathways in HCC

There were 188 overlapping dysregulated genes/proteins across the different types of data. Independently for each type of data, we obtained a list of pathways using pathDIP. We merged the list of dysregulated pathways in miRNA and methylation, given that these epigenetically regulate gene expression, in order to assess for overlapping pathways across the datasets.

This resulted in a list of 3 common, overlapping pathways among the different types of data: EGFR, β 1-integrin, and axon guidance pathways, as depicted in Figure 2. From the previous list of 188 common dysregulated elements in all different layers of data (Figure 3), we were able to identify 35/188 genes that were involved in these 3 shared pathways across the layers of data (Supplementary Table 1).

Prognostic value of pathways in HCC

We then examined the prognostic value of the deregulated genes associated to pathways of interest in HCC using TCGA RNA seq dataset, as listed in Table 2. Median survival of 364 patients in the TCGA, which was used for validation purposes regarding the prognostic value is reported. KMplotter HR results from TCGA RNA seq data reflected the altered modulation identified for these 9 genes in the 19 HCC papers relative to the gene expression data (Table 2). Among the five upregulated genes associated with positive HR values, CDK5, was reported with the highest HR value (1.85, $P = 0.0035$) and involved in cell cycle (Table 3). The other 4/9 genes reported as upregulated, COL2A1, LAMC1, RPS6KA3 and ITGB1 were identified with positive HR value by KM plotter analysis and involved in cellular migration (Table 2 and Table 3).

Four out of 9 genes were reported as downmodulated in the 19 HCC gene expression papers. Among these four, two genes, FGA and FGG, were identified as the top statistically significantly ($P = 0.0009$) associated with a protective role in HCC (HR values 0.52 and 0.59, respectively). FGA and FGG were consistently reported as downmodulated in about 45% of our 19 selected gene expression papers (Table 3). The other two downmodulated genes, EPHB1 and EFGR with negative HR values (Table 2) are reported to be affected by missense mutation leading to a loss of their protective role against cell migration.

Estradiol is a therapeutic agent that appropriately targets HCC genes

Using CTD, we found that estradiol was able to appropriately down- or upmodulate 4 out of 9 cancer-related genes (Table 2). Particularly, CTD reported estradiol capabilities to upregulated FGA, FGG and EGFR reported downmodulated in HCC (Table 2) and counteracting the upregulation of RPS6KA3 in HCC, suggesting a possible role for this hormone in HCC treatment.

DISCUSSION

In this study, we evaluate the molecular pathogenesis of HCC using a unique approach, that of combining all publicly available high-throughput data from patient HCC tumors. This encompasses all miRNA, methylation, genomic, transcriptomic and proteomic profiling data present in the literature, and represents the first effort to derive a consensus molecular model of HCC through analysis of these different types of data. Although these datasets originated from different patient cohorts, presented integrative analysis offers the opportunity to explore common key pathway dependencies of HCC. Starting with the initial generation of genomics and whole exome sequencing data, previous high-throughput studies have brought forth different lists of dysregulated genes, depending on the type of data evaluated. Dysregulated genes may affect different parts of a pathway. Therefore, a pathway-based approach when evaluating different types of high-throughput data offers the ability to assess the pathways most commonly affected in a given cancer. Additionally, the integrative analysis in our study encompasses a large number of patient samples.

Table 2 Prognostic value of the 9 dysregulated genes associated with the 3 common dysregulated pathways (EGFR, epidermal growth factor, β1-integrin and axon guidance) among the 4 types of data in obtained with KMplotter

Gene	Modulation in the 19 HCC papers	Probe-ID	HR	CI	Log-Rank *P* value	Median survival low (mo)	Median survival high (mo)	Estradiol gene modulation predicted by CTD
COL2A1	Up	1280	1.49	1.05-2.11	0.0229	61.7	54.1	N/A
FGA	Down	2243	0.52	0.35-0.77	0.0009	49.7	70.5	+
FGG	Down	2266	0.56	0.39-0.79	0.0009	38.3	70.5	+
LAMC1	Up	3915	1.43	0.98-2.09	0.06	56.5	38.3	N/A
CDK5	Up	1020	1.85	1.22-2.81	0.0035	81.9	6.2	N/A
EPHB1	Down	2047	0.72	0.048-1.08	0.1135	54.1	70.5	N/A
RPS6KA3	Up	6197	1.2	0.8-1.78	0.3743	54.1	56.5	-
EGFR	Down	1956	0.61	0.43-0.89	0.0085	31	70.5	+
ITGB1	Up	3688	1.37	0.95-1.97	0.0924	82.9	49.7	N/A

CTD based prediction identified Estradiol to efficiently affect the expression of the 4/9 genes based on their hazard ratios values. HR: Hazard ratios; HCC: Hepatocellular carcinoma; CI: Confidence interval; N/A: Not applicable.

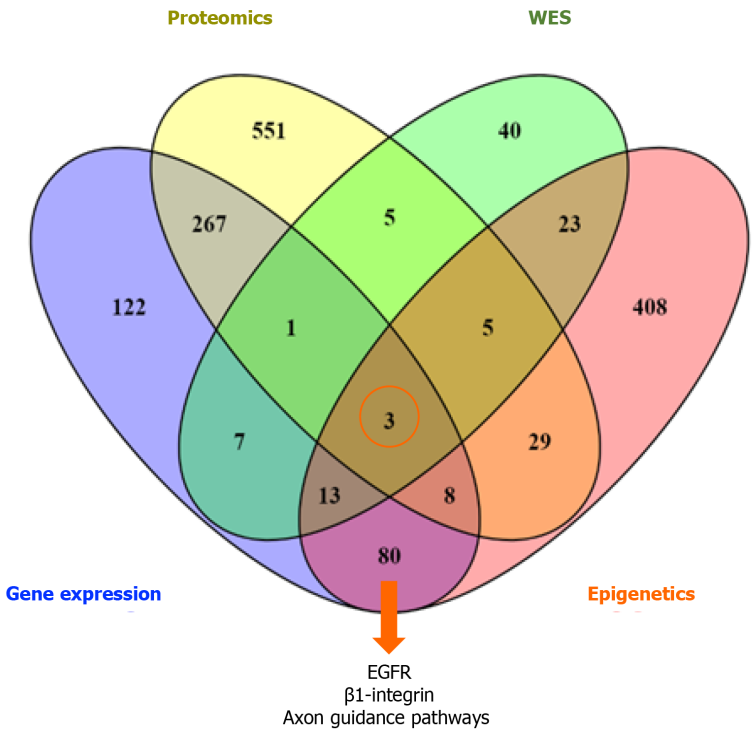

Figure 2 Venn diagram shows the three common pathways (EGFR, epidermal growth factor, β1-integrin, and axon guidance pathways) across the four different types of data.

Using this integrative approach, we confirm the importance of EGFR, β1-integrin and axon guidance as pathways critical in hepatocarcinogenesis. EGFR activates the signaling cascades of the Ras/Raf/MAPK and mTOR pathways, two pathways that were identified as key to HCC pathogenesis in the TCGA study[6]. The identification of β1-integrin as being commonly dysregulated in HCC is novel, and its significance is confirmed through its consistent dysregulation across types of data. β1-integrin is a cell surface receptor that senses the extracellular matrix, thereby modulating the hallmarks of cancer such as proliferative signaling with continuous activated cell replication, evasion of growth suppressors, resistance to angiogenesis as well as cancer cell invasion and metastasis[14].

Table 3 Modulation of the 9 dysregulated genes associated with the 3 common dysregulated pathways (EGFR, epidermal growth factor, β1-integrin and axon guidance) identified in the 19 hepatocellular carcinoma gene expression papers. Their genetic alteration in hepatocellular carcinoma and their mechanism in cancer are reported

Gene	Modulation in the 19 HCC papers	PMID	Mutation in HCC (PMID)	Role in cancer (PMID)
COL2A1	Up (2/19)	23800896/25666192	(rs3917) polymorphism is associated with higher risk of HCC (21665180)	COL2A1 promotes migration in HCC (29858962)
FGA	Down (9/19)	21320499/23800896/25093504/25536056/25141867/25376302/25666192/25645722/25666192	Deleted in HCC patients (27511114)	FGA is a positive predictor of survival in gastric cancer patients (15756001)
FGG	Down 8/19	21320499/23800896/25093504/25536056/25141867/25376302/25645722/24498002	Allelic loss (16980951)	FGG is involved in amino acid and redox metabolism pathway in HCC (28089356)
LAMC1	Up (4/19)	23800896/25536056/25141867/25645722	Not identified	LAMC1 promotes tumor cell invasion and migration in HCC (28928891)
CDK5	Up (2/19)	25141867/25376302	Not identified	CDK5 promotes proliferation in HCC (29312535)
EPHB1	Down (2/19)	23800896/25141867	Missense mutation (19469653)	EPHB1 inhibits cell migration(22242939)
RPS6KA3	Up 1/19	25141867	Somatic mutation and copy number variations (22561517)	RPS6KA3 increases cell proliferation (15833840)
EGFR	Down (2/19)	19098997/25141867	Missense mutation (26436086)	EGFR promotes cell adhesion (31465839)
ITGB1	Up (1/19)	25141867	Somatic number variations (24512821)	ITGB1 promotes migration (30664185)

HCC: Hepatocellular carcinoma.

Ras/Raf/MAPK and mTOR are established pathways in hepatocarcinogenesis, and are integrin-dependent signaling pathways[15]. Additionally, β1-integrin is known to crosstalk with EGFR. In fact, the downregulation of β1-integrin was found to decrease phosphorylation of EGFR and c-Met in hepatocytes during liver regeneration[16]. A synergistic relationship between integrins and EGFR has also been demonstrated in tumor progression[17]. The finding of axon guidance pathway-related proteins as being dysregulated across types of data, thereby establishing consistent dysregulation of this pathway in HCC, is also novel. Netrin-1 is the best studied protein in the axon guidance pathway, and is known to be overexpressed in various cancers[13]. It is responsible for regulation of apoptosis, with increased presence of netrin-1 leading to inhibition of apoptosis. The tumor suppressor p53, frequently mutated in the TCGA HCC study, regulates the cell cycle through netrin-1. The axon guidance pathway has previously been identified as a pathway that is significantly mutated in HCC based on integration of all genomic data in HCC[18]. This analysis revealed mutations along the axon guidance pathway as being prognostic of a higher rate of HCC metastasis. We were able to additionally validate the prognostic importance of dysregulated proteins in these pathways proteins using TCGA data.

HCC is a cancer that develops in the context of various chronic liver diseases, which may influence the molecular characteristics of HCC. Additionally, the underlying cirrhosis and liver dysfunction that are often concurrent may influence HCC development and behavior[2]. Patients are often diagnosed at an advanced stage of disease, when it is too late for curative treatment. A unique consideration in HCC is the inability to tolerate hepatotoxic chemotherapy in patients with liver dysfunction, as it is often patients with cirrhosis who develop HCC[19,20]. Therefore, liver function must be considered prior to, during, and after any form of treatment for HCC.

Thus, especially for HCC, it has been suggested that a multi-pronged approach to HCC therapy jointly targeting different pathways be adopted.

Omics technologies are essential in the progress towards elucidating the molecular basis of HCC. The current study represents the largest integration of all publicly available genomic, gene expression, methylation, miRNA and proteomic data in HCC, covering 85 studies and 3355 patient sample profiles. We identified consistently deregulated pathways associated with hepatocarcinogenesis across different types of data using integrative analysis tools, thereby confirming the importance of these genes in HCC pathogenesis. EGFR (activator of Ras/Raf/MAPK and mTOR) and β1-integrin (also modulator of the

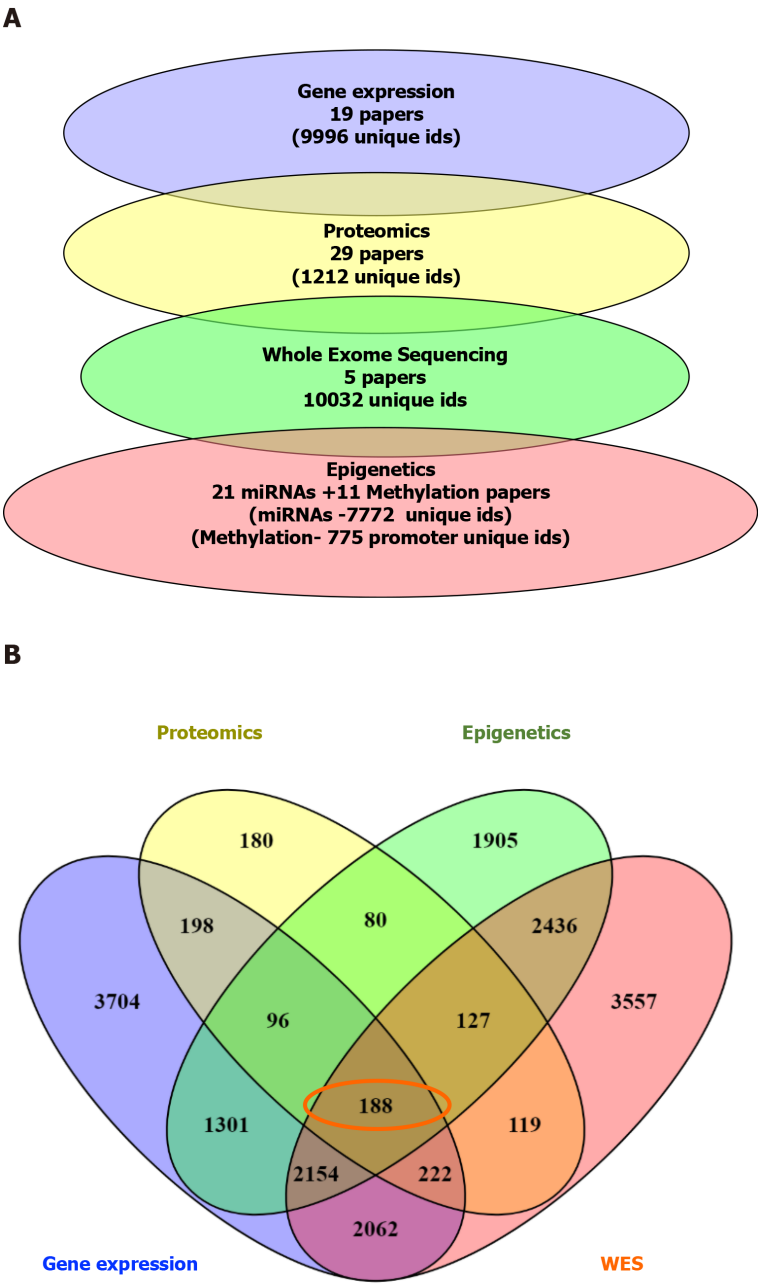

Figure 3 From the previous list of 188 common dysregulated elements in all different layers of data. A: Number of genes/proteins identified in each data type; B: Venn diagram showing the 188 genes identified as commonly deregulated across the 4 different type of data.

aforementioned pathways) were clearly identified as pivotal to HCC[5,21-23]. This is in keeping with the efficacy of the Ras/Raf/MAPK inhibitors sorafenib and regorafenib in HCC[24].

Even beyond this, we found these consistently deregulated genes across pathways to be appropriately modulated by estradiol. HCC is less common in women, and there have been clinical studies demonstrating that hormone therapy and female sex are protective against HCC as described earlier in this thesis.

Other integrative multi-omics studies have been recently performed for other tumors with high mortality such as breast and ovarian cancer[6,25]. Several breast cancer studies emphasizing how data integration of genomic/transcriptomic and proteomic has improved the molecular characterization of subtypes of breast cancer and elucidate its heterogeneity and its interaction with the microenvironment and aggressiveness[26,27]. A single source of data was used in the ovarian cancer multi-omics mathematical integration performed by Bhardwaj *et al*[25]. Copy number variation gene expression and methylation data from TCGA data portal were integrated using mathematical algorithm and identified 32 co-expressed genes and 6 pathways associated with survival.

The main limitation of our study is the different patient samples represented by the various types of data. Nonetheless, there is a large amount of high-throughput data, which allowed us to detect pathway dependency patterns that are compatible with the current HCC literature. Additionally, HCC tumors

arise in the setting of various chronic liver diseases. We could not assess for etiology-specific genes and pathways in this study, given that the clinical and genetic data to evaluate these differences were not fully available for all the studies. Therefore, we could only evaluate gene differences over whole datasets, rather than individual patients, due not complete individual annotation of the samples available on GEO for each specific dataset. The HCC samples in this integrative analysis all came from patients who had undergone hepatectomy. There were no specimens from patients who were candidates for ablation therapy (early stage), those who were undergoing liver transplantation, or those with advanced HCC. One might anticipate that the molecular features of such tumors differ, given the different stages of HCC captured, but there is unfortunately scarcity of data in this regard.

CONCLUSION

In conclusion, our study represents the largest integrative analysis of all publicly available data in HCC, spanning different types of high-throughput data. Pathway enrichment analysis elucidated EGFR, β1-integrin and axon guidance as pathway dependencies in HCC. These are proteins known to serve as master regulators of key pathways in HCC such as Ras/Raf/MAPK, Wnt/β-catenin and mTOR[28], and may serve as potential overarching therapeutic targets in HCC. The axon guidance pathway was identified as being of potential importance to HCC for the first time, with prognostic value suggested in patient sample validation with TCGA. Estradiol affects a large number of deregulated genes across data with appropriate modulation and may be a therapeutic agent that helps in HCC. A combined therapeutic approach conjointly targeting different pathways may be more optimal in the treatment of HCC, especially when underlying hepatic dysfunction compromises the ability to tolerate optimal chemotherapeutic doses.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is highly heterogeneous, difficult to characterize and the molecular basis of HCC has been elusive.

Research motivation

The Cancer Genome Atlas is a large-scale project that has enabled improved characterization of cancers with several layers of data. Elucidating the layers of data in a disease can provide additional insights into the pathways that drive cancer.

Research objectives

A novel integrative approach of all publicly available high-throughput data from patient HCC tumors was used to delineate critical pathway dependencies in HCC.

Research methods

A comprehensive analysis and characterization of all publicly available genomic, gene expression, methylation, miRNA and proteomic data in HCC covered 85 studies and 3355 patient sample profiles and identified the key overlapping dysregulated genes and pathways affected.

Research results

We identified the prognostic value of these genes in HCC genes, specifically with Netrin and Slit3 being novel proteins of prognostic importance to HCC.

Research conclusions

Our large integrative analysis of all publicly available data in HCC and our pathway enrichment analysis has elucidated epidermal growth factor, β1-integrin, and axon guidance as pathway dependencies in HCC.

Research perspectives

Based on our integrative analysis, epidermal growth factor, and β1-integrin are master regulators that could be considered as potential therapeutic targets in HCC.

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FOOTNOTES

Author contributions: Bhat M, Pasini E, Kotlyar M and Jurisica I study design, and writing of the manuscript; Bhat M, Pasini E, and Angeli M data collection, analysis and compilation; Ghanekar A, Jurisica I and Bhat M input into study design, data interpretation and final manuscript. All authors approved the final version of the manuscript.

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Conflict-of-interest statement: The authors do not have any conflict of interest to declare.

Data sharing statement: Technical appendix, statistical code available from the corresponding author at mamatha.bhat@uhn.ca all data sets are publicly available.

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REFERENCES

1 **Whittaker S**, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene* 2010; **29**: 4989-5005 [PMID: 20639898 DOI: 10.1038/onc.2010.236]
2 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226 DOI: 10.1053/j.gastro.2007.04.061]
3 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]
4 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
5 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
6 **Cancer Genome Atlas Research Network**. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341. e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]
7 **Wilk G**, Braun R. Integrative analysis reveals disrupted pathways regulated by microRNAs in cancer. *Nucleic Acids Res* 2018; **46**: 1089-1101 [PMID: 29294105 DOI: 10.1093/nar/gkx1250]
8 **Srivastava A**, Kumar S, Ramaswamy R. Two-layer modular analysis of gene and protein networks in breast cancer. *BMC Syst Biol* 2014; **8**: 81 [PMID: 24997799 DOI: 10.1186/1752-0509-8-81]
9 **Zhang H**, Liu T, Zhang Z, Payne SH, Zhang B, McDermott JE, Zhou JY, Petyuk VA, Chen L, Ray D, Sun S, Yang F, Chen L, Wang J, Shah P, Cha SW, Aiyetan P, Woo S, Tian Y, Gritsenko MA, Clauss TR, Choi C, Monroe ME, Thomas S, Nie S, Wu C, Moore RJ, Yu KH, Tabb DL, Fenyö D, Bafna V, Wang Y, Rodriguez H, Boja ES, Hiltke T, Rivers RC, Sokoll L, Zhu H, Shih IM, Cope L, Pandey A, Zhang B, Snyder MP, Levine DA, Smith RD, Chan DW, Rodland KD; CPTAC Investigators. Integrated Proteogenomic Characterization of Human High-Grade Serous Ovarian Cancer. *Cell* 2016; **166**: 755-765 [PMID: 27372738 DOI: 10.1016/j.cell.2016.05.069]
10 **Tokar T**, Pastrello C, Rossos AEM, Abovsky M, Hauschild AC, Tsay M, Lu R, Jurisica I. mirDIP 4.1-integrative database of human microRNA target predictions. *Nucleic Acids Res* 2018; **46**: D360-D370 [PMID: 29194489 DOI: 10.1093/nar/gkx1144]

11 **Kotlyar M**, Pastrello C, Sheahan N, Jurisica I. Integrated interactions database: tissue-specific view of the human and model organism interactomes. *Nucleic Acids Res* 2016; **44**: D536-D541 [PMID: 26516188 DOI: 10.1093/nar/gkv1115]

12 **Rahmati S**, Abovsky M, Pastrello C, Jurisica I. pathDIP: an annotated resource for known and predicted human gene-pathway associations and pathway enrichment analysis. *Nucleic Acids Res* 2017; **45**: D419-D426 [PMID: 27899558 DOI: 10.1093/nar/gkw1082]

13 **Arakawa H**. Netrin-1 and its receptors in tumorigenesis. *Nat Rev Cancer* 2004; **4**: 978-987 [PMID: 15573119 DOI: 10.1038/nrc1504]

14 **Davis AP**, Grondin CJ, Johnson RJ, Sciaky D, King BL, McMorran R, Wiegers J, Wiegers TC, Mattingly CJ. The Comparative Toxicogenomics Database: update 2017. *Nucleic Acids Res* 2017; **45**: D972-D978 [PMID: 27651457 DOI: 10.1093/nar/gkw838]

15 **Griffiths GS**, Grundl M, Leychenko A, Reiter S, Young-Robbins SS, Sulzmaier FJ, Caliva MJ, Ramos JW, Matter ML. Bit-1 mediates integrin-dependent cell survival through activation of the NFkappaB pathway. *J Biol Chem* 2011; **286**: 14713-14723 [PMID: 21383007 DOI: 10.1074/jbc.M111.228387]

16 **Speicher T**, Siegenthaler B, Bogorad RL, Ruppert R, Petzold T, Padrissa-Altes S, Bachofner M, Anderson DG, Koteliansky V, Fässler R, Werner S. Knockdown and knockout of β1-integrin in hepatocytes impairs liver regeneration through inhibition of growth factor signalling. *Nat Commun* 2014; **5**: 3862 [PMID: 24844558 DOI: 10.1038/ncomms4862]

17 **Ivaska J**, Heino J. Cooperation between integrins and growth factor receptors in signaling and endocytosis. *Annu Rev Cell Dev Biol* 2011; **27**: 291-320 [PMID: 21663443 DOI: 10.1146/annurev-cellbio-092910-154017]

18 **Zhang Y**, Qiu Z, Wei L, Tang R, Lian B, Zhao Y, He X, Xie L. Integrated analysis of mutation data from various sources identifies key genes and signaling pathways in hepatocellular carcinoma. *PLoS One* 2014; **9**: e100854 [PMID: 24988079 DOI: 10.1371/journal.pone.0100854]

19 **Mittal S**, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]

20 **Fitzmorris P**, Shoreibah M, Anand BS, Singal AK. Management of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2015; **141**: 861-876 [PMID: 25158999 DOI: 10.1007/s00432-014-1806-0]

21 **Zhu AX**, Abrams TA, Miksad R, Blaszkowsky LS, Meyerhardt JA, Zheng H, Muzikansky A, Clark JW, Kwak EL, Schrag D, Jors KR, Fuchs CS, Iafrate AJ, Borger DR, Ryan DP. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. *Cancer* 2011; **117**: 5094-5102 [PMID: 21538343 DOI: 10.1002/cncr.26165]

22 **Zhou Q**, Lui VW, Yeo W. Targeting the PI3K/Akt/mTOR pathway in hepatocellular carcinoma. *Future Oncol* 2011; **7**: 1149-1167 [PMID: 21992728 DOI: 10.2217/fon.11.95]

23 **Llovet JM**, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat Rev Clin Oncol* 2015; **12**: 436 [PMID: 26099984 DOI: 10.1038/nrclinonc.2015.121]

24 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]

25 **Bhardwaj A**, Van Steen K. Multi-omics Data and Analytics Integration in Ovarian Cancer. In: Maglogiannis I, Iliadis L, Pimenidis E, editors. *Artificial Intelligence Applications and Innovations* 2020; 347-57 [DOI: 10.1007/978-3-030-49186-4_29]

26 **Wagner J**, Rapsomaniki MA, Chevrier S, Anzeneder T, Langwieder C, Dykgers A, Rees M, Ramaswamy A, Muenst S, Soysal SD, Jacobs A, Windhager J, Silina K, van den Broek M, Dedes KJ, Rodríguez Martínez M, Weber WP, Bodenmiller B. A Single-Cell Atlas of the Tumor and Immune Ecosystem of Human Breast Cancer. *Cell* 2019; **177**: 1330-1345. e18 [PMID: 30982598 DOI: 10.1016/j.cell.2019.03.005]

27 **Bhatia S**, Monkman J, Blick T, Duijf PH, Nagaraj SH, Thompson EW. Multi-Omics Characterization of the Spontaneous Mesenchymal-Epithelial Transition in the PMC42 Breast Cancer Cell Lines. *J Clin Med* 2019; **8** [PMID: 31430931 DOI: 10.3390/jcm8081253]

28 **Bhat M**, Sonenberg N, Gores GJ. The mTOR pathway in hepatic malignancies. *Hepatology* 2013; **58**: 810-818 [PMID: 23408390 DOI: 10.1002/hep.26323]

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ORIGINAL ARTICLE

Case Control Study

Association of interferon lambda-4 rs12979860 polymorphism with hepatocellular carcinoma in patients with chronic hepatitis C infection

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Abstract

BACKGROUND

Hepatitis C virus (HCV) infection is a public health concern worldwide. Several factors, including genetic polymorphisms, may be evolved in the progression of HCV infection to liver diseases. Interferon lambdas (IFNLs) modulate the immune response during viral infections. IFNLs induce antiviral activity, interfering in the viral replication by promoting the expression of several genes that regulate immunological functions. The interferon lambda-4 (*IFNL4*) rs12979860 polymorphism, which is characterized by a C to T transition in intron 1, is associated with spontaneous and treatment-induced clearance of HCV infection and may play a role in the development of HCV-associated liver diseases, including hepatocellular carcinoma (HCC).

AIM

To investigate the association of *IFNL4* rs12979860 polymorphism with fibrosis, cirrhosis, and HCC in patients with chronic HCV infection.

METHODS

This study was comprised of 305 chronic HCV-infected patients (53 fibrosis, 154 cirrhosis, and 98 HCC cases). The control group was comprised of 260 HCV-negative healthy individuals. The *IFNL4* rs12979860 polymorphism was genotyped using the TaqMan assay. Fibrosis was diagnosed based on liver biopsy findings, while cirrhosis was diagnosed through clinical, laboratory, anatomo-

pathological, and/or imaging data. HCC was diagnosed through imaging tests, tumor, and/or anatomopathological markers.

RESULTS

The T allele was observed in the three groups of patients (fibrosis, cirrhosis, and HCC) at a significantly higher frequency when compared with the control group ($P = 0.047$, $P < 0.001$, and $P = 0.01$, respectively). Also, genotype frequencies presented significant differences between the control group and cirrhosis patients ($P < 0.001$) as well as HCC patients ($P = 0.002$). The risk analysis was performed using the codominant and dominant T allele models. In the codominant model, it was observed that the CT genotype showed an increased risk of developing cirrhosis in comparison with the CC genotype [odds ratio (OR) = 2.53; 95% confidence interval (CI): 1.55-4.15; $P < 0.001$] as well as with HCC (OR = 2.54; 95%CI: 1.44-4.56; $P = 0.001$). A similar result was observed in the comparison of the TT *vs* CC genotype between the control group and cirrhosis group (OR = 2.88; 95%CI: 1.44-5.77; $P = 0.001$) but not for HCC patients. In the dominant T allele model, the CT + TT genotypes were associated with an increased risk for progression to cirrhosis (OR = 2.60; 95%CI: 1.63-4.19; $P < 0.001$) and HCC (OR = 2.45; 95%CI: 1.42-4.31; $P = 0.001$).

CONCLUSION

These findings suggest that the T allele of *IFNL4* rs12979860 polymorphism is associated with the development of cirrhosis and HCC in chronic HCV-infected patients.

Key Words: Hepatitis C; Hepatitis C virus; Cirrhosis; Hepatocellular carcinoma; Genetic polymorphism; Interferon-lambda

Core Tip: Hepatitis C virus (HCV) infection is a major public health problem worldwide as the infection progresses to severe chronic liver diseases in many patients. Interferon lambdas modulate the immune responses against infections, including the antiviral activity by promoting the expression of several genes related to immunological functions. The interferon lambda-4 rs12979860 (C/T) polymorphism, which is associated with spontaneous and treatment-induced clearance of HCV, plays a pivotal role in the host response to HCV-associated liver diseases. In this case-control study, the rs12979860 T allele was found to be associated with the development of cirrhosis and hepatocellular carcinoma in chronic HCV-infected patients.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a public health concern worldwide as it is associated with increased morbidity and mortality[1,2]. HCV, a hepatotrophic virus, is the etiological factor for chronic hepatitis C. Patients with HCV infection can develop cirrhosis and hepatocellular carcinoma (HCC) and may need liver transplantation[2-4]. According to the World Health Organization report on viral hepatitis, 71 million people were infected with hepatitis C in 2015[2].

Generally, acute HCV infections are clinically silent infections. Among the patients with HCV infection, 15%-45% can eliminate the virus spontaneously, with the highest recovery rates observed in children and young women[5]. However, a vast majority of infected patients develop chronic hepatitis C, which is characterized by the persistence of HCV in the serum for more than 6 mo. Chronic HCV infection is associated with slow progression, and the patients may remain asymptomatic for several decades. Thus, the persistence of HCV in the organism can cause continuous damage to the liver and can progress to fibrosis, cirrhosis, and HCC[5,6].

HCC, which accounts for 80% of all primary liver cancers, is associated with high mortality rates. Globally, HCC is the third leading cause of cancer-related deaths. HCC is a complex disease with a variety of etiologies and may be associated with different risk factors, such as chronic hepatitis B virus (HBV) and HCV infections, alcoholic liver disease, and nonalcoholic steatohepatitis[7,8]. HCV infection,

which is the second most common risk factor for HCC, accounts for 10%-25% of all HCC cases. Additionally, 80%-90% of HCC cases are reported in patients with cirrhosis[9,10].

The pathogenesis of HCV infection and its progression to chronic liver disease vary among individuals. Several factors, including viral, environmental, and host characteristics, such as age, sex, ethnicity, and genetic factors, contribute to the pathogenesis of HCV[11]. The immune system-related genes, such as interferon lambdas (IFN-λs), are directly related to modulate viral infections with the ability to induce antiviral activity in target cells and interfere with HCV replication within the host cells. The binding of IFN-λ to its receptor activates the signal transducer and activator of transcription phosphorylation-dependent signaling cascade, inducing hundreds of IFN-stimulated genes and consequently regulating various immune functions[12-14].

The interferon lambda-3 gene (*IFNL3*), which is located on chromosome 19q13.13, encodes IFN-λ3 protein, a cytokine with antiviral properties. Genome-wide association studies have demonstrated the association of single nucleotide polymorphisms, such as rs12979860 and rs8099917, near the *IFNL3* gene (formerly known as interleukin-28B gene; *IL28B*), both with spontaneous virus elimination after acute infection and with sustained virological response in patients with chronic hepatitis C treated with pegylated interferon plus ribavirin combination therapy[15-18].

Prokunina-Olsson *et al*[19] demonstrated that the rs12979860 polymorphism, commonly referred as an *IL28B* or *IFNL3* variant, is in an independent loci and should be called an interferon lambda-4 (*IFNL4*) variant. The *IFNL4* gene is controlled by rs368234815 ΔG-TT polymorphism, in which the ΔG allele creates an open reading frame for *IFNL4*, while the TT allele does not. Furthermore, the ΔG allele (rs368234815) is reported to be in linkage disequilibrium with the T allele of rs12979860 polymorphism [13,19].

The rs12979860 polymorphism has a relevant and well-known role in the spontaneous and treatment-induced clearance of HCV infection[20]. However, the importance of this polymorphism in the progression of HCV-associated liver diseases is still unclear. Therefore, the objective of our study was to investigate the potential role of the variants from *IFNL4* rs12979860 polymorphism in the progression to hepatic fibrosis, cirrhosis, and HCC in chronic HCV-infected patients.

MATERIALS AND METHODS

Study population

This case-control study was conducted using a convenience sampling strategy. The case group was comprised of 305 patients who visited the outpatient clinic of the Gastroenterology-Hepatology Service of the Hospital de Clínicas de Porto Alegre in Brazil. HCV-positive patients diagnosed with fibrosis, cirrhosis, or HCC were included in the case group. Fibrosis (METAVIR F1-F3) was diagnosed based on liver biopsy findings, while cirrhosis was diagnosed based on liver biopsy or clinical evidence, such as liver imaging (abdominal ultrasonography, computed tomography, and magnetic resonance) abnormalities or endoscopic findings as well as current or past clinical evidence of decompensation, including Child-Pugh B or C classification (score of > 6), ascites on physical examination, hepatic encephalopathy, or variceal bleeding. HCC was diagnosed through liver biopsy (64/98; 65.3%) or in cirrhotic patients through dynamic computed tomography or magnetic resonance by the presence of a nodule of at least 1 cm featuring arterial phase enhancement with decreased enhancement during the portal venous phase as recommended by international guidelines. Patients with HCV/human immunodeficiency virus and/or HCV/HBV coinfection were excluded as well as patients with other causes of liver diseases such as HBV, metabolic associated fatty liver disease, alcohol abuse (more than 20 or 30 g daily consumption of ethanol for females and males, respectively), and/or hemochromatosis. The control group was comprised of 260 samples obtained from the donors at the Hospital de Clínicas de Porto Alegre blood bank. As Brazilian laws for blood donation requires, all have been tested negative for HBV, HCV, human immunodeficiency virus, syphilis, and Chagas disease. This study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (protocol number: 15-0126). All participants provided their written informed consent to participate in the study.

Molecular analysis

DNA was extracted from the blood samples using the salting-out method as described previously[21]. The polymorphism was genotyped using the validated pre-designed real-time PCR TaqMan® Assays (Applied Biosystems Inc., Foster City, CA, United States; catalog 4351376, assay ID: C___7820464_10) in the StepOnePlusTM Real-Time PCR Systems (Applied Biosystems Inc.). PCR was performed in an 18 μL reaction volume containing 10 mmol/L Tris-HCl (pH 8.5), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.0625 mmol/L dNTPs, 0.25 μM of each primer, 0.045 μM of each probe, 1 U Taq DNA polymerase (Cenbiot Enzimas, Porto Alegre, Brazil), and 1 μL extracted DNA (10-200 ng). The PCR conditions were as follows: 95 °C for 10 min (initial DNA denaturation), followed by 40 cycles of 95 °C for 15 s (denaturation) and 60 °C for 1 min (annealing and extension).

Statistical analyses

All statistical analyses were performed using SPSS® software (Statistical Package for the Social Sciences 17.0 version, Chicago, IL, United States). The normal distribution of the quantitative variables was examined using the Kolmogorov-Smirnov test with Lilliefors correction. The quantitative variables, which were expressed as mean ± SD, were analyzed using analysis of variance, followed by Tukey post-hoc test. For the categorical variables, the frequencies were calculated and expressed as percentages. Gene frequencies were determined by direct allele counting. Hardy-Weinberg equilibrium (HWE) deviation and the gene frequencies between groups were compared using the Chi-square test. Yates' correction for continuity was used to analyze the 2 × 2 contingency tables. Odds ratio (OR) was estimated with 95% confidence interval (CI). The differences were considered significant at $P < 0.05$ (two-tailed). Potential confounding factors were entered in the logistic regression models based on statistical criteria (only if the variable was associated with the study factor and with the outcome at $P < 0.20$). The statistical methods used in this study were reviewed by Dr. D. Simon from the Human Molecular Genetics Laboratory, Universidade Luterana do Brasil (Canoas, Brazil).

RESULTS

The sociodemographic and clinical characteristics of patients are described in Table 1. Patients were stratified into the following three groups: Fibrosis ($n = 53$), cirrhosis ($n = 154$), and HCC ($n = 98$). The mean age of the patients was 59.85 ± 8.83 years, with a statistically significant difference among the groups studied ($P = 0.019$). A significant statistical difference ($P = 0.024$) was also observed in the frequency of males in the HCC group (58.2%) when compared to the fibrosis (37.7%) and cirrhosis groups (43.5%). The mean value of body mass index presented a statistically significant difference between the groups with cirrhosis and HCC (27.80 ± 5.39 and 26.34 ± 4.15 kg/m^2, respectively; $P = 0.038$). Blood transfusion was the most frequent possible infection source among patients (41.0%). The frequencies of HCV 1 and 3 genotypes, which were the most common, were 40.7% and 36.7%, respectively.

Table 2 shows the allele and genotype frequencies of the *IFNL4* rs12979680 polymorphism in the patient and control groups. The success rate for genotyping *IFNL4* rs12979680 polymorphism was 100% in all studied groups. Statistically significant differences were observed regarding the allele frequencies, in which the frequency of the T allele was significantly higher in the three groups of patients analyzed when compared to the controls: [fibrosis group *vs* control group (OR = 1.57; 95%CI: 1.03-1.68; $P = 0.047$), cirrhosis group *vs* control group (OR = 1.75; 95%CI: 1.30-2.36; $P < 0.001$), and HCC group *vs* control group (OR = 1.57, 95%CI: 1.11-2.23; $P = 0.01$)].

Compared with those in the control group, the *IFNL4* genotype frequencies were significantly higher in the cirrhotic and ($P < 0.001$) HCC groups ($P = 0.002$). The genotype distribution in the control and fibrosis groups was in agreement with those expected from HWE ($P = 0.81$ and $P = 0.88$, respectively). In contrast, the genotype frequencies in the cirrhosis and HCC groups deviated from those expected from HWE ($P = 0.02$ and $P = 0.01$, respectively). When the genotype distribution was analyzed in the total sample of patients ($n = 305$), deviations from HWE were maintained ($P = 0.001$).

The risk of developing fibrosis, cirrhosis, and HCC was calculated using the following two genetic models: Codominant and dominant T allele models (Table 3). In the codominant model, it was observed that the CT *vs* CC genotype conferred an increased risk of developing cirrhosis in HCV patients when compared with the control group (OR = 2.53; 95%CI: 1.55-4.15; $P < 0.001$). Additionally, the CT *vs* CC genotype conferred an increased risk for HCC (OR = 2.54; 95%CI: 1.44-4.56; $P = 0.001$). A similar result was observed in the comparison of the TT *vs* CC genotype between cirrhosis patients and controls (OR = 2.88; 95%CI: 1.44-5.77; $P = 0.001$) but not for HCC. In the dominant T allele model, the CT + TT genotypes conferred an increased risk of developing cirrhosis (OR = 2.60; 95%CI: 1.63-4.19; $P < 0.001$) and HCC (OR = 2.45; 95%CI: 1.42-4.31; $P = 0.001$) when compared with the CC genotype. The observed associations remained significant when logistic regression models were analyzed controlling for potential confounding factors (data not shown).

Table 4 presents the distribution of the *IFNL4* rs12979680 polymorphism genotypes regarding clinical features of HCC patients. A significantly higher frequency of the T allele in the dominant T allele model was observed among patients with HCV genotypes 1 and 3 with a frequency of 92% and 67%, respectively ($P = 0.017$). In addition, a higher frequency of the TT genotype was observed among patients with hepatic encephalopathy ($P = 0.03$).

DISCUSSION

This study investigated the association of the *IFNL4* rs12979860 polymorphism with the development of fibrosis, cirrhosis, and HCC among patients with chronic HCV infection. The frequency of the T allele in the case group was higher than that in the control group. Additionally, the risk analyses indicated that

Table 1 Sociodemographic and clinical features of chronic hepatitis C virus positive patients

Characteristics	Total, *n* = 305	Fibrosis, *n* = 53	Cirrhosis, *n* = 154	HCC, *n* = 98	*P* value
Age in yr	59.85 ± 8.83	57.89 ± 10.43	59.29 ± 8.43	61.78 ± 8.22	0.019
Male	144 (47.2)	20 (37.7)	67 (43.5)	57 (58.2)	0.024
Ethnicity, Caucasian	218 (71.5)	35 (66.1)	110 (71.4)	73 (74.5)	0.547
BMI in kg/m²	27.08 ± 4.85	26.39 ± 4.14	27.80 ± 5.39	26.34 ± 4.15	0.038
Level of education					0.366
Completed primary education or less	196 (62.0)	31 (56.6)	100 (62.3)	65 (64.3)	
Secondary or higher education	102 (24.9)	20 (34.0)	51 (25.3)	31 (19.4)	
Smoker	59 (19.3)	16 (30.2)	31 (20.1)	12 (12.2)	0.001
Alcohol consumption					0.004
No	260 (85.2)	49 (92.5)	137 (89.0)	74 (75.5)	
Former	45 (14.8)	4 (7.5)	17 (11.0)	24 (24.5)	
Illicit drug use					0.164
No	243 (79.7)	43 (81.1)	122 (79.2)	78 (79.6)	
Yes	9 (3.0)	4 (7.5)	4 (2.6)	1 (1.0)	
Former user	53 (17.4)	6 (1.1)	28 (18.2)	19 (19.4)	
Coffee drinker	213 (69.8)	39 (73.6)	112 (72.7)	62 (63.3)	0.226
Age at infection of HCV in yr	27.43 ± 9.75	28.47 ± 9.12	27.48 ± 9.77	26.64 ± 10.26	0.735
Age at diagnosis of HCV in yr	49.11 ± 11.11	46.88 ± 12.99	49.17 ± 10.97	50.24 ± 10.11	0.223
HCV infection *via* blood transfusion	125 (41.0)	24 (45.3)	64 (41.6)	37 (37.8)	0.706
HCV-RNA as log_{10}UI/mL	6.05 ± 0.86	-	6.11 ± 0.87	5.86 ± 0.78	0.141
HCV genotypes					0.060
1	124 (40.7)	-	86 (55.8)	38 (38.8)	
2	7 (2.3)	-	4 (2.6)	3 (3.1)	
3	112 (36.7)	-	61 (39.6)	51 (52.0)	
Antiviral treatment	178 (58.4)	-	115 (74.7)	63 (64.3)	0.077
Diabetes	85 (27.9)	-	50 (32.5)	35 (35.7)	0.595
Steatosis	24 (7.9)	-	13 (8.4)	11 (11.2)	0.431
Ascites	66 (21.6)	-	31 (20.1)	35 (35.7)	0.005
Portal hypertension	146 (47.9)	-	72 (46.8)	74 (75.5)	< 0.001
Esophageal varices	156 (51.1)	-	91 (59.0)	65 (66.3)	0.231
Upper gastrointestinal bleeding	49 (16.0)	-	26 (16.9)	23 (23.5)	0.184
Spontaneous bacterial peritonitis	13 (4.3)	-	7 (4.5)	6 (6.1)	0.568
Hepatic encephalopathy	24 (7.9)	-	13 (8.4)	11 (11.2)	0.431
Child-Pugh					0.083
A	137 (44.9)	-	95 (61.7)	42 (42.9)	
B	43 (14.1)	-	28 (18.2)	15 (15.3)	
C	9 (3.0)	-	3 (1.9)	6 (6.1)	
Number of tumors					
1		-	-	62 (63.37)	
2		-	-	17 (17.35)	
≥ 3		-	-	18 (18.37)	

Tumor size in cm		-	-	2.8 ± 1.81	
Portal vein thrombosis		-	-	10 (10.20)	
Extrahepatic metastases		-	-	7 (7.14)	
Liver transplantation		-	-	47 (47.96)	
Deaths	14 (4.59)	-	8 (5.19)	6 (6.12)	0.754

Characteristics expressed as number and percentage or mean ± SD. BMI: Body mass index; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

Table 2 Allele and genotype frequencies of interferon lambda-4 rs12979860 polymorphism in patients with hepatitis C virus-associated liver diseases and healthy control subjects

rs12979860	Control, *n* = 260	Total patients, *n* = 305	Fibrosis, *n* = 53	Cirrhosis, *n* = 154	HCC, *n* = 98	*P* value				
						Fibrosis *vs* Control	Cirrhosis *vs* Control	HCC *vs* Control	Fibrosis *vs* Cirrhosis	Cirrhosis *vs* HCC
Allele						0.047	< 0.001	0.010	0.708	0.618
C	345 (66.3)	331 (54.3)	59 (55.7)	163 (52.9)	109 (55.6)					
T	175 (33.7)	279 (45.7)	47 (44.3)	145 (47.1)	87 (44.4)					
Genotype						0.113	< 0.001	0.002	0.541	0.665
CC	115 (44.2)	76 (24.9)	16 (30.2)	36 (23.4)	24 (24.5)					
CT	115 (44.2)	179 (58.7)	27 (50.9)	91 (59.1)	61 (62.2)					
TT	30 (11.6)	50 (16.4)	10 (18.9)	27 (17.5)	13 (13.3)					

Variables expressed as number (percentage). HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

patients with HCV infection harboring the T allele were more susceptible to develop cirrhosis and HCC.

The studies on the role of *IFNL4* rs12979860 polymorphism in HCV-related liver diseases have yielded controversial results. A recent meta-analysis of 18 studies involving different ethnicities attempted to elucidate the global association of this polymorphism with HCV and HBV[22]. The meta-analysis revealed that the *IFNL4* rs12979860 polymorphism is a risk factor for both HCV-and HBV-related HCC. Although the meta-analysis enhanced our understanding of the role of *IFNL4* rs12979860 polymorphism in the outcomes of liver diseases with viral etiologies, the results must be carefully analyzed. Some limiting factors, such as ethnic differences, discrepancies in clinical characteristics among different studies, genotyping methods, HCV genotypes, nonuniform controls in case-control studies, and the influence of confounding factors should be considered.

Various studies have evaluated the role of *IFNL4* rs12979860 polymorphism in the development of HCC. De la Fuente *et al*[23] examined the association of rs12979860 polymorphism with the development of HCC in both chronic HCV infection and nonviral cirrhosis. The authors reported that the TT genotype is highly prevalent in cirrhotic patients infected with HCV genotype 1 who were subjected to liver transplantation. However, there was no significant association between polymorphism variants and hepatocarcinogenesis.

The risk of developing HCC in patients responding to pegylated interferon plus ribavirin treatment is lower than that in nonresponders. Chang *et al*[24] evaluated 800 patients who received pegylated interferon plus ribavirin combination therapy but did not respond to treatment to evaluate the risk factors for HCC. The CT + TT genotypes of rs12979860 polymorphism were an independent risk factor for the development of HCC in these patients, which further indicated the importance of this polymorphism in the progression to HCC. Similarly, a study on 200 patients with advanced fibrosis revealed that the *IFNL4* rs12979860 TT genotype was significantly associated with HCC development after direct-acting antiviral therapy for chronic hepatitis C[25].

Table 3 Genetic models of association between interferon lambda-4 rs12979860 polymorphism and hepatitis C virus-associated liver diseases

rs12979860	Fibrosis *vs* Control		Cirrhosis *vs* Control		HCC *vs* Control		Fibrosis *vs* Cirrhosis		Cirrhosis *vs* HCC	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Codominant model										
CC	1.00 (Ref.)	-	1.00 (Ref.)	-	1.00 (Ref.)	-	1.00 (Ref.)	-	1.00 (Ref.)	-
CT	1.69 (0.82-3.54)	0.126	2.53 (1.55-4.15)	< 0.001	2.54 (1.44-4.56)	0.001	1.50 (0.67-3.28)	0.277	1.01 (0.52-1.95)	0.986
TT	2.40 (0.87-6.27)	0.053	2.88 (1.44-5.77)	0.001	2.08 (0.86-4.83)	0.068	1.20 (0.43-3.45)	0.702	0.72 (0.28-1.80)	0.447
T allele dominant model										
CC	1.00 (Ref.)	-	1.00 (Ref.)	-	1.00 (Ref.)	-	1.00 (Ref.)	-	1.00 (Ref.)	-
CT + TT	1.83 (0.94-3.71)	0.061	2.60 (1.63-4.19)	< 0.001	2.45 (1.42-4.31)	0.001	1.42 (0.66-2.97)	0.325	0.94 (0.50-1.79)	0.840

CI: Confidence interval; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; OR: Odds ratio; Ref.: Reference.

A large international study involving 2916 patients, mostly the European Caucasian population, revealed that the increased number of the T allele was significantly associated with the prevalence of cirrhosis/transition to cirrhosis in patients infected with HCV genotype 1. This association was evident in Caucasian European patients but not in Asian, Latin American, or Middle Eastern patients infected with HCV genotype 1[26].

The genetic background of populations can contribute to variable results among different studies as the allele frequencies of *IFNL4* rs12979860 polymorphism vary among populations. In this study, the minor allele frequencies of the *IFNL4* rs12979860 polymorphism, represented by the T allele, in the case and control groups were 0.46 and 0.34, respectively. The minor allele frequencies reported for European, Japanese, and Chinese populations in the 1000 Genomes database were 0.28, 0.10, and 0.06, respectively.

The role of IFN-λ4 in the pathophysiology of chronic HCV infection-mediated liver diseases is still under investigation. IFN-λ4 activates interferon-stimulated genes, induces cell death, and inhibits cell proliferation[27]. In the IFN-λ4-expressing cells, enhanced cell death may cause tissue inflammation, while the antiproliferative effect of IFN-λ4 could decrease the capacity of tissue remodeling[27,28]. In this sense, our study may provide significant information about the association of the genetic variants of the *IFNL4* rs12979860 polymorphism with disease progression and clinical features of hepatitis C, demonstrating that this polymorphism has relevance in the HCV spontaneous and treatment-induced clearance of HCV infection. Also, the present study can stimulate the clarification of this issue by the analyses of large samples as well as the correlation of genetic variants with gene expression and protein interactions.

This study has some limitations. The sample size of this study is relatively small. A more representative sample could enhance the statistical power to detect genetic differences. In this study, the fibrosis group, which had the lowest sample number, exhibited a trend of association with the TT genotype and the T allele when compared with the control group. A larger sample size could clarify the role of this polymorphism in the development of fibrosis. In addition, some data are missing in the liver fibrosis group (such as HCV RNA, HCV genotype, number of patients on antiviral treatment, diabetes, and steatosis), which precluded a more detailed comparison with the other groups. Besides, the analysis of a nonfibrotic (F0) HCV-infected group would be important because it makes the study more comprehensive. The analysis of a single polymorphism is insufficient to fully explain the genetic basis of HCC. In the cirrhosis and HCC groups, the genotype frequencies of the *IFNL4* rs12979860 polymorphism did not concur with those expected from HWE. The deviations from HWE can be due to the population stratification and selection or may indicate disease association[29,30]. As population stratification may have caused disequilibrium among the cirrhosis and HCC groups, HWE analysis was performed on the case group. However, the genotype frequency in the case group deviated from that expected from HWE. Thus, the observed imbalance could be explained by the effective role of this polymorphism in the sample of patients with HCV-related liver diseases.

Table 4 Distribution of the interferon lambda-4 rs12979860 genotypes based on the clinical features of patients with hepatocellular carcinoma, *n* = 98

		Genotypes		Codominant model	T allele dominant model
Variable	**CC, *n* = 24**	**CT, *n* = 61**	**TT, *n* = 13**	***P* value**	***P* value**
HCV genotypes				0.052	0.017
1	3 (14.3)	27 (46.6)	8 (61.5)		0.004
2	1(4.8)	2 (3.4)	-		
3	17 (81.0)	29 (50.0)	5 (38.5)		0.007
Diabetes	10 (41.7)	19 (31.1)	6 (46.2)	0.463	0.484
Steatosis	1(4.2)	8 (13.3)	2 (16.7)	0.409	0.195
Ascites	10 (41.7)	20 (32.8)	5 (41.7)	0.679	0.511
Portal hypertension	17 (70.8)	48 (78.7)	9 (75.0)	0.741	0.469
Esophageal varices	17 (70.8)	39 (63.9)	9 (75.0)	0.682	0.646
Upper gastrointestinal bleeding	8 (33.3)	10 (16.4)	5 (41.7)	0.075	0.201
Spontaneous bacterial peritonitis	1 (4.2)	5 (8.2)	-	0.500	0.636
Hepatic encephalopathy	3 (12.5)	2 (3.3)	3 (25.0)	**0.030**	0.383
Child-Pugh				0.209	0.156
A	8 (61.5)	26 (63.4)	8 (88.9)		
B	2 (15.4)	12 (29.3)	1 (11.1)		
C	3 (23,1)	3 (7.3)	-		
Number of tumors				0.325	0.684
1	17 (70.8)	39 (65.0)	6 (46.2)		
2	3 (12.5)	12 (20.0)	2 (15.4)		
≥ 3	4 (16.7)	9 (15.0)	5 (38.5)		
Portal vein thrombosis	4 (16.7)	4 (6.6)	2 (16.7)	0.286	0.238
Extrahepatic metastases	1 (4.2)	5 (8.6)	1 (7.7)	0.780	0.487

Variables expressed as number (percentage). HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

CONCLUSION

The findings of this study suggest that the T allele of *IFNL4* rs12979860 polymorphism is a potential genetic factor that determines the susceptibility to cirrhosis and HCC development among patients with chronic HCV.

ARTICLE HIGHLIGHTS

Research background

As a serious public health problem worldwide, hepatitis C virus (HCV) infection has unfavorable trends in morbidity and mortality. Due to high hepatotrophic potential, HCV may cause chronic complications, such as fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Progression to chronic liver disease usually varies and is influenced by different factors, including genetic factors. The interferon lambda-4 (*IFNL4*) rs12979860 polymorphism, characterized by a C to T transition in the intron 1, has been associated with spontaneous and treatment-induced clearance of HCV infection and may play a role in HCV-associated liver diseases, including HCC.

Research motivation

Although the rs12979860 polymorphism has a relevant and well-known role in the spontaneous and treatment-induced clearance of HCV infection, the importance of genetic variants of this polymorphism

in the progression of HCV-associated liver diseases is still unclear.

Research objectives

We aimed to investigate the potential role of the variants in the progression to hepatic fibrosis, cirrhosis, and HCC in chronic HCV-infected patients. In addition, the distribution of the rs12979860 *IFNL4* genetic variants was analyzed in accordance with clinical features of patients.

Research methods

This case-control study included 305 patients with chronic HCV infection patients (53 with fibrosis, 154 with cirrhosis, and 98 with HCC), and 260 HCV-negative healthy individuals as controls. Diagnosis of fibrosis (METAVIR F1-F3) was performed by liver biopsy findings, while the diagnosis of cirrhosis was performed through clinical, laboratorial, anatomopathological, and/or imaging data. Lastly, diagnosis of HCC was performed through dynamic imaging tests, and/or anatomopathological markers. Patients with HCV/human immunodeficiency virus and/or HCV/ hepatitis B virus coinfection were excluded. Molecular analysis was performed using validated pre-designed real-time PCR TaqMan® Assays.

Research results

A higher frequency of the T allele was observed among the groups of patients (fibrosis, cirrhosis, and HCC) as compared to the controls: (P = 0.047; P < 0.001; and P = 0.01, respectively). Also, significant differences were observed concerning genotype frequencies between HCC (P = 0.002) and cirrhosis patients (P < 0.001) in comparison with controls. Two genetic models were tested in the risk analysis: Codominant model and dominant T allele model. In the codominant model, it was observed that the CT genotype was related to an increased risk of cirrhosis [odds ratio (OR) = 2.53; 95% confidence interval (CI): 1.55-4.15; P < 0.001] and HCC (OR = 2.54; 95%CI: 1.44-4.56; P = 0.001) as compared to CC genotype. In the comparison of the TT *vs* CC genotype, a significant difference was observed between the control group and cirrhosis group (OR = 2.88; 95%CI: 1.44-5.77; P = 0.001) but not the HCC group. In the dominant T allele model, the CT + TT genotypes confer an increased risk for the progression to cirrhosis (OR = 2.60; 95%CI: 1.63-4.19; P < 0.001) and HCC (OR = 2.45; 95%CI: 1.42-4.31; P = 0.001). Finally, a significant higher frequency of the T allele among patients with HCV genotypes 1 and 3 (92% and 67%, respectively; P = 0.017) and a higher frequency of TT genotype among patients with hepatic encephalopathy (P = 0.03) was observed.

Research conclusions

This study suggests that the T allele from *IFNL4* rs12979860 polymorphism is associated with the development of cirrhosis and HCC in chronic HCV-infected patients.

Research perspectives

As an important factor related to spontaneous and treatment-induced clearance of HCV infection, the analysis of *IFNL4* rs12979860 polymorphism in the present study may provide a better understanding of the genetic variants with disease progression and clinical features. In order to clarify this issue, large samples are needed to verify the association of genetic polymorphisms with hepatitis C as well as the correlation of genetic variants with gene expression and protein interactions.

FOOTNOTES

Author contributions: de Bitencorte JT, Álvares-da-Silva MR, and Simon D were involved with conception and design of the study; de Bitencorte JT, Rech TF, and dos Santos DC were involved with acquisition of the samples and data; de Bitencorte JT performed the molecular analysis; de Bitencorte JT, Rech TF, Lunge VR, and Simon D performed the statistical analysis and interpretation of data; de Bitencorte JT, Rech TF, and Simon D drafted the manuscript; All authors read and approved the final version of the manuscript.

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REFERENCES

1 **Petruzziello A**, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol* 2016; **22**: 7824-7840 [PMID: 27678366 DOI: 10.3748/wjg.v22.i34.7824]
2 **World Health Organization**. Global hepatitis report, 2017 [Internet]. [cited 6 July 2020]. Available from: https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/
3 **Webster DP**, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]
4 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]
5 **Lingala S**, Ghany MG. Natural History of Hepatitis C. *Gastroenterol Clin North Am* 2015; **44**: 717-734 [PMID: 26600216 DOI: 10.1016/j.gtc.2015.07.003]
6 **Maasoumy B**, Wedemeyer H. Natural history of acute and chronic hepatitis C. *Best Pract Res Clin Gastroenterol* 2012; **26**: 401-412 [PMID: 23199500 DOI: 10.1016/j.bpg.2012.09.009]
7 **Makarova-Rusher OV**, Altekruse SF, McNeel TS, Ulahannan S, Duffy AG, Graubard BI, Greten TF, McGlynn KA. Population attributable fractions of risk factors for hepatocellular carcinoma in the United States. *Cancer* 2016; **122**: 1757-1765 [PMID: 26998818 DOI: 10.1002/cncr.29971]
8 **Zhu RX**, Seto WK, Lai CL, Yuen MF. Epidemiology of Hepatocellular Carcinoma in the Asia-Pacific Region. *Gut Liver* 2016; **10**: 332-339 [PMID: 27114433 DOI: 10.5009/gnl15257]
9 **Bertuccio P**, Turati F, Carioli G, Rodriguez T, La Vecchia C, Malvezzi M, Negri E. Global trends and predictions in hepatocellular carcinoma mortality. *J Hepatol* 2017; **67**: 302-309 [PMID: 28336466 DOI: 10.1016/j.jhep.2017.03.011]
10 **Bosetti C**, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. *Best Pract Res Clin Gastroenterol* 2014; **28**: 753-770 [PMID: 25260306 DOI: 10.1016/j.bpg.2014.08.007]
11 **Matsuura K**, Tanaka Y. Host genetic variants influencing the clinical course of hepatitis C virus infection. *J Med Virol* 2016; **88**: 185-195 [PMID: 26211651 DOI: 10.1002/jmv.24334]
12 **Hemann EA**, Gale M Jr, Savan R. Interferon Lambda Genetics and Biology in Regulation of Viral Control. *Front Immunol* 2017; **8**: 1707 [PMID: 29270173 DOI: 10.3389/fimmu.2017.01707]
13 **O'Brien TR**, Prokunina-Olsson L, Donnelly RP. IFN-λ4: the paradoxical new member of the interferon lambda family. *J Interferon Cytokine Res* 2014; **34**: 829-838 [PMID: 24786669 DOI: 10.1089/jir.2013.0136]
14 **Syedbasha M**, Egli A. Interferon Lambda: Modulating Immunity in Infectious Diseases. *Front Immunol* 2017; **8**: 119 [PMID: 28293236 DOI: 10.3389/fimmu.2017.00119]
15 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]
16 **Rauch A**, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY; Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; **138**: 1338-1345, 1345.e1-1345. e7 [PMID: 20060832 DOI: 10.1053/j.gastro.2009.12.056]
17 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]
18 **Tanaka Y**, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-

1109 [PMID: 19749757 DOI: 10.1038/ng.449]

19 **Prokunina-Olsson L**, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, Hergott D, Porter-Gill P, Mumy A, Kohaar I, Chen S, Brand N, Tarway M, Liu L, Sheikh F, Astemborski J, Bonkovsky HL, Edlin BR, Howell CD, Morgan TR, Thomas DL, Rehermann B, Donnelly RP, O'Brien TR. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet* 2013; **45**: 164-171 [PMID: 23291588 DOI: 10.1038/ng.2521]

20 **Matsuura K**, Watanabe T, Tanaka Y. Role of IL28B for chronic hepatitis C treatment toward personalized medicine. *J Gastroenterol Hepatol* 2014; **29**: 241-249 [PMID: 24325405 DOI: 10.1111/jgh.12475]

21 **Lahiri DK**, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**: 5444 [PMID: 1681511 DOI: 10.1093/nar/19.19.5444]

22 **Qin S**, Wang J, Zhou C, Xu Y, Zhang Y, Wang X, Wang S. The influence of interleukin 28B polymorphisms on the risk of hepatocellular carcinoma among patients with HBV or HCV infection: An updated meta-analysis. *Medicine (Baltimore)* 2019; **98**: e17275 [PMID: 31568008 DOI: 10.1097/MD.0000000000017275]

23 **de la Fuente S**, Citores MJ, Duca A, Cisneros E, Baños I, Vilches C, Cuervas-Mons V. Interleukin-28B TT genotype is frequently found in patients with hepatitis C virus cirrhosis but does not influence hepatocarcinogenesis. *Clin Exp Med* 2017; **17**: 217-223 [PMID: 27083168 DOI: 10.1007/s10238-016-0418-1]

24 **Chang KC**, Ye YH, Wu CK, Lin MT, Tsai MC, Tseng PL, Hu TH. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C without sustained response to combination therapy. *J Formos Med Assoc* 2018; **117**: 1011-1018 [PMID: 29254684 DOI: 10.1016/j.jfma.2017.11.008]

25 **Simili A**, Mazzella G, Ravaioli F, Festi D, Bacchi-Reggiani ML, Porro A, Bazzoli F, Azzaroli F. Interleukin 28 Polymorphisms and Hepatocellular Carcinoma Development after Direct Acting Antiviral Therapy for Chronic Hepatitis C. *J Gastrointestin Liver Dis* 2019; **28**: 449-456 [PMID: 31826071 DOI: 10.15403/jgld-309]

26 **Mangia A**, De Ledinghen V, Bailly F, Brahm J, Keiss J, Valantinas J, Rasmann N, Messinger D, Tatsch F, Bakalos G, Foster GR; Gen-C study group. *IL28B* genotype is associated with cirrhosis or transition to cirrhosis in treatment-naive patients with chronic HCV genotype 1 infection: the international observational Gen-C study. *Springerplus* 2016; **5**: 1990 [PMID: 27917361 DOI: 10.1186/s40064-016-3663-6]

27 **Bantel H**, Schulze-Osthoff K. Apoptosis in hepatitis C virus infection. *Cell Death Differ* 2003; **10** Suppl 1: S48-S58 [PMID: 12655346 DOI: 10.1038/sj.cdd.4401119]

28 **Eslam M**, Hashem AM, Leung R, Romero-Gomez M, Berg T, Dore GJ, Chan HL, Irving WL, Sheridan D, Abate ML, Adams LA, Mangia A, Weltman M, Bugianesi E, Spengler U, Shaker O, Fischer J, Mollison L, Cheng W, Powell E, Nattermann J, Riordan S, McLeod D, Armstrong NJ, Douglas MW, Liddle C, Booth DR, George J, Ahlenstiel G; International Hepatitis C Genetics Consortium (IHCGC). Interferon-λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. *Nat Commun* 2015; **6**: 6422 [PMID: 25740255 DOI: 10.1038/ncomms7422]

29 **Balding DJ**. A tutorial on statistical methods for population association studies. *Nat Rev Genet* 2006; **7**: 781-791 [PMID: 16983374 DOI: 10.1038/nrg1916]

30 **Wittke-Thompson JK**, Pluzhnikov A, Cox NJ. Rational inferences about departures from Hardy-Weinberg equilibrium. *Am J Hum Genet* 2005; **76**: 967-986 [PMID: 15834813 DOI: 10.1086/430507]

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Immunization status and hospitalization for vaccine-preventable and non-vaccine-preventable infections in liver-transplanted children

Palittiya Sintusek, Yong Poovorawan

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Abstract

BACKGROUND

Infections and associated morbidity and mortality may be more frequent in children who have undergone liver transplant than in healthy children. Immunization strategies to prevent vaccine-preventable infections (VPIs) can effectively minimize this infection burden. However, data on age-appropriate immunization and VPIs in children after liver transplant in Asia are limited.

AIM

To evaluate the immunization status, VPIs and non-VPIs requiring hospitalization in children who have undergone a liver transplant.

METHODS

The medical records of children who had a liver transplant between 2004 and 2018 at King Chulalongkorn Memorial Hospital (Bangkok, Thailand) were retrospectively reviewed. Immunization status was evaluated *via* their vaccination books. Hospitalization for infections that occurred up to 5 years after liver transplantation were evaluated, and divided into VPIs and non-VPIs. Hospitalizations for cytomegalovirus and Epstein-Barr virus were excluded. Severity of infection, length of hospital stay, ventilator support, intensive care unit requirement, and mortality were assessed.

RESULTS

Seventy-seven children with a mean age of 3.29 ± 4.17 years were included in the study, of whom 41 (53.2%) were female. The mean follow-up duration was 3.68 ± 1.45 years. Fortyeight children (62.3%) had vaccination records. There was a significant difference in the proportion of children with incomplete vaccination according to Thailand's Expanded Program on Immunization (52.0%) and accelerated vaccine from Infectious Diseases Society of America (89.5%) ($P < 0.001$). Post-liver transplant, 47.9% of the children did not catch up with age-appropriate immunizations. There were 237 infections requiring hospitalization during the 5 years of follow-up. There were no significant differences in hospitalization for VPIs or non-VPIs in children with complete and incomplete immunizations. The risk of serious infection was high in the first year after receiving a liver transplant, and two children died. Respiratory and gastrointestinal systems were common sites of infection. The most common pathogens that caused VPIs were rotavirus, influenza virus, and varicella-zoster virus.

CONCLUSION

Incomplete immunization was common pre- and post-transplant, and nearly all children required hospitalization for non-VPIs or VPIs within 5 years post-transplant. Infection severity was high in the first year post-transplant.

Key Words: Children; Hospitalization; Immunization; Liver transplant; Thailand; Vaccine-preventable infection

Core Tip: Incomplete age-appropriate immunization in children waiting for a liver transplant was expected, and nearly half of them had not caught up with age-appropriate vaccinations post-transplant. Though there was no significant difference in hospitalization from vaccine-preventable infections (VPIs) and non-VPIs in children with complete and incomplete immunizations. At least 13.1% required hospitalization within 5 years post-transplant, and > 10% were admitted to the intensive care unit and required respiratory support. The severity of infections was high during the first year post-transplant. Complete immunization and robust infection control should be prioritized in children both pre and post-liver transplant.

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INTRODUCTION

Infection after a liver transplant is a serious concern due to potential associated morbidity and mortality [1-4], as well as the standard complications and severe symptoms that can be experienced by immunocompetent patients. Such infections can give rise to graft rejection, thus affecting short- or long-term graft survival[4]. Accordingly, strategies to reduce overall post-transplant infection are warranted. Immunization is considered an effective, relatively noninvasive, and affordable way to reduce vaccine-preventable infections (VPIs)[5] such as measles, varicella, influenza, and viral hepatitis A and B, among others. The Infectious Diseases Society of America (IDSA)[6] and the American Society of Transplantation Infectious Disease Community of Practice[7] encourage accelerated vaccination, particularly with regard to live vaccines in immunocompromised children awaiting for solid organ transplantation.

Children awaiting a liver transplant can be at a disproportionate risk of VPIs because they tend not to have undergone a complete series of age-appropriate immunizations, because their serious illness has taken medical priority over vaccination[8]. Verma and Wade[9] reported that in their experience at King's College Hospital, only 20%-30% of children had undergone a complete series of age-appropriate immunizations prior to liver transplantation. Diana *et al*[10] reported that less than half of a cohort of children who underwent liver transplant at the Children's Hospital of Geneva in Switzerland had undergone a complete series of age-appropriate vaccinations, with rates of 43% for diphtheria-tetanus-acellular pertussis-polio vaccine, 44% for measles-mumps-rubella (MMR) vaccine, 13% for hepatitis B vaccine, and 5% for hepatitis A vaccine at the time of liver transplantation. Feldman *et al*[4,11] investigated morbidity, mortality, and costs associated with VPIs in children after solid organ transplants, and reported a significantly higher rate of VPIs in these children than in the general pediatric population.

Studies conducted in the United States and other western countries have highlighted the effects of VPIs in children after solid organ transplantation[4,9-11], but published data on VPIs in children after liver transplantation in the East are scarce. To improve the quality of life of liver-transplanted children by minimizing the serious complications associated with post-liver transplant infections, strategies to avoid VPIs based on strong evidence should be initiated worldwide, including in Asia.

The aim of the present study was to evaluate immunization status in Thai children at the time of liver transplantation, and for up to 5 years post-liver transplantation. The prevalence and effects of VPIs and non-VPIs during hospitalization were also assessed.

MATERIALS AND METHODS

The current study was a retrospective review of all children who received a liver transplant at King Chulalongkorn Memorial Hospital in Thailand from January 2004 to August 2018. Demographic data, patient characteristics, and immunization records from vaccination books were collated. Hospitalization records pertaining to the liver transplant operation and admission due to infections for up to 5 years post-transplant were included. Hospitalizations for Epstein-Barr virus (EBV) and cytomegalovirus were excluded from the study. Infection etiology and source were investigated by the doctors in charge. Culture from specimens was available for all bacterial origins, and immunological and molecular techniques were available for the diagnosis of both viral and bacterial infections, including polymerase chain reaction panel analysis for respiratory tract infections and gastrointestinal infections, and antibody titers for hepatitis A/B/E, dengue, and measles.

Infections were divided into VPIs and non-VPIs. Length of hospital stay, severity of infections, and mortality from infections were collated and classified into three groups: Intensive care unit (ICU) requirement, ventilator support, and death. Complete immunization was defined as that conducted in accordance with the Expanded Program on Immunization (EPI) in Thailand (Table 1) and the accelerated vaccination recommendations described in the 2013 IDSA Clinical Practice Guideline for Vaccination of the Immunocompromised Host[6], which notes: "... children aged 6-12 mo can receive MMR and varicella vaccine and the second dose should be administered at 12 mo for MMR and ≥ 3 mo apart for varicella vaccine. However, the last MMR or varicella vaccine injection should not be within 4 wk of a liver transplant schedule."

Statistical analysis

Continuous and categorical data are presented as the mean ± SD, medians and interquartile ranges, proportions, or percentages as appropriate. The Mann Whitney *U* test and unpaired *t*-test were used to compare continuous data, and Fisher's exact test and the χ^2 test were used to compare discrete data. $P < 0.05$ was considered statistically significant. Data analyses were performed using Statistical Package for the Social Sciences version 24.0.0 (SPSS, Inc.; Chicago, IL, United States). A biomedical statistician employed at the Department of Statistics Science, Kasetsart University (Bangkok, Thailand) reviewed the statistical analyses conducted in the study.

RESULTS

Patient characteristics and history of immunization

Seventy-seven children with a mean age of 3.29 ± 4.17 years were included in the study, of whom 41 (53.2%) were female. The indications for liver transplantation were biliary atresia ($n = 63$), indeterminate acute liver failure ($n = 3$), progressive familial intrahepatic cholestasis ($n = 2$), Alagille syndrome ($n = 2$), cryptogenic cirrhosis ($n = 1$), citrin deficiency ($n = 1$), Budd-Chiari syndrome ($n = 1$), hepatoblastoma ($n = 1$), autoimmune hepatitis ($n = 1$), glycogen storage disease type IV ($n = 1$), and bile acid deficiency ($n = 1$). The mean follow-up time was 3.68 ± 1.45 years, and 32 children were followed up for a full 5 years after liver transplantation. Vaccinations were noted in the vaccination books of 48/77 children (62.3%). Substantial proportions of children did not have complete vaccinations in accordance with Thailand's EPI ($n = 25$, 52%) (Table 1) or accelerated vaccinations in accordance with the IDSA recommendations ($n = 43$, 89.5%) ($P < 0.001$). Post-liver transplant, 23 children (47.9%) could not catch up with the appropriate immunizations for age. All children were revaccinated with hepatitis B vaccine if hepatitis B surface antibody was < 10 mIU/mL. Other vaccines they received after liver transplantation included those for influenza ($n = 12$), invasive pneumococcal disease ($n = 10$), Japanese encephalitis ($n = 6$), diphtheria/ tetanus/pertussis-inactivated polio vaccine ($n = 6$), and hepatitis A ($n = 3$). A minority of children were not up-to-date with influenza vaccination ($n = 18$, 37.5%) and pneumococcal conjugate vaccine ($n = 22$, 45.8%) post-liver transplant compared with pre-liver transplant ($n = 30$, 62.5% for influenza and $n = 36$, 75% for pneumococcal conjugate vaccine) ($P < 0.001$; Table 2). With regard to live vaccines, three individuals were inadvertently vaccinated with MMR at their local hospitals without any serious side effects.

Table 1 The immunization schedule in Thailand and accelerated vaccines by the Infectious Disease Society of America

	Vaccine	Birth	1 mo	2 mo	4 mo	6 mo	7 mo	9 mo	12 mo	18 mo	24 mo	4 yr	9 yr	11 yr
Thai's EPI vaccines	BCG	1												
	HBV	1	(For positive maternal HBsAg)	2		3								
	DTP, OPV/IPV			1	2	3				4		5		
	MMR					Acc[1]		1	Acc[1]		2			
	JE							1			2			
	Influenza					1	2							
	Tdap													1
	HPV												Acc	1-2[2]
Optional vaccine in Thailand	Rota			1	2	(3)								
	PCV			1	2	3			4					
	Varicella					Acc[1]		Acc[1]	1	2				
	HAV								1	2				
	Dengue												1-3[3]	

[1]Acc denotes accelerated vaccines from the 2013 Infectious Diseases Society of America Clinical Practice Guideline for Vaccination of the Immunocompromised Host in which measles-mumps-rubella (MMR) at 6 and 12 mo of age and varicella at 6 mo of age and 3 mo apart from the first dose.

[2]Indicates 0 and 6 mo.

[3]Indicates 0, 6, 12 mo.

BCG: Bacillus Calmette-Guerin vaccine; DTP: Diphtheria-tetanus-pertussis; EPI: Expanded Program on Immunization; HAV: Hepatitis A vaccine; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B vaccine; HPV: Human papillomavirus vaccine; JE: Japanese encephalitis; OPV/IPV: Oral polio vaccine/inactivated polio vaccine; PCV: Pneumococcal conjugate vaccine; Tdap: Tetanus-diphtheria-acellular pertussis.

Infections during and after liver transplant

Infection severity and mortality were highest during the first year post-liver transplant. The respiratory and gastrointestinal systems were the most common sites of infection (Table 3). Two children died within 3 mo after liver transplantation, and both had underlying post-transplant lymphoproliferative disorder. One of these two children had mixed infection with bocavirus, mycoplasma, and parvovirus B19. The other exhibited EBV viremia that progressed to respiratory failure with an unidentified infectious origin. Of the 31 hospitalizations for VPIs recorded during the study period the median length of hospital stay was 6 d (range: 3-8 d), and in three cases ICU admission and ventilator support were required; two with influenza and one with *Streptococcus pneumoniae* infection. When the children were divided into complete and incomplete immunization groups based on Thailand's EPI, there were no significant differences in the numbers of hospitalizations for VPIs or non-VPIs (Table 4).

Table 2 Vaccination history in children at liver transplant and up to 5 years follow-up (*n* = 48)

Vaccines	Incomplete vaccination for age at transplantation		Incomplete vaccination for age after liver transplant, *n* (%)
	Thai EPI program, *n* (%)	Accelerated vaccine from IDSA, *n* (%)	
DTP-OPV/IPV	12 (25)	N/A	6 (12.5)
HBV	6 (12.5)		0
MMR	12 (25)	30 (62.5)[b]	27 (56.3)[b]
JE	16 (33.3)	N/A	10 (20.8)
Varicella	16 (33.3)	34 (70.8)[b]	34 (70.8)[b]
HAV	26 (54)		23 (47.9)
Influenza	30 (62.5)		18 (37.5)[a]
PCV	36 (75)		22 (45.8)[b]
Rota	37 (77)	N/A	37 (77)
All	25 (52)	43 (89.5)[b]	23 (47.9)
	(not included rota vaccine)		(not included lived vaccine)

[a]$P < 0.05$ *vs* Thai Expanded Program on Immunization (EPI).
[b]$P < 0.001$ *vs* Thai EPI program.
DTP: Diphtheria-tetanus-pertussis; HAV: Hepatitis A vaccine; HBV: Hepatitis B vaccine; IDSA: Infectious Diseases Society of America; JE: Japanese encephalitis; MMR: Measles-mumps-rubella; N/A: Not applicable; OPV/IPV: Oral polio vaccine/inactivated polio vaccine; PCV: Pneumococcal conjugate vaccine.

Pathogens causing hospitalization in children post-liver transplant

A total of 237 infections requiring hospitalization were recorded during the study period. The most commonly identified bacterial pathogens were *Escherichia coli* (13.1%), *Salmonella* sp. (8.1%), and *Klebsiella pneumoniae* (6.8%), and the most commonly identified viral pathogens were parainfluenza (5.9%), rotavirus (3.4%), and respiratory syncytial virus (3.4%). In cases of VPIs, the most common pathogens were rotavirus (3.4%), influenza virus (2.5%), and varicella-zoster virus (2.1%) (Table 5 and 6).

DISCUSSION

In this study, incomplete age-appropriate immunization before liver transplantation in children was common, particularly with regard to live vaccines that can be accelerated before liver transplantation. Post-liver transplant in nearly half of the children in the study did not catch up with all age-appropriate vaccines. At least 13.1% of the children in the study required hospitalization for VPIs during the 5 years post-liver transplant, and in these cases, the lengths of hospital stays were up to 1 wk. More than 10% of the children required admission to the ICU and respiratory support from VPIs, reflecting the burden of VPIs during the post-transplant period. With regard to non-VPIs, both bacterial and viral infections of the respiratory and gastrointestinal systems played major roles in hospitalizations with severe infections and mortality, especially during the first year post-transplant.

To the best of our knowledge, the current study is the first to investigate immunization status and infections requiring hospitalization in Asian children who underwent a liver transplant. Compared to previous studies in Europe[9,10] and the United States[4,11], in the present study, there was a higher rate of incomplete age-appropriate immunization before liver transplantation, particularly with respect to the accelerated MMR and varicella vaccination. However, the number of hospitalizations with VPIs (13.1%) was comparable to that in a study conducted in the United States by Feldman *et al*[4,11] (11.3%). Moreover, the VPIs in that study were more severe and required longer hospital stays than those in the current study. Genetic risk factors may explain this phenomenon, as with the more contagious and severe coronavirus disease 2019 infections in Europe and the United States than in Thailand.

Prior to liver transplantation, physicians frequently do not offer patient immunization, particularly with respect to live vaccines[8,12,13]. There is solid evidence of adequate immune responses to varicella and measles vaccination in children aged < 1 year[14-16]; hence, the policy to promote accelerated vaccination in children before immunosuppressant therapy was initiated[6,7,17,18]. It is probable that this is not standard practice in healthy children. Moreover, children waiting for a liver transplant may have had complex and serious illnesses that needed to be given priority. Some physicians may not be

Table 3 Characteristics of hospitalization from vaccine-preventable infections and non-vaccine-preventable infections up to 5 years follow-up

Time	Type of infections				Organ specific infections, *n* (%)						The severity of infections, *n* (%)		
	VPIs		Non-VPIs		RS	GI	Blood	Renal	Skin	Others	ICU	Ventilator dependence	Death
	Times, *n* (%)	LOS (d)[1]	Times, *n* (%)	LOS (d)[1]									
During transplant	4 (5.2)	51 (24,79)	73 (94.8)[b]	35 (27,49)	25 (35.2)	24 (31.2)	20 (26)	6 (7.8)	2 (2.6)	0	All	All	0
< 3 mo	2 (6.9)	3 (3,3)	27 (93.1)[b]	12 (7,28)[a]	13 (44.8)	10 (34.5)	2 (6.9)	2 (6.9)	1 (3.4)	1 (3.4)	6 (20.7)	5 (17.2)	2 (6.9)
3-6 mo	5 (17.9)	8 (5,39)	23 (82.1)[b]	10 (4,15)	11 (39.3)	13 (46.4)	2 (7.1)	1 (3.6)	0	1 (3.6)	8 (28.6)	6 (21.4)	0
> 6-12 mo	3 (8.3)	5 (3,5)	33 (91.7)[b]	7 (6,17)	15 (41.7)	11 (30.6)	6 (16.7)	0	2 (5.6)	2 (5.6)	10 (27.8)	6 (8.3)	0
> 12-24 mo	6 (15)	5 (4,9)	34 (85)[b]	7.5 (5,10)	18 (45)	12 (30)	1 (2.5)	1 (2.5)	4 (10)	4 (10)	11 (27.5)	9 (22.5)	0
> 2-5 yr	11 (40.7)	6 (3,8)	16 (59.3)	5 (4,9)	7 (25.9)	10 (37)	1 (3.7)	0	6 (22.2)	3 (1.9)	5 (18.5)	1 (3.7)	0
Total	31 (13.1)	6 (3,8)	206 (86.9)[b]	8 (5,15)	89 (37.6)	80 (33.8)	32 (13.5)	10 (4.2)	15 (6.3)	11 (4.6)	40 (16.9)	27 (11.4)	2 (0.84)

[a]$P < 0.05$ *vs* vaccine-preventable infection (VPI) group.
[b]$P < 0.001$ *vs* VPI group.
[1]Data are presented as median (interquartile range).
GI: Gastrointestinal; ICU: Intensive care unit; LOS: Length of stay; RS: Respiratory system.

familiar with the accelerated immunization program[8,13], and therefore may decide to postpone vaccination. A specific protocol and concerted focus on educational interventions, or the development of specialized team care that is responsible for these issues is crucial to ensure that all candidates receive appropriate vaccinations to minimize complications associated with VPIs[6]. One great benefit of pre-liver transplant vaccination is higher immunogenicity compared with revaccination post-liver transplant[18]. Moreover, pretransplant vaccination of children will likely lead to herd immunity that will be beneficial for other transplant children in inpatient and outpatient clinics during their visits[13].

In the present study, the rate of incomplete age-appropriate immunization after liver transplantation was high, and there was no significant difference between the pretransplant rate (52.0%) and the post-transplant rate (47.9%). In theory, children's vaccination schedules should be postponed for more than 2 mo after liver transplantation because of the possibility of an inadequate immune responses[6]. The high level of immunosuppressants is another factor to consider. In the present study almost half of the children were not up-to-date with their age-appropriate immunizations during up to 5 years of follow-up. The reasons might be relatively low concern over children in a stable condition post-transplant, and a level of immunosuppression that is not low enough to warrant immunization. Notably, only 62.3% of the children's guardians brought vaccination books to visits to the doctor. As well as unawareness, financial problems would likely be a major concern for the children's guardians, especially with regard to vaccines that are not included in Thailand's EPI such as pneumococcal conjugate vaccine, influenza vaccine, hepatitis A vaccine, and varicella vaccine. Fortunately the infectious diseases unit in our department conducted a campaign to promote the administration of pneumococcal conjugate vaccine

Table 4 Children with vaccination records who developed vaccine-preventable or non-vaccine-preventable diseases

Age-appropriate immunization	Thai's Expanded Program on Immunization				2013 Infectious Diseases Society of America			
	Infection and hospitalization, *n*			Total	Infection and hospitalization, *n*			Total
	None	VPIs and non-VPIs	Non-VPIs		None	VPIs and non-VPIs	Non-VPIs	
Complete immunization	5	5	12	22	9	9	25	43
Incomplete immunization	5	6	15	26	1	2	2	5
Total	10	11	27	48	10	11	27	48

VPIs: Vaccine-preventable infections.

and influenza vaccine to all immunocompromised children every year at no charge. This afforded the children in the present study the opportunity to access these vaccines, and there was a significant increase in the proportion of children that received these vaccines post-transplant ($P < 0.001$). Long-term provision of these high-cost vaccines by the authorities would be a worthwhile venture. With respect to live vaccines, there has been controversy about whether they should be administered to children after liver transplantation[17,19,20-23]. Thus, further reports and large cohort studies are required in order to clarify the safety of live vaccines in these vulnerable patients, before they are routinely vaccinated posttransplant.

In this study, the rate of hospitalization for VPIs up to 5 years post-transplant was similar to those reported in previous studies[9-11], but significantly higher than that in the normal population[9]. There was the mortality report of VPIs in children with immunocompromised hosts[1,2,22,24,25], but in this study, there was no mortality from VPIs. The VPIs requiring hospitalization in the current study were due to rotavirus, influenza, varicella, dengue fever, measles, *Streptococcus pneumoniae*, hepatitis B/E, and *Vibrio cholera*. These data should emphasize the value of complete immunization and robust infection control to physicians.

Viral hepatitis is endemic in Thailand, but interestingly in the present study there were no reports of hospitalization for hepatitis A post-liver transplant, and only one case of hepatitis E infection that required hospitalization. Viral hepatitis can be symptomatic and severe in older children and adults, and older children and adults may ingest more contaminated food and water than young children. Consequently, serology testing and immunization may be valuable in these groups. There is a reported case in which *de novo* hepatitis B infection was diagnosed 3 years after a liver transplant despite the recipient having undergone complete hepatitis B immunization pre-transplant[26]. This demonstrates that complete hepatitis B immunization pre-liver transplant does not guarantee post-transplant protection. That case prompted us to instigate a protocol for reimmunization and hepatitis B surface antibody monitoring every 3-6 mo to maintain a protective level of > 100 mIU/mL. *De novo* hepatitis B in the aforementioned boy who had hepatitis B surface antibody > 1000 mIU/mL pretransplant[26] may reflect waning immunity post-liver transplant. As well as vaccination, research evaluating the humoral and cellular immunity evoked by each vaccine should be conducted to determine vaccination schedules and the antibody parameters required to prevent VPIs more effectively. In the present study, the overall infection rate was high in the first year post-transplant, hence vaccination should be initiated as soon as possible after liver transplanted children are sufficiently stable. Predictors of high immunogenic responsivity to vaccination are needed to enable physicians to decide on optimal timepoints for reimmunization.

The current study had some limitations. It was a single-center study with a relatively small sample size. The true prevalence of VPIs may be lower than the frequency in the study, because the study only included children with severe enough illness to require hospitalization. Almost all children in the present study were referred from distant and rural areas, and it is possible that some of them subsequently attended more local hospitals due to infections. The main strength of the study was the reliable vaccination records obtained directly from the patients' vaccination books, which facilitated comparisons of vaccination status pre-transplant and post-transplant.

CONCLUSION

Incomplete immunization was common in children pre-liver transplant and post-liver transplant. Almost all of the children in the study required hospitalization due to VPIs or non-VPIs within 5 years post-liver transplant. The severity of infections was highest in the first year post-liver transplant.

Table 5 Pathogen causing hospitalization in children after liver transplantation

Time	The rank of the pathogen, *n* (%)			
	Bacteria	Total	Virus, fungus, and unidentified	Total
During transplant	*E. coli* (*n* = 19, 24.7), *K. pneumoniae* (*n* = 12, 15.6), *A. baumannii* (*n* = 11, 14.3), *Enterococcus*/*Staphylococcus* (*n* = 4, 5.2), *Salmonella* (*n* = 3, 3.9), *P. aeruginosa* (*n* = 2, 2.6), *B. cereus*/*Corynebacterium*/*S. pneumoniae*/*Elizabethkingia meningoseptica*/*Stenotrophomonas*/*Streptococcus mirabilis*/*C. difficile* (*n* = 1, 1.3)	62	Rotavirus/adenovirus/bocavirus (*n* = 2, 2.6), parainfluenza/fungus/varicella-zoster virus (*n* = 1, 1.3)	9[b]
< 3 mo	*E. coli*/*K. pneumoniae*/*Enterococcus*/*Salmonella*/*Aeromonas* (*n* = 2, 6.9), *Corynebacterium*/*C. difficile*/*Plesiomonas* (*n* = 1, 3.4)	13	Parainfluenza (*n* = 3, 10.3), coronavirus (*n* = 2, 6.9), rotavirus/bocavirus/RSV/dengue/fungus/norovirus/rhinovirus/parvovirus B19 (*n* = 1, 3.4), unidentified (*n* = 6, 20.7)	19
3-6 mo	*Salmonella*/*E. coli* (*n* = 2, 7.1), *K. pneumoniae*/*Enterococcus*/*S. pneumoniae*/*Staphylococcus* (*n* = 1, 3.6)	8	RSV (*n* = 4, 14.3), influenza (*n* = 2, 7.1), rotavirus/parainfluenza/rhinovirus/measles/HHV6 (*n* = 1, 3.6), unidentified (*n* = 9, 32.1)	20
> 6-12 mo	*E. coli* (*n* = 4, 11.1), *Salmonella* (*n* = 3, 8.3), *A. baumannii*/*Enterococcus*/mycoplasma/*C. difficile* (*n* = 2, 5.6), *Stenotrophomonas*/*Staphylococcus*/*Aeromonas*/*Pseudomonas*/*Plesiomonas*/*P. jirovecii* (*n* = 1, 2.8)	21	*P*arainfluenza (*n* = 3, 8.3), norovirus/herpes simplex virus (*n* = 2, 5.6), fungus/RSV/rhinovirus/influenza/measles (*n* = 1, 2.8), unidentified (*n* = 3, 8.3)	15
> 12-24 mo	*Salmonella* (*n* = 8, 12.5), *E. coli* (*n* = 3, 7.5), *Aeromonas*/*Pseudomonas*/mycoplasma/*Plesiomonas* (*n* = 1, 2.5)	15	Parainfluenza (*n* = 6, 15), rotavirus (*n* = 2, 5), adenovirus/varicella-zoster virus/dengue/rhinovirus/influenza/measles/metapneumovirus/hepatitis E/coxakie AB (*n* = 1, 2.5) unidentified (*n* = 11, 27.5)	28
> 2-5 yr	*Salmonella*/mycoplasma (*n* = 2, 7.4), *E. coli*/*K. pneumoniae*/*Staphylococcus*/*Vibrio cholera*/*B. cereus* (*n* = 1, 3.7)	9	Varicella-zoster virus (*n* = 3, 11.1), rotavirus/RSV/dengue/influenza (*n* = 2, 7.4), fungus/norovirus/herpes simplex virus/hepatitis B (*n* = 1, 3.7), unidentified (*n* = 3, 11.1)	18
Overall	*E. coli* (*n* = 31, 13.1), *Salmonella* (*n* = 20, 8.1), *K. pneumoniae* (*n* = 16, 6.8), *A. baumannii* (*n* = 13, 5.5), *Enterococcus* (*n* = 9, 3.8), *Staphylococcus* (*n* = 8, 3.3), mycoplasma (*n* = 5, 2.1), *C. difficile* (*n* = 4, 1.7), *Plesiomonas Shigelloides*/*Aeromonas* (*n* = 3, 1.3), *Corynebacterium*/*S. pneumononiae*/*Stenotrophomonas*/*P. aeruginosa*/*Aeromonas* (*n* = 2, 0.8), *Bacillus*/*Elizabethkingia meningoseptica*/*Streptococcus mirabilis*/*P. jirovecii*/*Vibrio cholera*/*B. cereus* (*n* = 1, 0.4)	128	Parainfluenza (*n* = 14, 5.9), rotavirus/RSV (*n* = 8, 3.4), influenza (*n* = 6, 2.5), varicella-zoster virus (*n* = 5, 2.1), dengue/norovirus/fungus/rhinovirus (*n* = 4, 1.7), adenovirus/bocavirus/herpes simplex virus/measles (*n* = 3, 1.3), coronavirus (n=2, 0.8), HHV6/metapneumovirus/hepatitis E/coxakie AB/hepatitis B (*n* = 1, 0.4), unidentified (*n* = 32, 13.5)	109[b]

[b]*P* < 0.001; virus *vs* bacterial causes of infections at each time point. *A. baumannii*: *Acinetobacter baumannii*; *B. cereus*: B*acillus* cereus; *C. difficile*: *Clostridium difficile*; *E. coli*: *Escherichia coli*; HHV6: Human herpes virus 6; *K. pneumoniae*: *Klebsiella pneumoniae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. jirovecii*: *Pneumocystis jirovecii*; RSV: Respiratory syncytial virus; *S. pneumoniae*: *Streptococcus pneumoniae*.

Table 6 Vaccine-preventable infections causing hospitalization in children after liver transplantation

Time	During transplant	< 3 mo	3-6 mo	> 6-12 mo	> 12-24 mo	> 2-5 yr	Overall
Rota	2	1	1	0	2	2	8
Influenza	0	0	2	1	1	2	6
Varicella	1	0	0	0	1	3	5
Dengue	0	1	0	0	1	2	4
Measles	0	0	1	1	1	0	3
Streptococcus pneumoniae	1	0	1	0	0	0	2
Hepatitis B	0	0	0	0	0	1	1
Hepatitis E	0	0	0	0	1	0	1
Vibrio cholera	0	0	0	0	0	1	1

ARTICLE HIGHLIGHTS

Research background

Infection after liver transplantation is a serious concern due to potential morbidity and mortality, thus strategies to reduce overall post-transplant infection are warranted. Immunization is an effective and relatively noninvasive and affordable way to reduce vaccine-preventable infections (VPIs).

Research motivation

There is strong evidence that VPIs and non-VPIs post-transplant cause high fatality and increase graft rejection, but published data on VPIs and their effects in children post-liver transplant in Asia are scarce.

Research objectives

To investigate immunization status in children at the time of liver transplantation and up to 5 years thereafter. The prevalence and impact of VPIs and non-VPIs during hospitalization were also evaluated.

Research methods

The current retrospective study included 77 children who underwent liver transplantation and were followed up for up to 5 years thereafter. Demographic data, patient characteristics, immunization details derived from vaccination records, and hospitalizations for VPIs and non-VPIs were analyzed.

Research results

The mean follow-up duration after liver transplantation was 3.68 ± 1.45 years. Of the 77 children in the study, 48 (62.3%) had vaccination records in their vaccination books. There was a significant difference in the proportion of children with incomplete vaccination according to Thailand's Expanded Program on Immunization (n = 25, 52%) and accelerated vaccine from Infectious Diseases Society of America recommendations (n = 43, 89.5%) (P < 0.001). Post-liver transplant, almost half of the children in the study did not catch up with appropriate immunizations for age. There were 237 infections requiring hospitalization during up to 5 years of follow-up post-liver transplant at our hospital. The risks of VPIs and non-VPIs were highest during the first year after liver transplantation, and 2 children died. Respiratory and gastrointestinal systems were common sites of infection. The most commonly identified pathogens that caused VPIs were rotavirus, influenza virus, and varicella-zoster virus.

Research conclusions

Incomplete age-appropriate immunization in children pre-liver transplant and post-liver transplant were common. At least 13.1% of the children in the study required hospitalization for a VPI during a follow-up period of up to 5 years post-transplantation. There was high morbidity, especially during the first year after transplantation. Hence, complete immunization and robust infection control should be considered in such children.

Research perspectives

The current study suggests that incomplete age-appropriate immunization is a major concern, because a large number of patients with VPIs requiring hospitalization were recorded. Interestingly, waning immunity post-liver transplant can evidently lead to VPIs, as evidenced by a case in which *de novo* hepatitis B infection developed 3 years postliver transplantation in a child who had a hepatitis B surface antibody titer of > 1000 mIU/mL pre-liver transplantation. As well as policies to increase pre- and post-

transplant immunization rates, studies investigating humoral and cellular immunity induced by vaccination after liver transplantation are needed.

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FOOTNOTES

Author contributions: Sintusek P designed and oversaw the study, drafted the manuscript, collected, interpreted, and analyzed the data, and made critical revisions related to important intellectual content; Poovorawan Y suggested critical intellectual content and approved the final manuscript.

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REFERENCES

1 **Kaplan LJ**, Daum RS, Smaron M, McCarthy CA. Severe measles in immunocompromised patients. *JAMA* 1992; **267**: 1237-1241 [PMID: 1538561]
2 **Fitts SW**, Green M, Reyes J, Nour B, Tzakis AG, Kocoshis SA. Clinical features of nosocomial rotavirus infection in pediatric liver transplant recipients. *Clin Transplant* 1995; **9**: 201-204 [PMID: 7549061]
3 **McGregor RS**, Zitelli BJ, Urbach AH, Malatack JJ, Gartner JC Jr. Varicella in pediatric orthotopic liver transplant recipients. *Pediatrics* 1989; **83**: 256-261 [PMID: 2536479]
4 **Feldman AG**, Sundaram SS, Beaty BL, Kempe A. Hospitalizations for Respiratory Syncytial Virus and Vaccine-Preventable Infections in the First 2 Years After Pediatric Liver Transplant. *J Pediatr* 2017; **182**: 232-238 [PMID: 28088400 DOI: 10.1016/j.jpeds.2016.12.021]
5 **Feldman AG**, Sundaram SS, Beaty BL, Torres R, Curtis DJ, Kempe A. Immunization Status at the Time of Liver Transplant in Children and Adolescents. *JAMA* 2019; **322**: 1822-1824 [PMID: 31714979 DOI: 10.1001/jama.2019.14386]
6 **Rubin LG**, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, Bousvaros A, Dhanireddy S, Sung L, Keyserling H, Kang I; Infectious Diseases Society of America. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. *Clin Infect Dis* 2014; **58**: 309-318 [PMID: 24421306 DOI: 10.1093/cid/cit816]

7 **Danziger-Isakov L**, Kumar D; AST ID Community of Practice. Vaccination of solid organ transplant candidates and recipients: Guidelines from the American society of transplantation infectious diseases community of practice. *Clin Transplant* 2019; **33**: e13563 [PMID: 31002409 DOI: 10.1111/ctr.13563]

8 **Feldman AG**, Kempe A, Beaty BL, Sundaram SS; Studies of Pediatric Liver Transplantation (SPLIT) Research Group. Immunization practices among pediatric transplant hepatologists. *Pediatr Transplant* 2016; **20**: 1038-1044 [PMID: 27449120 DOI: 10.1111/petr.12765]

9 **Verma A**, Wade JJ. Immunization issues before and after solid organ transplantation in children. *Pediatr Transplant* 2006; **10**: 536-548 [PMID: 16856989 DOI: 10.1111/j.1399-3046.2006.00527.x]

10 **Diana A**, Posfay-Barbe KM, Belli DC, Siegrist CA. Vaccine-induced immunity in children after orthotopic liver transplantation: a 12-yr review of the Swiss national reference center. *Pediatr Transplant* 2007; **11**: 31-37 [PMID: 17239121 DOI: 10.1111/j.1399-3046.2006.00596.x]

11 **Feldman AG**, Beaty BL, Curtis D, Juarez-Colunga E, Kempe A. Incidence of Hospitalization for Vaccine-Preventable Infections in Children Following Solid Organ Transplant and Associated Morbidity, Mortality, and Costs. *JAMA Pediatr* 2019; **173**: 260-268 [PMID: 30640369 DOI: 10.1001/jamapediatrics.2018.4954]

12 **Olarte L**, Lin PL, Barson WJ, Romero JR, Tan TQ, Givner LB, Hoffman JA, Bradley JS, Hultén KG, Mason EO, Kaplan SL. Invasive pneumococcal infections in children following transplantation in the pneumococcal conjugate vaccine era. *Transpl Infect Dis* 2017; **19**: e12630 [PMID: 27862712 DOI: 10.1111/tid.12630]

13 **Feldman AG**, Hsu EK, Mack CL. The Importance of Prioritizing Pre and Posttransplant Immunizations in an Era of Vaccine Refusal and Epidemic Outbreaks. *Transplantation* 2020; **104**: 33-38 [PMID: 31876696 DOI: 10.1097/TP.0000000000002936]

14 **Redd SC**, King GE, Heath JL, Forghani B, Bellini WJ, Markowitz LE. Comparison of vaccination with measles-mumps-rubella vaccine at 9, 12, and 15 months of age. *J Infect Dis* 2004; **189** Suppl 1: S116-S122 [PMID: 15106100 DOI: 10.1086/378691]

15 **Kanra G**, Ceyhan M, Ozmert E. Safety and immunogenicity of live attenuated varicella vaccine in 9-month-old children. *Pediatr Int* 2000; **42**: 674-677 [PMID: 11192526 DOI: 10.1046/j.1442-200x.2000.01300.x]

16 **van der Maas NA**, Woudenberg T, Hahné SJ, de Melker HE. Tolerability of Early Measles-Mumps-Rubella Vaccination in Infants Aged 6-14 Months During a Measles Outbreak in The Netherlands in 2013-2014. *J Infect Dis* 2016; **213**: 1466-1471 [PMID: 26690343 DOI: 10.1093/infdis/jiv756]

17 **Pittet LF**, Verolet CM, McLin VA, Wildhaber BE, Rodriguez M, Cherpillod P, Kaiser L, Siegrist CA, Posfay-Barbe KM. Multimodal safety assessment of measles-mumps-rubella vaccination after pediatric liver transplantation. *Am J Transplant* 2019; **19**: 844-854 [PMID: 30171797 DOI: 10.1111/ajt.15101]

18 **L'Huillier AG**, Wildhaber BE, Belli DC, Diana A, Rodriguez M, Siegrist CA, Posfay-Barbe KM. Successful serology-based intervention to increase protection against vaccine-preventable diseases in liver-transplanted children: a 19-yr review of the Swiss national reference center. *Pediatr Transplant* 2012; **16**: 50-57 [PMID: 22093802 DOI: 10.1111/j.1399-3046.2011.01600.x]

19 **Khan S**, Erlichman J, Rand EB. Live virus immunization after orthotopic liver transplantation. *Pediatr Transplant* 2006; **10**: 78-82 [PMID: 16499592 DOI: 10.1111/j.1399-3046.2005.00403.x]

20 **Posfay-Barbe KM**, Pittet LF, Sottas C, Grillet S, Wildhaber BE, Rodriguez M, Kaiser L, Belli DC, McLin VA, Siegrist CA. Varicella-zoster immunization in pediatric liver transplant recipients: safe and immunogenic. *Am J Transplant* 2012; **12**: 2974-2985 [PMID: 22994936 DOI: 10.1111/j.1600-6143.2012.04273.x]

21 **Shinjoh M**, Hoshino K, Takahashi T, Nakayama T. Updated data on effective and safe immunizations with live-attenuated vaccines for children after living donor liver transplantation. *Vaccine* 2015; **33**: 701-707 [PMID: 25510391 DOI: 10.1016/j.vaccine.2014.11.052]

22 **Fishman JA**. Infection in solid-organ transplant recipients. *N Engl J Med* 2007; **357**: 2601-2614 [PMID: 18094380 DOI: 10.1056/NEJMra064928]

23 **Shinjoh M**, Miyairi I, Hoshino K, Takahashi T, Nakayama T. Effective and safe immunizations with live-attenuated vaccines for children after living donor liver transplantation. *Vaccine* 2008; **26**: 6859-6863 [PMID: 18930096 DOI: 10.1016/j.vaccine.2008.09.076]

24 **Kumar D**, Humar A, Plevneshi A, Green K, Prasad GV, Siegal D; Toronto Invasive Bacterial Diseases Network; McGeer A. Invasive pneumococcal disease in solid organ transplant recipients--10-year prospective population surveillance. *Am J Transplant* 2007; **7**: 1209-1214 [PMID: 17286615 DOI: 10.1111/j.1600-6143.2006.01705.x]

25 **Apalsch AM**, Green M, Ledesma-Medina J, Nour B, Wald ER. Parainfluenza and influenza virus infections in pediatric organ transplant recipients. *Clin Infect Dis* 1995; **20**: 394-399 [PMID: 7742447 DOI: 10.1093/clinids/20.2.394]

26 **Sintusek P**, Posuwan N, Wanawongsawad P, Jitraruch S, Poovorawan Y, Chongsrisawat V. High prevalence of hepatitis B-antibody loss and a case report of *de novo* hepatitis B virus infection in a child after living-donor liver transplantation. *World J Gastroenterol* 2018; **24**: 752-762 [PMID: 29456414 DOI: 10.3748/wjg.v24.i6.752]

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ORIGINAL ARTICLE

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Endoscopic retrograde cholangiopancreatography and liver biopsy in the evaluation of elevated liver function tests after liver transplantation

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Abstract

BACKGROUND

Abnormal liver function tests (LFTs) in post-liver transplant (LT) patients pose a challenge in the timing and selection of diagnostic modalities. There are little data regarding the accuracy of endoscopic retrograde cholangiopancreatography (ERCP) and liver biopsy (LB) in diagnosing post-transplant complications.

AIM

To evaluate the diagnostic performance of ERCP and LB in patients with non-vascular post-LT complications.

METHODS

This single-center retrospective study evaluated patients undergoing both ERCP and LB for evaluation of elevated LFTs within 6 mo of LT from 2000 to 2017. Diagnostic operating characteristics including accuracy, sensitivity and specificity for various diagnoses were calculated for ERCP and LB. The R factor (ratio of alkaline phosphatase to alanine aminotransferase) was also calculated for each patient.

RESULTS

Of the 1284 patients who underwent LT, 91 patients (74.7% males, mean age of 51) were analyzed. Anastomotic strictures (AS, 24.2%), acute cellular rejection (ACR, 11%) and concurrent AS/ACR (14.3%) were the most common diagnoses. ERCP

carried an accuracy of 79.1% (95%CI: 69.3-86.9), LB had an accuracy of 93.4% (95%CI: 86.2-97.5), and the combination of the two had an accuracy of 100% (95%CI: 96-100). There was no difference between patients with AS and ACR in mean R factor (AS: 1.9 *vs* ACR: 1.1, $P = 0.24$). Adverse events did not differ between the two tests (ERCP: 3.1% *vs* LB: 1.1%, $P = 0.31$).

CONCLUSION
In patients with abnormal LFTs after LT without vascular complications, the combination of LB and ERCP carries low risk and improves diagnostic accuracy over either test alone.

Key Words: Liver transplantation; Endoscopic retrograde cholangiopancreatography; Liver biopsy; Abnormal liver tests; Acute cellular rejection; Anastomotic biliary stricture

Core Tip: Patients commonly develop unexplained elevations in liver function tests after liver transplantation. After cross sectional imaging and basic lab tests, endoscopic retrograde cholangiopancreatography (ERCP) and liver biopsy (LB) are both performed in arbitrary fashion since the diagnostic capacity of each test remains unclear. In this study we found that ERCP and LB are both effective diagnostic tests in the setting of the 2 most common diagnoses, anastomotic biliary stricture and acute cellular rejection. Combining these tests increases the overall diagnostic accuracy to 100%, and both tests carried adverse event rates of < 5%. This study justifies combining ERCP and LB when the diagnosis remains elusive.

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INTRODUCTION

Since 2012, the number of liver transplants (LTs) performed annually in the United States has increased each year, reaching a record number of 8250 in 2018[1]. Just as the field of transplantation has evolved over the past 5 decades, so too have the nuances of post-transplant clinical care. Clinicians commonly face the conundrum of abnormal liver function tests (LFTs) soon after LT which often indicates a transplant-related complication. Practice guidelines provided by the American Association for the Study of Liver Diseases (AASLD), American Society of Transplantation, and the European Association for the Study of the Liver note that the frequency of monitoring LFTs after LT and the subsequent work-up should be individualized to the patient and time after LT, prior complications, stability of serial testing, and the suspected underlying pathology[2,3].

The underlying cause, however, can be challenging to discern. Depending on the pattern of abnormal LFTs, evaluation of the biliary system with transabdominal ultrasound, MRI, CT, and/or endoscopic retrograde cholangiopancreatography (ERCP) may be most appropriate when the LFT pattern is cholestatic, whereas liver biopsy (LB) should be performed first when parenchymal injury is suspected [2]. To date, there are insufficient data regarding the relative accuracy of ERCP and LB in diagnosing specific post-LT complications. Current societal guidelines strongly support both of these tests (Grade 1A recommendations) but provide little guidance on which should be performed initially[2]. The decision to choose LB, ERCP, or both (and in which order) is therefore left to the discretion of the transplant surgeon, hepatologist, or interventional endoscopist. The primary aim of this study was to evaluate the diagnostic performance of ERCP and LB in patients with non-vascular post-LT complications.

MATERIALS AND METHODS

This was a single-center, retrospective review of all patients who underwent LT followed by both LB and ERCP at the University of Colorado Hospital from January 2000 to June 2017.

Patients

Patients undergoing deceased or living donor LT at our center during the study period were identified

using the LT database. Inclusion criteria included adult patients post-LT who underwent both LB and ERCP within 6 mo after LT with a primary indication of elevated LFTs. Patients with a clearly identifiable cause of elevated LFTs–such as drug or medication-related hepatitis, vascular liver disease or infectious hepatitis based on the initial history, labs, or imaging studies-were excluded from the analysis. Patients who did not receive post-LT care at our institution were also excluded. Post-LT biliary anatomy types included duct-to-duct (DD) anastomosis and Roux-en-Y hepaticojejunostomy (RYHJ).

Patients with a mixed pattern of liver injury based on LFTs underwent either LB or ERCP initially at the discretion of the provider. ERCP was the first invasive diagnostic test performed when patients had symptoms suggestive of cholangitis or a predominantly cholestatic pattern of elevated LFTs. LB was performed after labs and cross-sectional imaging when hepatocellular disease was suspected. It is our practice to monitor immunosuppressant levels on all post-LT patients. Approval from the Colorado Multi-Institutional Review Board was obtained prior to beginning the study.

ERCP

ERCP was performed under conscious sedation, monitored anesthesia care, or general anesthesia by one of 7 advanced endoscopists who have performed > 1000 ERCPs each. Endoscopists utilized the standard technique in cannulating the bile duct and performing cholangiography. Occlusion cholangiography was used to visualize the entire native and donor biliary tree with particular attention paid to the anastomosis. Biliary sphincterotomy was performed in select cases at the discretion of the endoscopist. If present, strictures were treated with the placement of plastic or fully covered metal stents were placed across strictures according to the endoscopist's judgment. Dilation of strictures *via* balloon or catheter was performed prior to stenting in select cases.

Conventional techniques such as balloon and basket sweeping were used to remove bile duct stones and/or casts, and single or multiple stents were placed across anastomotic bile duct leaks. For patients with DD biliary anastomosis, a standard duodenoscope was used to reach the ampulla. For patients with RYHJ anatomy either a pediatric colonoscope or small bowel enteroscope (single-balloon, double-balloon, or rotational overtube) was used to reach the biliary anastomosis.

LB

While percutaneous (ultrasound-guided) LB represented the preferred route of biopsy, transjugular LB was generally performed in patients with an International Normalized Ratio > 1.5, when intravascular pressure measurements were needed, or when the abdominal anatomy precluded a safe percutaneous approach. Both percutaneous and transjugular LB were performed under conscious sedation. LB techniques are described in detail in an AASLD position paper[4]. Board certified GI pathologists examined all histology samples.

Outcomes and definitions

The study's primary outcome was the accuracy of ERCP and LB in making the ultimate final diagnosis or diagnoses driving the abnormal LFTs, as determined by the GI and Hepatology services. Secondary outcomes included sensitivity and specificity for ERCP and LB in the final diagnosis. Acute cellular rejection (ACR) was defined and graded using a 1-9 scale based on histopathologic findings using the rejection activity index, which was based on inflammatory changes in the portal triads, bile ducts, and venous endothelium (with scores of 1-3 for each of the 3 categories)[5]. A score of 3 or more was classified as definite ACR (Figure 1)[5]. Recurrent hepatitis C infection (HCV) after LT was defined by detectable serum HCV RNA. Anastomotic stricture (AS) was defined as a benign-appearing narrowing in the region of the biliary anastomosis during ERCP, typically within 5-6 mm from the suture line, usually associated with delayed contrast drainage and/or moderate resistance to passage of an inflated 12 mm balloon (Figure 2).

True positive results for LB or ERCP were defined by findings supportive of at least one of the final diagnosis/es as defined above. True negative results were defined by ERCP or LB results that failed to support the final diagnosis/es with or without supporting an alternative diagnosis. For example, if LB showed signs of a large bile duct obstruction or cholangitis, this was considered a true positive for a final diagnosis of anastomotic stricture or cholangitis, respectively. Conversely, if ERCP did not show biliary pathology, this was considered a false negative when the final diagnosis was a hepatocellular disorder such as ACR or recurrent HCV.

Statistical analysis

Descriptive statistics were used to depict patient demographics, symptoms and laboratory data. An R factor was calculated as the ratio between the degree of elevation of alkaline phosphatase and the degree of elevation of alanine aminotransferase[6]. R factors > 5 were considered to be consistent with hepatocellular damage and R factors < 2 suggested cholestatic patterns of injury, with R factors between 2 and 5 suggesting a mixed pattern of injury. Diagnostic operating characteristics including sensitivity, specificity, and accuracy [(true positive + true negative)/(true positive + false negative + false positive + true negative)] were calculated for both ERCP and LB. Fisher's exact test or the chi square test were used to compare categorical variables between patients with ACR and AS. The student's *t*-test was used to

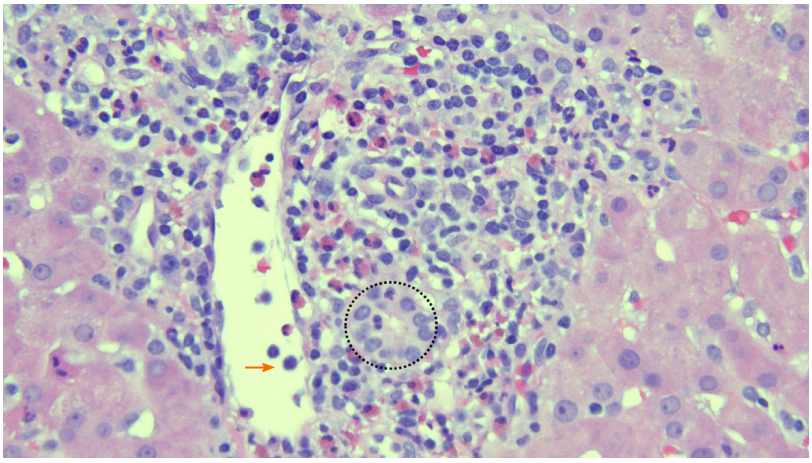

Figure 1 Photomicrograph of representative portal tract in acute cellular rejection. Mixed, lymphocyte predominant portal-based inflammation, bile duct inflammation characterized by lymphocyte infiltration (circle), and a large portal venule with subendothelial lymphocyte infiltration and intraluminal lymphocyte tethering[24] (hematoxylin and eosin stain, 40 ×).

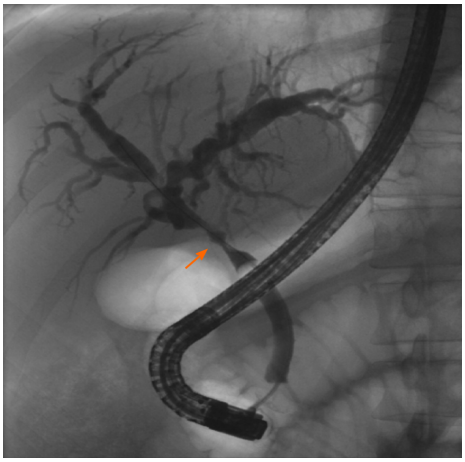

Figure 2 Cholangiogram during endoscopic retrograde cholangiopancreatography demonstrating an anastomotic stricture (arrow).

compare continuous variables between patients with ACR and AS. Adverse event rates were compared between ERCP and LB using the Fisher's exact test. All statistical analysis was performed using STATA 15.1 (StataCorp, College Station, TX, United States).

RESULTS

Patients

A total of 1284 patients underwent LT at our center during the study period (Figure 3). Of these, 96 patients (7.5%) received both an ERCP and LB for evaluation of persistently elevated LFTs within the first 6 mo after LT. Ninety-one patients received long-term follow-up at our institution and were included in the final analysis. The mean time interval between the 2 procedures was 9.1 d (SD 6.9).

The mean age of the cohort was 51 (SD 12.1) and 74.7% (n = 68) were male (Table 1). Deceased donor transplants (n = 73, 80.2%) accounted for the majority of transplants, and 73.6% (n = 67) had DD biliary anatomy. Presenting symptoms included jaundice (23.1%, n = 21), abdominal pain (15.4%, n = 14), and fever (12.1%, n = 11), and 21 (25%) patients were asymptomatic. Initial imaging consisted of ultrasound (74.7%), CT (18.7%), and magnetic resonance cholangiopancreatography (MRCP, 6.6%) with a mean donor bile duct diameter of 4.6 (SD 1.9) mm. Imaging revealed a dilated duct in 9 (9.9%, 8 with ultrasound, 1 with MRCP) of patients. LB was performed as the first of the 2 tests in 51 (56%) patients, and 71.4% (n = 65) of LBs were performed *via* the percutaneous route. Nearly 75% of patients were on dual immunosuppression therapy (n = 68) with 22% of patients on monotherapy (n = 20) with the combination of tacrolimus and mycophenolate sodium being the most common combination therapy (n = 21).

Table 1 Baseline characteristics reported as *n* (%) or mean (SD)

Variable	Overall cohort (*n* = 91)
Age	51 (12.1)
Sex (male)	68 (74.7)
Presenting symptom	
Jaundice	21 (23.1)
Fever	11 (12.1)
Abdominal pain	14 (15.4)
Asymptomatic	21 (25)
Liver biopsy performed first	51 (56)
Percutaneous liver biopsy	65 (71.4)
Bile duct diameter (mm)	4.6 (1.9)
R factor	2 (2.4), Range: 0.1-6.4
Alkaline phosphatase (international units/liter)	392.6 (248.4)
AST (units/liter)	200.5 (674.8)
ALT (units/liter)	205.4 (444.2)
Total bilirubin (mg/dL)	4.5 (5.4)
Deceased donor	73 (80.2)
Transplant biliary anatomy	
Duct-to-duct	67 (73.6)
Roux-en-Y hepaticojejunostomy	24 (26.4)
Tacrolimus	66 (73.3)
Sirolimus	20 (22.2)
Everolimus	6 (6.6)
Mycophenolate sodium	28 (31.1)
Mycophenolate mofetil	13 (14.4)
Cyclosporine	16 (17.8)
Prednisone	20 (22.2)
Immunosuppression monotherapy	20 (22)
Dual immunosuppression therapy	68 (74.7)
Triple immunosuppression therapy	3 (3.3)

AST: Aspartate aminotransferase; ALT: Alanine transaminase.

Technically, all LB and ERCP procedures were performed successfully. The most common single diagnosis ultimately was AS (34.1%), followed by ACR (11%) with all diagnoses displayed in Table 2. A total of 29 (31.9%) patients had multiple concurrent diagnoses contributing to the elevation in LFTs (and included as final diagnoses), and the most common was a dual diagnosis of AS with ACR (14.3%, *n* = 13). Four (4.4%) patients had 3 concurrent diagnoses, all of which included ACR and AS (Table 2).

Diagnostic operating characteristics

The diagnostic operating characteristics of LB and ERCP are shown in Table 3. The overall accuracy of ERCP was 79.1% (95%CI: 69.3-86.9). The overall accuracy of LB was 93.4% (95%CI: 86.2-97.5). Combined, the 2 tests had an overall accuracy of 100% (95%CI: 96-100).

For AS, ERCP had an accuracy of 100% (95%CI: 84.6-100) while LB had an accuracy of 72.7% (95%CI: 49.8-89.3). For ACR, LB had an accuracy of 100% (95%CI: 69.2-100) while ERCP had an accuracy of 0% (95%CI: 0-30.9). Sensitivities carried the same values as the accuracy in all cases due to the lack of false positive results. For the same reason, specificity could not be calculated for any of the diagnostic tests.

Table 2 Etiologies of liver function test elevation reported as *n* (%)

Single diagnosis	*n* (%)
Anastomotic stricture	31 (34.1)
Acute cellular rejection	10 (11)
Recurrent primary sclerosing cholangitis	6 (19.4)
Recurrent HCV	5 (5.5)
Biliary cast syndrome	3 (3.3)
Ischemic cholangiopathy	2 (2.2)
Papillary stenosis	1 (1.1)
Posterior reversible encephalopathy syndrome	1 (1.1)
Cholestatic hepatitis	1 (1.1)
Recurrent PBC	1 (1.1)
Venous outflow obstruction	1 (1.1)
Two diagnoses	
Anastomotic stricture and acute cellular rejection	13 (14.3)
Recurrent HCV and anastomotic stricture	6 (19.4)
Bile leak and acute cellular rejection	2 (2.2)
Congestive hepatopathy and anastomotic stricture	1 (1.1)
Anastomotic stricture and suprahepatic cava stenosis	1 (1.1)
Recurrent PBC and anastomotic stricture	1 (1.1)
CMV hepatitis and bile leak	1 (1.1)
Three diagnoses	
Acute cellular rejection, anastomotic stricture, and recurrent HCV	2 (2.2)
Acute cellular rejection, anastomotic stricture, and de novo autoimmune hepatitis	1 (1.1)
Acute cellular rejection, anastomotic stricture, and CMV hepatitis	1 (1.1)

HCV: Hepatitis C virus; PBC: Primary biliary cholangitis; CMV: Cytomegalovirus.

Table 3 Operating characteristics for endoscopic retrograde cholangiopancreatography and liver biopsy in diagnosing post-liver transplant complications

	ERCP	LB	ERCP + LB
Overall accuracy % (95%CI)	79.1 (69.3-86.9)	93.4 (86.2-97.5)	100 (96-100)
Overall sensitivity % (95%CI)	79.1 (69.3-86.9)	93.4 (86.2-97.5)	100 (96-100)
Acute cellular rejection accuracy % (95%CI)	0 (0-30.9)	100 (69.2-100)	100 (91.9-100)
Anastomotic stricture accuracy % (95%CI)	100 (84.6-100)	72.7 (49.8-89.3)	100 (89.4-100)

ERCP: Endoscopic retrograde cholangiopancreatography; LB: Liver biopsy.

Liver function tests

The mean R factor (ratio of alkaline phosphatase and alanine aminotransferase) was 2 (SD 2.4), with a mean alkaline phosphatase (AP) level of 392.6 (SD 248.4) IU/L and mean total bilirubin (TB) level of 4.5 (SD 5.4) mg/dL. The mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 200.5 (SD 674.8) and 205.4 (444.2), respectively. Between patients with AS and patients with ACR, there was no significant difference in R factor (AS: 1.9 *vs* ACR: 1.1, $P = 0.24$), AP (AS: 376.3 *vs* ACR: 452.2, $P = 0.48$), TB (AS: 4.1 *vs* ACR: 5.5, $P = 0.41$), AST (AS: 130.9 *vs* ACR: 127.9, $P = 0.94$), or ALT (AS: 203.1 *vs* ACR: 169.5, $P = 0.58$). There was also no difference between the 2 diagnoses in terms of bile duct diameter (AS: 4.8 mm *vs* ACR: 3.8 mm, $P = 0.36$). Patients with concurrent AS and ACR had a mean R

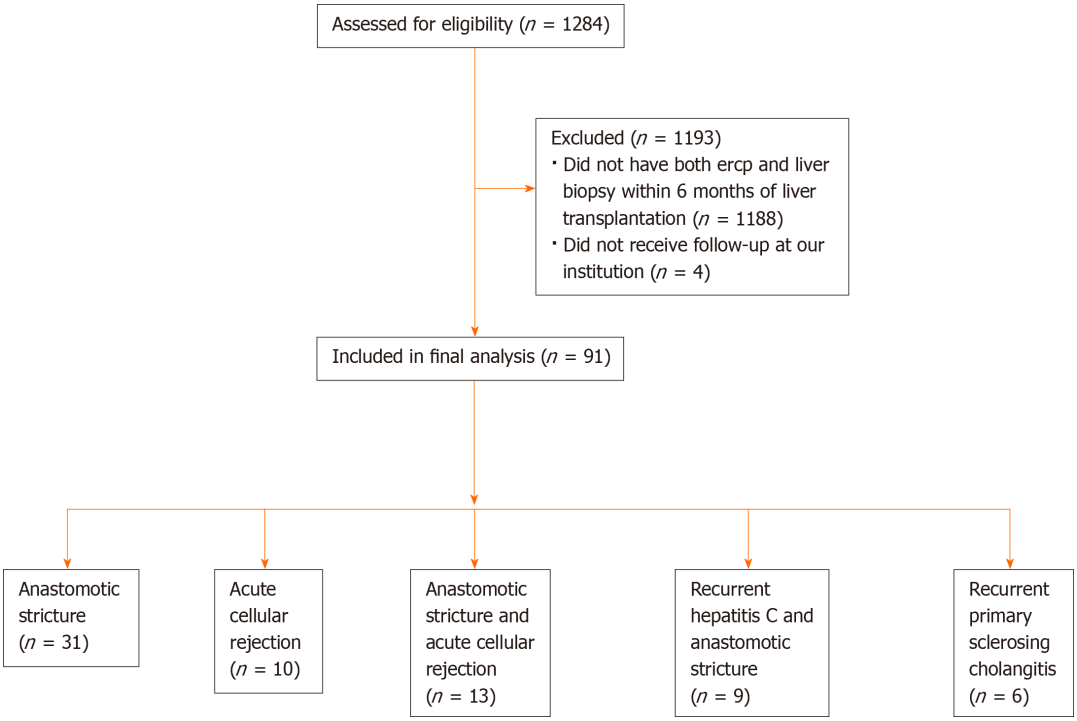

Figure 3 Flow diagram of patients. ERCP: Endoscopic retrograde cholangiopancreatography.

factor of 1.06 (0.7).

Adverse events

A total of 3 adverse events occurred after 96 ERCPs (3.1%): 1 case of mild post-ERCP pancreatitis treated conservatively, and 2 cases of post-procedure abdominal pain requiring overnight hospitalization and supportive care. One adverse event occurred after LB, a hepatoportal fistula that required hospitalization and angiography with embolization by Interventional Radiology. There was no significant difference in the adverse event rates due to ERCP or LB (3.1% *vs* 1.1%, $P = 0.31$).

DISCUSSION

It is common to encounter asymptomatic patients with abnormal LFTs in the post-LT setting, as well as symptomatic patients with normal LFTs. It is also common for patients to undergo multiple invasive diagnostic tests as part of the work-up. Abnormal LFTs post-LT are a major cause of unplanned hospital readmissions, and the ensuing work-up may consume significant resources[7]. ERCP is the accepted diagnostic and therapeutic test for suspected biliary pathology and LB is the accepted test for suspected hepatocellular pathology. But in reality, because of the poor specificity of LFT patterns and the limitations of cross-sectional imaging, patients with post-LT LFT elevations will too often undergo both procedures. The timing and order of these procedures is left to the discretion of the transplant surgeon, hepatologist and advanced endoscopist, with little evidence to guide them. Despite the high incidence of immune-mediated and biliary complications following LT, the usual clinical tools (*e.g.*, clinical history, LFT patterns, bile duct diameter on imaging) are poorly specific for any single diagnosis. Besides the main finding of our study, this study demonstrated that patients with AS had no significant difference from patients with ACR in terms of R factor, alkaline phosphatase level, total bilirubin level, AST level, ALT level, or bile duct diameter. Hence, additional testing with LB and ERCP was justified.

Ultrasound and MRCP have variable accuracy in diagnosing biliary pathology post-LT, since obstructive ductal dilation in the transplanted liver is variable. Several studies have demonstrated poor sensitivity and specificity of bile duct diameter post-LT[8-11]. While both modalities can detect biliary dilatation, MRCP offers an advantage over ultrasound in being able to detect biliary strictures with a sensitivity ranging from 64%-79%[9,12]. While both of these modalities are first-line options for imaging in the diagnostic work-up of elevated LFTs after LT, we have found that MRCP both under-estimates and over-estimates stenosis size and severity. Additionally, ERCP permits a real-time accurate assessment of strictures, based on contrast drainage and balloon passage, and the ability to perform stricture therapy. For these reasons, we generally go straight to ERCP and bypass MRCP when there is significant ductal dilation, a cholestatic pattern of LFTs, or a negative LB.

To our knowledge, this is the largest study evaluating the diagnostic performance of combined LB and LT in patients with abnormal LFTs after LT. Our novel finding in this study is the high diagnostic accuracy for ERCP and LB, in contrast to standard laboratory tests or cross-sectional imaging. Diagnostic accuracy was 79.1% overall for ERCP and 93.4% overall for LB. Combined, the 2 tests study had an overall diagnostic accuracy of 100%.

ACR and AS were the most frequent final diagnoses in our patients. These are commonly encountered diseases in the LT population, but the differential diagnosis remains broad (Figure 4) and includes de novo autoimmune hepatitis, recurrent liver disease (HCV, PSC, others), drug toxicity, de novo infection, biliary stones or casts, hepatic artery thrombosis, and more[2]. We recognize that a previously common clinical dilemma–differentiating recurrent HCV from ACR or other etiologies–is less common in the current direct-acting antiviral (DAA) era, and our study included patients in the current and pre-DAA eras.

In the early days of LT, ACR was a near-universal complication resulting in long-term graft failure [13,14]. Advances in immunosuppression have subsequently led to reduced rates of allograft rejection, though the incidence still ranges from 20% to 40% after LT, with most occurring within the first month [15-17]. In addition, ACR remains clinically significant, impacting long-term graft survival and mortality [18]. The incidence of biliary complications after LT is highly variable but still relatively common. The estimated incidence of AS post-LT is up to 20% for patients following deceased donor LT and 19%-40% after living donor liver transplantation. Risk factors include graft ischemia, DD anastomosis, reperfusion injury, deceased donor, and hepatic artery thrombosis. The incidence of non-anastomotic stricture is 0.5% to 10%, while stones/sludge are seen post-LT in approximately 5% of patients. Biliary cast syndrome is less common (2.5%-3%)[19-22].

It is critical to make a prompt and diagnosis when a transplanted patient presents with abnormal LFTs, since graft survival depends on timely and appropriate treatment. While ACR is successfully treated with various combinations of immunosuppressive medication, the management of biliary complications is procedural. AS may be treated successfully with endoscopic placement of multiple plastic stents or a covered metal stent. Recent data suggests that metal stents incur fewer procedures and costs while leading to stricture resolution similarly to plastic stents[23].

Our study sheds light on the frequency of dual diagnoses in patients with abnormal LFTs post-LT, which is an under-studied phenomenon. In this study, 34 (37.4%) patients had multiple diagnoses, of which the most common combination was AS plus ACR (14.3%). Four patients (4.4%) ultimately received 3 final diagnoses. In practice, patients receive therapy for multiple diseases concurrently (*e.g.* stenting for AS plus corticosteroid bursts for AS), so knowing which diagnosis is dominant can be challenging. Previous studies assessing abnormal LFTs in the post-LT population mostly included patients undergoing LB or ERCP but not both, so our study may represent more complex, sicker patients[7]. Alternatively, some of the various diagnoses in our patients may be clinically silent. AS, for example, is quite subjective and may be diagnosed or treated by endoscopists even though the stricture may not be high-grade or impede bile flow.

Our findings suggest that physicians managing post-LT patients can have a lower threshold to perform both LB and ERCP when evaluating abnormal LFTs within the context of the patient's clinical presentation. While one modality alone has high diagnostic accuracy over lab tests and imaging, LB and ERCP combined have a very high diagnostic accuracy. Ultimately the decision to perform one test over the other depends on clinician experience, but both tests improve the diagnostic accuracy over one test alone. However, despite the high prevalence of multiple final diagnoses (37.4%), only 96 of 1284 transplanted patients at our center underwent both ERCP and LB during the study period, suggesting they are used sparingly overall. Finally, the adverse event rates of ERCP and LB are low, and we demonstrated no significant difference between the two.

This study was limited by its size and design. It was performed at a single, United States tertiary care hospital with experienced endoscopists and transplant hepatologists, so the results may not be generalizable to other centers. The final diagnosis was determined by review of the medical record and hence may be affected by bias or subjectivity amongst the various treating physicians. Moreover, a reproducible, objective grading score for AS has not been established. The study was also limited by its retrospective nature and by limiting the analysis to patients undergoing ERCP and LB early after LT during the 17-year study period. An additional limitation is the variable time gap between ERCP and LB, although across the entire study population the mean time interval between both procedures was relatively short (9.1 d) suggesting that the diagnostic evaluation typically occurred during a single clinical episode. Despite these limitations, our cohort represents the modern-day practice of ERCP and LB after LT, and the study permits a comparison between the 2 key diagnostic tests in the most common clinical scenarios. Future studies may include a prospective evaluation of abnormal LFTs post-LT or outcomes of post-LT patients who undergo empiric treatment without LB or ERCP.

CONCLUSION

In summary, these results offer insight into the diagnostic and etiology of abnormal LFTs after LT, in

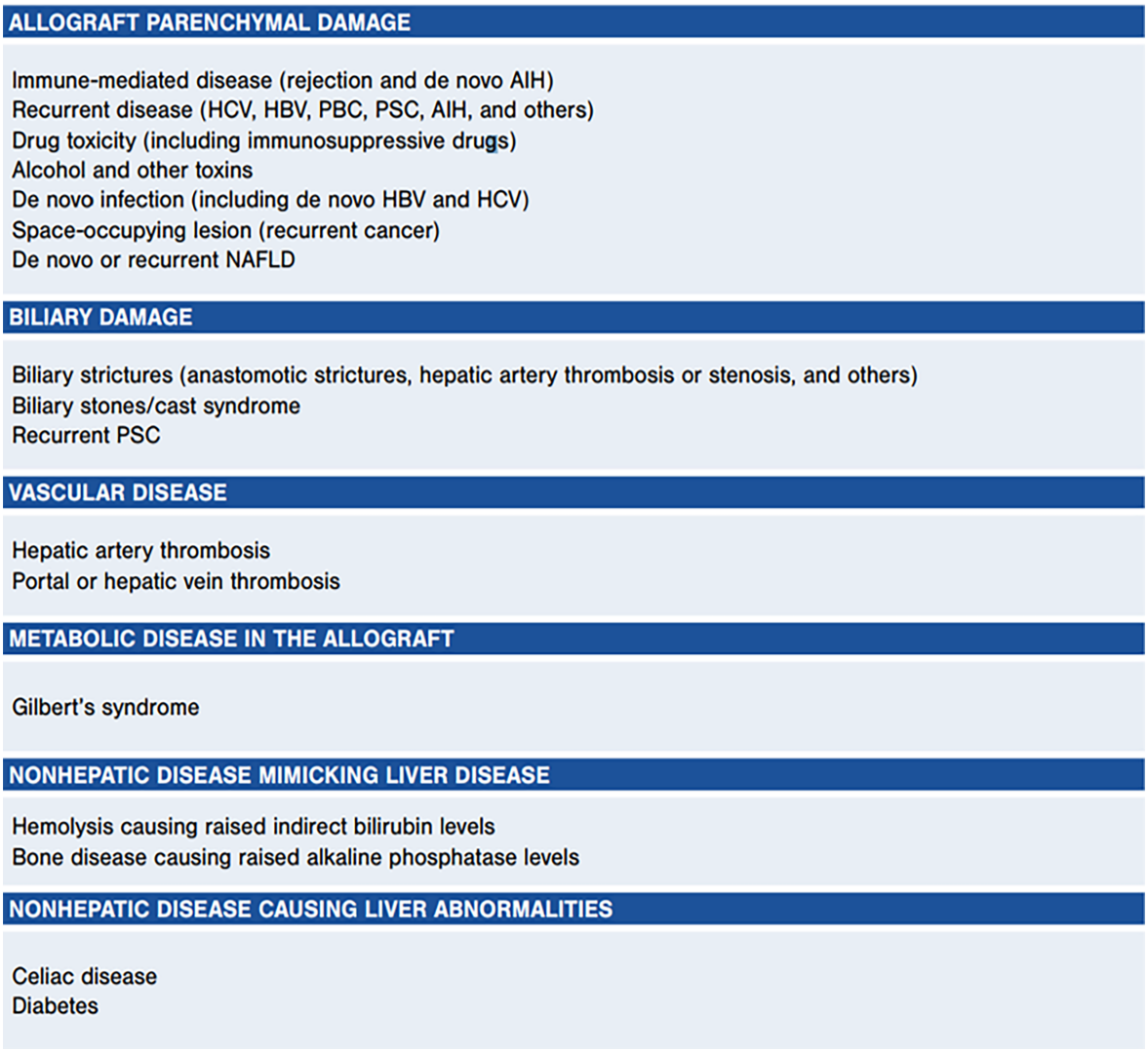

Figure 4 Causes of liver test abnormalities after liver transplantation. Legend: Used with permission from Lucey *et al*[2], 2013. HBV: Hepatitis B virus; HCV: Hepatitis C virus; PBC: Primary biliary cholangitis; PSC: Pulmonary scar cancer.

which standard lab and imaging studies have poor specificity. Our study shows that LB and ERCP improve diagnostic accuracy over either test alone and carry low risk. Dual diagnoses are relatively common in this population. In the future, prospective and multicenter studies should include patients undergoing LB and ERCP beyond the early post-LT period and establish reproducible, objective criteria for the ultimate diagnosis.

ARTICLE HIGHLIGHTS

Research background

Elevated liver function tests (LFTs) are commonly encountered in the post-liver transplant (LT) setting. When a diagnosis is not made by history, labs, and cross-sectional imaging, endoscopic retrograde cholangiopancreatography (ERCP) and liver biopsy (LB) are commonly performed. However, the diagnostic performance of each of these tests individually and in combination remains unknown.

Research motivation

We first hoped to determine what are the most common diagnoses in the population of patients with elevated LFTs after LT. At the same time, we want to assess the diagnostic performance of both ERCP and LB in these patients so that we can decide which of these tests is safer and more effective at clinching the diagnosis.

Research objectives

We aimed to assess the diagnostic accuracy and safety of ERCP and LB together and in isolation for a final diagnosis in patients with unexplained LFT elevations after LT.

Research methods

In this single-center, retrospective study we evaluated patients undergoing both ERCP and LB for the evaluation of elevated LFTs within 6 mo of LT based on review of existing medical records. Diagnostic accuracy, sensitivity and specificity for the various final diagnoses were calculated for each test.

Research results

Anastomotic strictures (AS), acute cellular rejection (ACR) and concurrent AS and ACR were the most common diagnoses. ERCP carried an accuracy of 79.1%, LB had an accuracy of 93.4%, and the combination of the 2 had an accuracy of 100% (95%CI: 96-100). The pattern of liver chemistries (R Factor) did not diagnostic accuracy of either test. Adverse event rates did not differ between the 2 tests.

Research conclusions

While LB had a higher accuracy than ERCP, the combination of the 2 tests had an accuracy of 100% and a low adverse event rate, suggesting that physicians can have a low threshold in utilizing both modalities for the evaluation of elevated LFTs.

Research perspectives

In patients with elevated LFTs after LT without a diagnosis, neither LB nor ERCP is clearly superior. Both tests can be used and the decision to use one over the other will depend on the clinical context and physician preference. However, when necessary both tests can be used safely together to reach a final diagnosis in nearly all patients.

FOOTNOTES

Author contributions: Han S designed and performed and research and wrote the manuscript; Kriss M designed the research and contributed to the analysis and revised the manuscript; Attwell A supervised the study and organized and edited the manuscript.

Institutional review board statement: This study was reviewed and approved by the Colorado Multiple Institutional Review Board.

Informed consent statement: As this was a retrospective study, informed consent was not required from patients.

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Data sharing statement: Certain additional data from the study results are available from the corresponding author at augustin.attwell@cuanschutz.edu. Otherwise no additional data is available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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REFERENCES

1 **Services USDoHH**. Transplants by Donor Type, Organ Procurement and Transplantation Network (OPTN). 2019. Available from: https://optn.transplant.hrsa.gov/

2 **Lucey MR**, Terrault N, Ojo L, Hay JE, Neuberger J, Blumberg E, Teperman LW. Long-term management of the successful adult liver transplant: 2012 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Liver Transpl* 2013; **19**: 3-26 [PMID: 23281277 DOI: 10.1002/lt.23566]

3 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [PMID: 26597456 DOI: 10.1016/j.jhep.2015.10.006]

4 **Rockey DC**, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology* 2009; **49**: 1017-1044 [PMID: 19243014 DOI: 10.1002/hep.22742]

5 Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; **25**: 658-663 [PMID: 9049215 DOI: 10.1002/hep.510250328]

6 **DeLeve LD**, Kaplowitz N. Mechanisms of drug-induced liver disease. *Gastroenterol Clin North Am* 1995; **24**: 787-810 [PMID: 8749899]

7 **Russell TA**, Angarita SAK, Showen A, Agopian V, Busuttil RW, Kaldas FM. Optimizing the Management of Abnormal Liver Function Tests after Orthotopic Liver Transplant: A Systems-Based Analysis of Health Care Utilization. *Am Surg* 2017; **83**: 1152-1156 [PMID: 29391114 DOI: 10.1177/000313481708301028]

8 **St Peter S**, Rodriquez-Davalos MI, Rodriguez-Luna HM, Harrison EM, Moss AA, Mulligan DC. Significance of proximal biliary dilatation in patients with anastomotic strictures after liver transplantation. *Dig Dis Sci* 2004; **49**: 1207-1211 [PMID: 15387348 DOI: 10.1023/B:DDAS.0000037814.96308.7a]

9 **Akbar A**, Tran QT, Nair SP, Parikh S, Bilal M, Ismail M, Vanatta JM, Eason JD, Satapathy SK. Role of MRCP in Diagnosing Biliary Anastomotic Strictures After Liver Transplantation: A Single Tertiary Care Center Experience. *Transplant Direct* 2018; **4**: e347 [PMID: 29796418 DOI: 10.1097/TXD.0000000000000789]

10 **Romagnuolo J**, Bardou M, Rahme E, Joseph L, Reinhold C, Barkun AN. Magnetic resonance cholangiopancreatography: a meta-analysis of test performance in suspected biliary disease. *Ann Intern Med* 2003; **139**: 547-557 [PMID: 14530225 DOI: 10.7326/0003-4819-139-7-200310070-00006]

11 **Jorgensen JE**, Waljee AK, Volk ML, Sonnenday CJ, Elta GH, Al-Hawary MM, Singal AG, Taylor JR, Elmunzer BJ. Is MRCP equivalent to ERCP for diagnosing biliary obstruction in orthotopic liver transplant recipients? *Gastrointest Endosc* 2011; **73**: 955-962 [PMID: 21316670 DOI: 10.1016/j.gie.2010.12.014]

12 **Beswick DM**, Miraglia R, Caruso S, Marrone G, Gruttadauria S, Zajko AB, Luca A. The role of ultrasound and magnetic resonance cholangiopancreatography for the diagnosis of biliary stricture after liver transplantation. *Eur J Radiol* 2012; **81**: 2089-2092 [PMID: 21906897 DOI: 10.1016/j.ejrad.2011.07.008]

13 **Nacif LS**, Pinheiro RS, Pécora RA, Ducatti L, Rocha-Santos V, Andraus W, D'Albuquerque LC. Late acute rejection in liver transplant: a systematic review. *Arq Bras Cir Dig* 2015; **28**: 212-215 [PMID: 26537150 DOI: 10.1590/S0102-67202015000300017]

14 **Thurairajah PH**, Carbone M, Bridgestock H, Thomas P, Hebbar S, Gunson BK, Shah T, Neuberger J. Late acute liver allograft rejection; a study of its natural history and graft survival in the current era. *Transplantation* 2013; **95**: 955-959 [PMID: 23442806 DOI: 10.1097/TP.0b013e3182845f6c]

15 **Campsen J**, Zimmerman MA, Mandell S, Kaplan M, Kam I. A Decade of Experience Using mTor Inhibitors in Liver Transplantation. *J Transplant* 2011; **2011**: 913094 [PMID: 21461386 DOI: 10.1155/2011/913094]

16 **Wang YC**, Wu TJ, Wu TH, Lee CF, Chou HS, Chan KM, Lee WC. The risk factors to predict acute rejection in liver transplantation. *Transplant Proc* 2012; **44**: 526-528 [PMID: 22410062 DOI: 10.1016/j.transproceed.2012.01.041]

17 **González MG**, Madrazo CP, Rodríguez AB, Gutiérrez MG, Herrero JI, Pallardó JM, Ortiz de Urbina J, Paricio PP. An open, randomized, multicenter clinical trial of oral tacrolimus in liver allograft transplantation: a comparison of dual vs. triple drug therapy. *Liver Transpl* 2005; **11**: 515-524 [PMID: 15838889 DOI: 10.1002/Lt.20382]

18 **Levitsky J**, Goldberg D, Smith AR, Mansfield SA, Gillespie BW, Merion RM, Lok AS, Levy G, Kulik L, Abecassis M, Shaked A. Acute Rejection Increases Risk of Graft Failure and Death in Recent Liver Transplant Recipients. *Clin Gastroenterol Hepatol* 2017; **15**: 584-593. e2 [PMID: 27567694 DOI: 10.1016/j.cgh.2016.07.035]

19 **Albert JG**, Filmann N, Elsner J, Moench C, Trojan J, Bojunga J, Sarrazin C, Friedrich-Rust M, Herrmann E, Bechstein WO, Zeuzem S, Hofmann WP. Long-term follow-up of endoscopic therapy in stenosis of the bilio-biliary anastomosis associated with orthotopic liver transplantation. *Liver Transpl* 2013 [PMID: 23526624 DOI: 10.1002/lt.22643]

20 **Akamatsu N**, Sugawara Y, Hashimoto D. Biliary reconstruction, its complications and management of biliary complications after adult liver transplantation: a systematic review of the incidence, risk factors and outcome. *Transpl Int* 2011; **24**: 379-392 [PMID: 21143651 DOI: 10.1111/j.1432-2277.2010.01202.x]

21 **Sharma S**, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. *Liver Transpl* 2008; **14**: 759-769 [PMID: 18508368 DOI: 10.1002/lt.21509]

22 **Moy BT**, Birk JW. A Review on the Management of Biliary Complications after Orthotopic Liver Transplantation. *J Clin Transl Hepatol* 2019; **7**: 61-71 [PMID: 30944822 DOI: 10.14218/JCTH.2018.00028]

23 **Martins FP**, De Paulo GA, Contini MLC, Ferrari AP. Metal versus plastic stents for anastomotic biliary strictures after liver transplantation: a randomized controlled trial. *Gastrointest Endosc* 2018; **87**: 131.e1-131. e13 [PMID: 28455159 DOI: 10.1016/j.gie.2017.04.013]

24 **Cotton PB**, Garrow DA, Gallagher J, Romagnuolo J. Risk factors for complications after ERCP: a multivariate analysis of 11,497 procedures over 12 years. *Gastrointest Endosc* 2009; **70**: 80-88 [PMID: 19286178 DOI: 10.1016/j.gie.2008.10.039]

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META-ANALYSIS

Effectiveness of entecavir in preventing hepatocellular carcinoma development is genotype-dependent in hepatitis B virus-associated liver cirrhosis

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Abstract

BACKGROUND

The oral nucleos(t)ide analogue, entecavir (ETV) was demonstrated to reduce the rate of hepatocellular carcinoma (HCC) in patients with hepatitis B virus (HBV)-associated liver cirrhosis. However, the reduction of HCC differs in various regions of the world.

AIM

To investigate the reduction of HCC development due to ETV therapy by meta-analysis.

METHODS

We surveyed the differences in HCC development following ETV treatment based on published articles using PubMed (2004-2019).

RESULTS

The regions with the most marked reduction in HCC development due to ETV therapy were Spain (1.0%/year) and Canada (Southern part, 1.3%/year), and the most ineffective areas were South Korea (3.6%-3.8%/year), China (3.3%/year), Taiwan (2.4%-3.1%/year), and Hong Kong (2.8%/year). Following ETV administration, the incidence of HCC in genotype D regions (1.89% ± 0.28%/year, mean ±

SE) was significantly lower than that in genotype C regions (2.91% ± 0.24%/year, $P < 0.01$). With regard to the initial HBV-DNA level, in genotype C patients (average: 5.61 Log_{10}IU/mL) this was almost the same as that in genotype D patients (average: 5.46 Log_{10}IU/mL). Moreover, there was no association between the prevalence ratio of HBV and the incidence of HCC on ETV treatment.

CONCLUSION

The effectiveness of ETV in preventing HCC development in HBV-associated liver cirrhosis is genotype-dependent.

Key Words: Hepatocellular carcinoma; Entecavir; Genotype of hepatitis B virus; Oral nucleos(t)ide analogue

Core Tip: Entecavir was demonstrated to reduce the rate of hepatocellular carcinoma (HCC) in patients with hepatitis B virus (HBV)-associated liver cirrhosis. The reduction of HCC differs in various regions of the world. We surveyed these differences based on published articles using PubMed (2004-2019). Following entecavir administration, the incidence of HCC in genotype D regions (1.89% ± 0.28%/year, mean ± SE) was significantly lower than that in genotype C regions (2.91% ± 0.24%/year, $P < 0.01$). The initial HBV-DNA level in genotype C patients was almost the same as that in genotype D patients. The effectiveness of entecavir in preventing HCC development in patients with HBV-associated liver cirrhosis is genotype-dependent.

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INTRODUCTION

The third-generation nucleos(t)ide analogue, entecavir (ETV) is currently recommended as one of the first-line antiviral therapies for chronic hepatitis B virus (HBV) infection. Moreover, it is generally accepted that long-term ETV treatment may reduce the incidence of hepatocellular carcinoma (HCC) in HBV-infected patients. Wong *et al*[1] demonstrated that the 5-year cumulative incidence of HCC was 13.8% in an ETV cohort *vs* 26.4% in a control cohort.

However, on surveying published reports, the effect of ETV in preventing HCC differed in various regions of the world. In this study, we examined the reduction of HCC development in various regions of the world, and the possible reasons for these differences.

MATERIALS AND METHODS

The PubMed database was searched (2004-2019) for studies published in English regarding the follow-up results of the development of HCC in patients with HBV-associated liver cirrhosis after treatment with ETV for more than 2 years. Studies with follow-up periods shorter than 3 years after ETV treatment were excluded.

In this study, we included only HBV cirrhotic cases. Furthermore, we surveyed the possible reasons for the differences in HCC reduction. We examined the association between the reduction in HCC development and initial HBV-DNA levels, which is a strong accelerating factor for HCC development [2], the prevalence of HBV in these regions, and HBV genotypes.

To compare the incidence of HCC between the main genotypes C and D, we calculated the weighted mean of the HCC incidence rate for each genotype using the random effect model (ref: Dersimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clinical Trials 1986; 7: 177-188). To assess whether the incidence rate among genotype D patients was lower than that among genotype C patients, we calculated the P value using a Z test. All reported P values correspond to two-sided tests, and those with $P < 0.05$ were considered significant. All analyses were performed with JMP version 12 (SAS Institute, Cary, NC, United States).

RESULTS

The results of HBV-associated cirrhotic patients administered ETV are presented in Table 1.

The regions where HCC development was markedly reduced by ETV therapy were Spain (1.0%/year)[3] and Canada (Southern part) (1.3%/year)[4]. The most ineffective regions were South Korea (3.6%-3.8%/year)[5,6], China (3.3%/year)[7], Taiwan (2.4%-3.1%/year)[8,9], Japan (Ehime, southern part of Japan 2.9%/year)[10], and Hong Kong (2.8%/year)[1]. The regions with a moderate reduction were Turkey (2.2%-2.7%/year)[11,12], the Caucasus (2.2%/year)[13], and Greece (1.8%/year)[14].

With regard to the genotype of HBV, the incidence of HCC in regions where the main prevalent type is D (1.89% ± 0.28%/year, mean ± SE) was significantly lower than that in regions where the main prevalent genotype is C (2.91% ± 0.24%/year, $P < 0.01$) (Table 2).

Moreover, the incidence of HCC in regions where the main prevalent genotype is C was significantly higher than that in regions where the main prevalent genotype was other than C (D + A, 1.61% ± 0.21%/year, $P < 0.0001$).

The initial HBV-DNA levels in genotype C patients (average 5.61 Log_{10}IU/mL) was almost the same as that in genotype D patients (average 5.46 Log_{10}IU/mL) (Table 3).

The association between the prevalence ratio of HBV in various countries and the incidence of HCC with ETV treatment was as follows (Table 1): The incidence of HCC with ETV treatment with a prevalence ratio of HBV of more than 8% was 2.64% ± 0.16%/year (mean ± SE), as compared with 2.39% ± 0.14%/year in regions where the prevalence ratio of HBV was 2%-7% (not significant, $P = 0.576$).

DISCUSSION

We demonstrated that there were marked differences in the impact of ETV treatment on reducing the risk of HCC in patients with HBV-associated cirrhosis in many countries of the world. We must consider why such differences exist.

Firstly, the genotypes of HBV should be considered. Genotype C is seen mostly in Asia, and genotype A in Northwest Europe, North America, India, and Africa. Genotype D is seen in Southern Europe, Middle Eastern Europe, and India. Various cross-sectional studies have found that patients with genotype C have more severe liver disease including cirrhosis or HCC than those with other genotypes [15,16].

In cohort studies of 426 chronic hepatitis B patients from Hong Kong[17] and of 4841 HBsAg-positive men from Taiwan[18], genotype C was associated with a 3-to 5-fold increased risk of HCC, respectively, compared with other HBV genotypes. Moreover, it was reported that the estimated 5-year cumulative incidence of HCC was 17% in East Asia where HBV genotype C is predominant and 10% in Western regions where HBV genotype D or A is predominant[19].

It is considered that the same tendency exists even on long-term treatment with ETV, and the incidence of HCC is higher in genotype C regions than in regions with other genotypes (especially genotype D).

In our studies, we demonstrated that ETV treatment of HBV cirrhotic patients with genotype C was less effective at preventing the occurrence of HCC than in those with other genotypes (chiefly genotype D).

In support of our findings, Kao *et al*[20] demonstrated differences in the response to lamivudine between HBV genotypes. They reported that genotype B showed a better virological response to lamivudine than genotype C in Taiwan.

Another factor that must be taken into account is the association between the prevalence ratio of HBV in various places and the incidence of HCC under ETV treatment. The incidence of HCC under ETV treatment where the prevalence ratio of HBV is more than 8% was 2.64% ± 0.16%/year, as compared with 2.39% ± 0.14%/year in regions where the prevalence ratio of HBV was 2%-7% (not significant, $P = 0.576$).

Another important factor that must be taken into consideration is the initial HBV-DNA level. However, we demonstrated that the initial HBV-DNA level in genotype C patients was almost the same as that in genotype D patients.

CONCLUSION

The impact of long-term ETV treatment on reducing the risk of HCC in patients with HBV cirrhosis differs in many countries of the world[1-13,21]. Moreover, it was demonstrated that effectiveness of ETV in preventing HCC development is genotype-dependent in HBV-associated liver cirrhosis.

Table 1 Difference in the impact of entecavir treatment on the risk of hepatocellular carcinoma in patients with hepatitis B virus-associated cirrhosis in various regions of the world

Ref.	Region	Main genotype	Prevalence ratio	Entecavir administered to HBV cirrhotics patients	Observation period (yr)	Incidence of HCC (%/yr)
Riveiro-Barciela *et al*[3]	Spain (Caucasian)	D	2%-7%	64	4.6	1.0
Coffin et al[4]	Canada (South)	D	< 2%	25	3.2	1.3
Hosaka *et al*[21]	Japan (Tokyo)	C	< 2%	79	5.0	1.4
Papatheodoridis *et al*[14]	Greece	A	2%-7%	69	3.3	1.8
Idilman *et al*[11]	Turkey	D	2%-7%	72	4.0	2.2
Arends *et al*[13]	Caucasus	D	> 8%	155	3.5	2.2
Su *et al*[8]	Taiwan	C	> 8%	1315	4.0	2.4
Köklü *et al*[12]	Turkey	D	2%-7%	73	3.0	2.7
Wong *et al*[1]	Hong Kong	C	> 8%	482	5.0	2.8
Watanabe et al [10]	Japan (Ehime)	C	2%-7%	86	5.0	2.9
Chen et al[9]	Taiwan	C	> 8%	586	4.9	3.1
Chen *et al*[2]	China (Chinese)	C	> 8%	61	4.0	3.3
Kim et al[5]	Korea	C	2%-7%	367	5.0	3.6
Choi et al[6]	Korea	C	2%-7%	510	4.0	3.8

HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus.

Table 2 Difference in the incidence of hepatocellular carcinoma under long-term treatment with entecavir between genotype C and genotype D cirrhotic patients

	Incidence of HCC (%/yr)	*P* value
Genotype C group (n = 8)	2.91 ± 0.24 (SE)	$P < 0.01$
Genotype D group (n = 5)	1.89 ± 0.28 (SE)	$P < 0.01$

HCC: Hepatocellular carcinoma.

Table 3 Comparison of initial hepatitis B virus deoxyribonucleic acid levels (log_{10} IU/mL) between genotype C and D cirrhotic patients treated with entecavir

Main genotype	Ref.	Entecavir administered to HBV cirrhotic patientssis	Initial HBV DNA	Average
C	Su *et al*[8]	1315	5.5	5.61
C	Wong et al[1]	482	5.0	
C	Watanabe *et al*[10]	86	6.4	
C	Chen et al[9]	586	5.9	
C	Chen *et al*[2]	61	5.8	
C	Kim et al[5]	367	4.6	
C	Choi et al[6]	510	6.7	
D	Riveiro-Barciela *et al*[3]	64	4.9	5.46
D	Coffin et al[4]	25	6.5	
D	Idilman *et al*[11]	72	5.5	
D	Arends *et al*[13]	155	5.4	
D	Köklü *et al*[12]	73	5.7	

HBV DNA: Hepatitis B virus deoxyribonucleic acid.

ARTICLE HIGHLIGHTS

Research background

The oral nucleos(t)ide analogue, entecavir (ETV) was demonstrated to reduce the rate of hepatocellular carcinoma (HCC) in patients with hepatitis B virus (HBV)-associated liver cirrhosis. However, the reduction in HCC is different in various countries of the world.

Research motivation

The relationship between the reduction of HCC and HBV genotypes is interesting.

Research objectives

We surveyed the differences in the reduction of HCC development following ETV administration in many countries.

Research methods

We surveyed the differences in the reduction of HCC development following long-term administration of ETV based on already published articles using PubMed (2004-2019).

Research results

The countries which showed the greatest reduction in HCC development following ETV administration were Spain, Canada, and most ineffective countries or regions were South Korea, China, Taiwan, and Hong Kong. With ETV administration, the incidence of HCC in genotype D regions was significantly lower than that in genotype C regions. The initial HBV-DNA levels in genotype C patients was almost the same as that in genotype D patients. No relationship was observed between the prevalence ratio of HBV and the incidence of HCC following ETV treatment.

Research conclusions

The effectiveness of ETV in preventing HCC development in HBV-associated liver cirrhosis is genotype-dependent.

Research perspectives

In countries with low effectiveness of ETV in the prevention of HCC development, frequent surveillance using imaging modalities will be necessary.

FOOTNOTES

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REFERENCES

1 **Wong GL**, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Lai JW, Lo AO, Chan HY, Wong VW. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. *Hepatology* 2013; **58**: 1537-1547 [PMID: 23389810 DOI: 10.1002/hep.26301]
2 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]
3 **Riveiro-Barciela M**, Tabernero D, Calleja JL, Lens S, Manzano ML, Rodríguez FG, Crespo J, Piqueras B, Pascasio JM, Comas C, Gutierrez ML, Aguirre A, Suárez E, García-Samaniego J, Rivero M, Acero D, Fernandez-Bermejo M, Moreno D, Sánchez-Pobre P, de Cuenca B, Moreno-Palomares JJ, Esteban R, Buti M. Effectiveness and Safety of Entecavir or Tenofovir in a Spanish Cohort of Chronic Hepatitis B Patients: Validation of the Page-B Score to Predict Hepatocellular Carcinoma. *Dig Dis Sci* 2017; **62**: 784-793 [PMID: 28078526 DOI: 10.1007/s10620-017-4448-7]
4 **Coffin CS**, Rezaeeaval M, Pang JX, Alcantara L, Klein P, Burak KW, Myers RP. The incidence of hepatocellular carcinoma is reduced in patients with chronic hepatitis B on long-term nucleos(t)ide analogue therapy. *Aliment Pharmacol Ther* 2014; **40**: 1262-1269 [PMID: 25312649 DOI: 10.1111/apt.12990]
5 **Kim HS**, Kim BK, Kim SU, Park JY, Kim DY, Song KJ, Park JW, Kim YJ, Baatarkhuu O, Han KH, Ahn SH. Association Between Level of Fibrosis, Rather Than Antiviral Regimen, and Outcomes of Patients With Chronic Hepatitis B. *Clin Gastroenterol Hepatol* 2016; **14**: 1647-1656. e6 [PMID: 27305847 DOI: 10.1016/j.cgh.2016.05.039]
6 **Choi J**, Kim HJ, Lee J, Cho S, Ko MJ, Lim YS. Risk of Hepatocellular Carcinoma in Patients Treated with Entecavir *vs* Tenofovir for Chronic Hepatitis B: A Korean Nationwide Cohort Study. *JAMA Oncol* 2019; **5**: 30-36 [PMID: 30267080 DOI: 10.1001/jamaoncol.2018.4070]
7 **Tsuzuki S**, Orita H, Sato N. Intermolecular interactions of oligothienoacenes: Do S□S interactions positively contribute to crystal structures of sulfur-containing aromatic molecules? *J Chem Phys* 2016; **145**: 174503 [PMID: 27825221 DOI: 10.1063/1.4966580]
8 **Su TH**, Hu TH, Chen CY, Huang YH, Chuang WL, Lin CC, Wang CC, Su WW, Chen MY, Peng CY, Chien RN, Huang YW, Wang HY, Lin CL, Yang SS, Chen TM, Mo LR, Hsu SJ, Tseng KC, Hsieh TY, Suk FM, Hu CT, Bair MJ, Liang CC, Lei YC, Tseng TC, Chen CL, Kao JH; C-TEAM study group and the Taiwan Liver Diseases Consortium. Four-year entecavir therapy reduces hepatocellular carcinoma, cirrhotic events and mortality in chronic hepatitis B patients. *Liver Int* 2016; **36**: 1755-1764 [PMID: 27634134 DOI: 10.1111/Liv.13253]
9 **Chen YC**, Peng CY, Jeng WJ, Chien RN, Liaw YF. Clinical outcomes after interruption of entecavir therapy in HBeAg-negative chronic hepatitis B patients with compensated cirrhosis. *Aliment Pharmacol Ther* 2015; **42**: 1182-1191 [PMID: 26381928 DOI: 10.1111/apt.13409]
10 **Watanabe T**, Tokumoto Y, Joko K, Michitaka K, Mashiba T, Hiraoka A, Ochi H, Koizumi Y, Tada F, Hirooka M, Yoshida O, Imai Y, Abe M, Hiasa Y. Effects of long-term entecavir treatment on the incidence of hepatocellular carcinoma in chronic hepatitis B patients. *Hepatol Int* 2016; **10**: 320-327 [PMID: 26198757 DOI: 10.1007/s12072-015-9647-8]
11 **Idilman R**, Gunsar F, Koruk M, Keskin O, Meral CE, Gulsen M, Elhan AH, Akarca US, Yurdaydin C. Long-term

entecavir or tenofovir disoproxil fumarate therapy in treatment-naïve chronic hepatitis B patients in the real-world setting. *J Viral Hepat* 2015; **22**: 504-510 [PMID: 25431108 DOI: 10.1111/jvh.12358]

12 **Köklü S**, Tuna Y, Gülşen MT, Demir M, Köksal AŞ, Koçkar MC, Aygün C, Coban S, Ozdil K, Ataseven H, Akin E, Pürnak T, Yüksel I, Ataseven H, Ibiş M, Yildirim B, Nadir I, Küçükazman M, Akbal E, Yüksel O, Başar O, Alkan E, Baykal O. Long-term efficacy and safety of lamivudine, entecavir, and tenofovir for treatment of hepatitis B virus-related cirrhosis. *Clin Gastroenterol Hepatol* 2013; **11**: 88-94 [PMID: 23063679 DOI: 10.1016/j.cgh.2012.10.003]

13 **Arends P**, Sonneveld MJ, Zoutendijk R, Carey I, Brown A, Fasano M, Mutimer D, Deterding K, Reijnders JG, Oo Y, Petersen J, van Bömmel F, de Knegt RJ, Santantonio T, Berg T, Welzel TM, Wedemeyer H, Buti M, Pradat P, Zoulim F, Hansen B, Janssen HL; VIRGIL Surveillance Study Group. Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians. *Gut* 2015; **64**: 1289-1295 [PMID: 25011935 DOI: 10.1136/gutjnl-2014-307023]

14 **Papatheodoridis GV**, Manolakopoulos S, Touloumi G, Nikolopoulou G, Raptopoulou-Gigi M, Gogos C, Vafiadis-Zouboulis I, Karamanolis D, Chouta A, Ilias A, Drakoulis C, Mimidis K, Ketikoglou I, Manesis E, Mela M, Hatzis G, Dalekos GN; HepNet. Greece Study Group. Hepatocellular carcinoma risk in HBeAg-negative chronic hepatitis B patients with or without cirrhosis treated with entecavir: HepNet.Greece cohort. *J Viral Hepat* 2015; **22**: 120-127 [PMID: 25040685 DOI: 10.1111/jvh.12283]

15 **Kim BK**, Revill PA, Ahn SH. HBV genotypes: relevance to natural history, pathogenesis and treatment of chronic hepatitis B. *Antivir Ther* 2011; **16**: 1169-1186 [PMID: 22155900 DOI: 10.3851/IMP1982]

16 **Kramvis A**, Kew MC. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. *J Viral Hepat* 2005; **12**: 456-464 [PMID: 16108759 DOI: 10.1111/j.1365-2893.2005.00624.x]

17 **Chan HL**, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, Sung JJ. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; **53**: 1494-1498 [PMID: 15361502 DOI: 10.1136/gut.2003.033324]

18 **Yu MW**, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, Shih WL, Kao JH, Chen DS, Chen CJ. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; **97**: 265-272 [PMID: 15713961 DOI: 10.1093/jnci/dji043]

19 **Fattovich G**, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48**: 335-352 [PMID: 18096267 DOI: 10.1016/j.jhep.2007.11.011]

20 **Kao JH**, Liu CJ, Chen DS. Hepatitis B viral genotypes and lamivudine resistance. *J Hepatol* 2002; **36**: 303-304 [PMID: 11830346 DOI: 10.1016/s0168-8278(01)00246-x]

21 **Hosaka T**, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 2013; **58**: 98-107 [PMID: 23213040 DOI: 10.1002/hep.26180]

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CASE REPORT

Living-donor liver transplantation in Budd-Chiari syndrome with inferior vena cava complete thrombosis: A case report and review of the literature

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Abstract

BACKGROUND

Budd-Chiari syndrome (BCS) is a challenging indication for liver transplantation (LT) due to a combination of massive liver, increased bleeding, retroperitoneal fibrosis and frequently presents with stenosis of the inferior vena cava (IVC). Occasionally, it may be totally thrombosed, increasing the complexity of the procedure, as it should also be resected. The challenge is even greater when performing living-donor LT as the graft does not contain the retrohepatic IVC; thus, it may be necessary to reconstruct it.

CASE SUMMARY

A 35-year-old male patient with liver cirrhosis due to BCS and hepatocellular carcinoma beyond the Milan criteria underwent living-donor LT with IVC reconstruction. It was necessary to remove the IVC as its retrohepatic portion was completely thrombosed, up to almost the right atrium. A right-lobe graft was retrieved from his sister, with outflow reconstruction including the right hepatic vein and the branches of segment V and VIII to the middle hepatic vein. Owing to

massive subcutaneous collaterals in the abdominal wall, venovenous bypass was implemented before incising the skin. The right atrium was reached *via* a transdiaphragramatic approach. Hepatectomy was performed *en bloc* with the retrohepatic vena cava. It was reconstructed with an infra-hepatic vena cava graft obtained from a deceased donor. The patient remains well on outpatient clinic follow-up 25 mo after the procedure, under an anticoagulation protocol with warfarin.

CONCLUSION

Living-donor LT in BCS with IVC thrombosis is feasible using a meticulous surgical technique and tailored strategies.

Key Words: Liver transplantation; Living donors; Budd-Chiari syndrome; Hepatic veno-occlusive disease; Inferior vena cava; Case report

Core Tip: A right-lobe living-donor liver transplantation (LT) with inferior vena cava (IVC) resection and reconstruction was performed in a patient with liver cirrhosis due to Budd-Chiari syndrome and hepatocellular carcinoma beyond the Milan criteria. It was necessary to remove the IVC because its retrohepatic portion was completely thrombosed, up to almost the right atrium. It was reconstructed with an infra-hepatic vena cava graft obtained from a deceased donor. The patient remains well 25 mo after the procedure. This case highlights the meticulous surgical technique and tailored strategies required for dealing with these challenging procedures in living-donor LT.

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INTRODUCTION

Budd-Chiari Syndrome (BCS) is characterized by the obstruction of hepatic venous drainage that leads to progressive hepatic congestion and, ultimately, portal hypertension and liver cirrhosis[1]. This blockage may be present in the hepatic venules, main hepatic veins, inferior vena cava (IVC) or right atrium[2]. Several nonsurgical therapeutics have been described, such as anticoagulation therapy, percutaneous transluminal angioplasty and interventional radiologic placement of a transjugular intrahepatic portosystemic shunt (TIPS) or direct intrahepatic portocaval shunt[1-3]. Liver transplantation (LT) is indicated in acute cases of fulminant hepatic failure or chronic cases with cirrhosis, which commonly evolve with gastrointestinal bleeding, untreatable ascites, sarcopenia, encephalopathy and hepatocellular carcinoma (HCC)[4]. In such scenarios, TIPS is often unfeasible due to extensive venous thrombosis or advanced liver disease[5].

Venous thrombosis can affect not only the hepatic veins but also a prolonged segment of the retrohepatic IVC, occasionally very close to the right atrium. The association between the severity of the disease, the extension of the venous thrombosis and the massive liver that is frequently present in BCS makes LT a particularly difficult procedure in these cases[1]. The hypercoagulative nature of the syndrome further increases the challenge, owing to vascular complications[6].

The challenge is even greater when considering living donor liver transplantation (LDLT) since the graft does not contain the retrohepatic IVC, as in deceased-donor liver transplantation (DDLT). Therefore, hepatic venous reconstruction is more complex, especially if the IVC is also obliterated[7]. That is the reason why only approximately 70 patients with BCS underwent LDLT worldwide between 1989 and 2015[1,8]. When LDLT is performed and HCC is also present, DDLT may not be possible in case of postoperative complications if the patient is beyond the Milan criteria[9], depending on local legislation in some countries, such as Brazil. Thus, performing LDLT for BSC in such a scenario is even more risky.

We report a case of a complex retrohepatic IVC thrombosis due to BCS in a patient with HCC beyond the Milan criteria. As the patient had a good response to transarterial chemoembolization (TACE) and his alfa fetoprotein levels decreased, we decided to perform LDLT.

CASE PRESENTATION

Chief complaints

A 35-year-old cirrhotic male patient was referred for LT evaluation due to BCS and HCC.

History of present illness

The patient had been diagnosed with cirrhosis and BCS four years previously, after presenting with ascites and hematemesis due to esophageal varices. Abdominal computed tomography (CT) scan on this occasion showed hepatic veins thrombosis and signs of chronic hepatopathy with paraumbilical vein recanalization and extensive collateral circulation in the splenic hilum, around the stomach, and in the anterior and lateral abdominal walls. The liver also showed multiple hepatic nodules of up to 1.5 cm in diameter, some them hypervascularized, which in the context of BCS, were compatible with regenerative hepatic nodules. Hepatic biopsy revealed chronic hepatic outflow obstruction. Laboratory testing for autoimmune hepatitis was negative, as were serological markers for hepatitis C and B viruses. The patient also denied previous alcohol abuse. No thrombophilia was diagnosed, despite extensive hematological investigation. The patient was then maintained on oral anticoagulation with warfarin.

History of past illness

The patient had no previous medical history.

Personal and family history

The patient was a smoker (10 cigarettes/day for 20 years). There was no relevant family history concerning this case.

Physical examination

The patient exhibited mild jaundice and extensive subcutaneous collateral veins in the anterior abdominal wall (Figure 1). Further physical examination was unremarkable.

Laboratory examinations

Blood analysis revealed normal hemoglobin, mild leukopenia and mild thrombocytopenia with mildly elevated total bilirubin, direct bilirubin and gamma-glutamyl-transferase (Table 1). Kidney function and electrolytes were normal as well as serum albumin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. The patient's prothrombin time was elevated even without warfarin (Table 1). Considering that the patient did not present encephalopathy or ascites, his Child-Pugh score was A6, and his Model of End-Stage Liver Disease (MELD) score was 15. His alpha-fetoprotein level was 58.7 ng/mL (normal range < 10 ng/mL), although 6 mo earlier, it was 9.4 ng/mL.

Imaging examinations

During outpatient follow-up, an abdominal CT scan showed a heterogeneously vascularized nodule in segment V, which increased from 2 cm to 4 cm in three years (Figure 2A and B). He also showed complete thrombosis of the retrohepatic IVC, up to almost the right atrium, with large subcutaneous veins in his abdominal wall (Figure 2C). Further evaluation with abdominal liver magnetic resonance imaging with hepatobiliary contrast showed two hypervascularized nodules with hypocaptation in the biliary phase in segments V and II, 4 and 2.3 cm in size, respectively (Figure 3). Considering the previous CT scans with multiple regenerative nodules, these 2 specific nodules were classified as indeterminate lesions. Given their growth, the atypical pattern of contrast uptake and the rise in alpha-fetoprotein serum levels, further investigation with biopsy of these nodules was indicated due to the suspicion of HCC.

FINAL DIAGNOSIS

Percutaneous ultrasound-guided biopsy of the largest nodule confirmed a moderately differentiated HCC (grade 3 Edmondson-Steiner grading system). Therefore, the patient presented liver cirrhosis due to BCS with retrohepatic vena cava thrombosis and multicentric HCC beyond the Milan criteria.

TREATMENT

According to Brazilian legislation, the patient could not be listed for DDLT due to being beyond the Milan criteria. He underwent 2 TACE procedures in order to downstage the lesions to within the Milan criteria so that he could be listed. Even though the serum alfa-fetoprotein level decreased from 58.7 to 18

Table 1 Laboratory tests results and normal range

Laboratory test	Result	Normal range
Hemoglobin	12.6 g/dL	12.5-17.5 g/dL
Leukocytes	3.5 × 10^9/L	4-11 × 10^9/L
Platelets	80 × 10^3/mm^3	150-400 × 10^3/mm^3
Total bilirubin	1.73 mg/dL	0.2-1 mg/dL
Direct bilirubin	0.85 mg/dL	< 0.3 mg/dL
Alanine aminotransferase	20 U/L	< 41 U/L
Aspartate aminotransferase	35 U/L	< 37 U/L
Alkaline phosphatase	78 U/L	40-129 U/L
Gamma-glutamyl-transferase	115 U/L	8-91 U/L
Creatinine	0.79 mg/dL	0.7-1.2 mg/dL
Blood urea nitrogen	31 mg/dL	10-50 mg/dL
Sodium	143 mEq/L	135-145 mEq/L
Potassium	3.9 mEq/L	3.5-4.5 mEq/L
Albumin	4.4 g/dL	3.4-4.8 g/dL
Prothrombin time	21.8 s	9.4-12.5 s
International normalized ratio	1.75	0.95-1.2

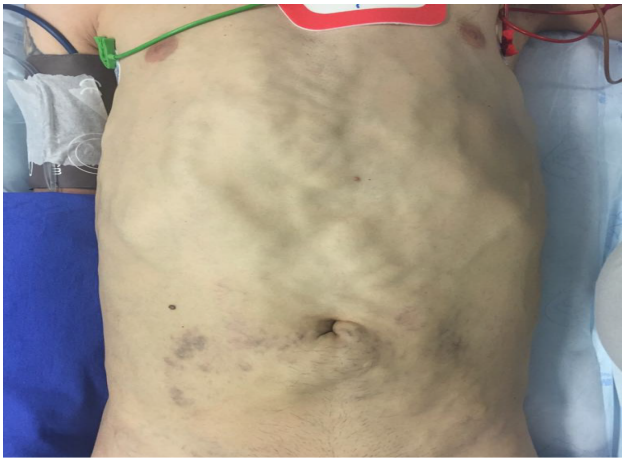

Figure 1 Massive blood return by subcutaneous veins in the anterior abdominal wall, which required the use of venovenous bypass prior to the abdominal incision.

ng/mL, the nodules did not decrease in size and the patient remained beyond the Milan criteria. His sister then volunteered for liver donation and the patient was selected for LDLT. She was a healthy 51-year-old female with a body mass index of 22.6 kg/m^2. Liver volumetry revealed a right lobe of 724 cm^3 (66% of the entire organ), and usual biliary tree anatomy was found on magnetic resonance cholangiopancreatography. Liver parenchyma also showed simple cysts.

The patient weighed 71 kg, resulting in a predicted graft-to-recipient weight ratio (GRWR) of 0.81%. Donor operation consisted of a right hepatectomy with middle hepatic vein preservation. The procedure was uneventful, resulting in a 560 g right lobe graft with usual anatomy (GRWR of 0.79%). In the backtable operation, the right hepatic vein and the V5 and V8 branches of the middle hepatic vein were reconstructed to avoid outflow blockage.

For the recipient, the surgical strategy included the use of a venovenous bypass prior to incising the abdomen due to very large subcutaneous collaterals in the abdominal and thoracic walls. The left femoral and left axillary veins were used to implement the venovenous bypass. Hepatectomy was performed with the retrohepatic vena cava, close to the right atrium. The explanted liver weighed 1880 g. The portal vein was then cannulated and added to the venovenous bypass. As the right lobe graft did not include the retrohepatic vena cava, it was reconstructed using an infra-hepatic IVC from a deceased

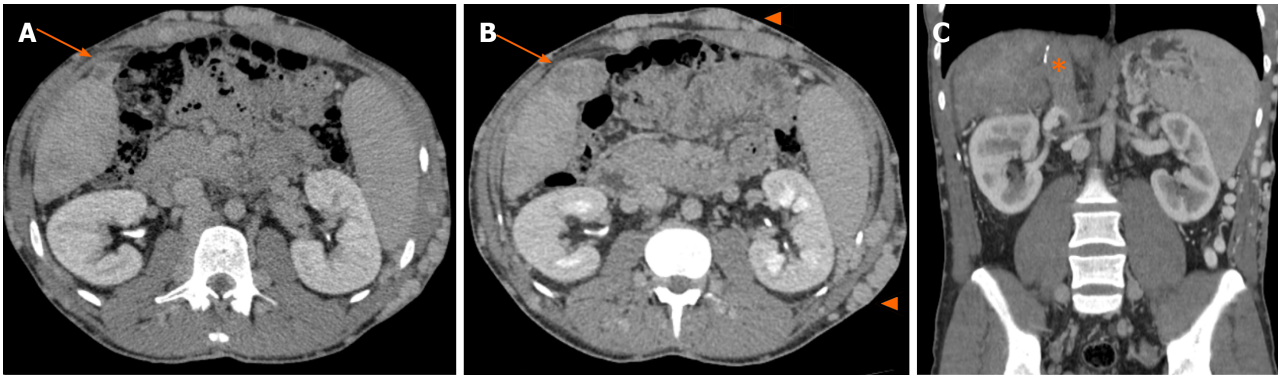

Figure 2 Abdominal computed tomography scans, with a 3-year interval. A: Heterogeneously vascularized nodule in segment V, of 2 cm, more visible in delayed phase due to hypocaptation (arrow); B: Same nodule in segment V in an exam scan performed 3 years later, with 4 cm (arrow). Massive subcutaneous veins in the abdominal wall are noted (arrowhead); C: The retrohepatic vena cava is completely thrombosed, up to almost the right atrium (asterisk).

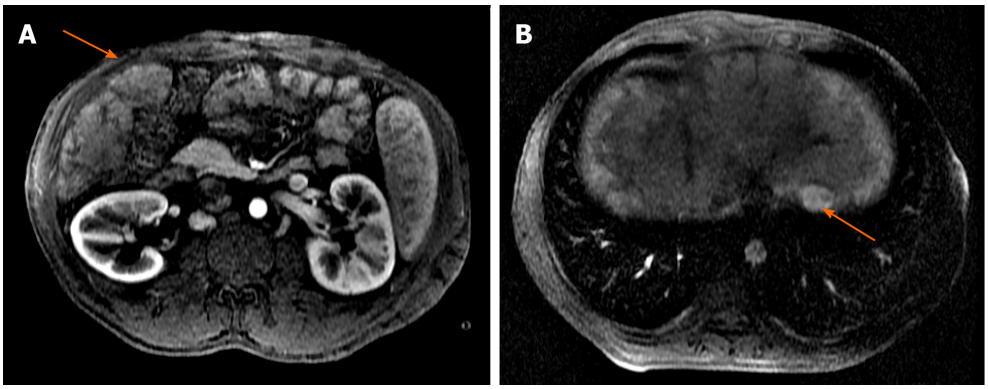

Figure 3 Liver magnetic resonance imaging with hepatobiliary contrast (arterial phase). A: Hypervascularized nodule in segment V of 4 cm (arrow); B: Hypervascularized nodule in segment II of 2.3 cm (arrow).

donor (Figure 4A). The graft was then implanted using this newly formed IVC to be anastomosed with the graft venous conduit for the outflow reconstruction. The right portal vein, right hepatic artery and right hepatic duct of the graft were then respectively anastomosed to their counterparts in the recipient (Figure 4B and C). Total and warm ischemia times were 370 and 30 min, respectively.

OUTCOME AND FOLLOW-UP

The donor's postoperative course was uneventful, and she was discharged home on postoperative day (POD) 5. The recipient was extubated on POD 2, and anticoagulation with enoxaparin was restarted, as well as low-dose aspirin. Liver Doppler ultrasound on POD 1 and 15 showed preserved graft vascularization. Renal function remained preserved, and the patient's condition progressively improved. The patient's immunosuppression regimen included intraoperative corticoid bolus and tapering associated with tacrolimus. The patient was discharged home on POD 19. Everolimus was added to the tacrolimus regimen 3 mo after the transplantation. Low-dose corticoid was maintained for 6 mo.

On histopathological analysis, the explanted liver confirmed hepatic cirrhosis related to chronic BCS and two moderately differentiated HCCs in segment V (4.5 cm) and segment II (2.5 cm).

Routine abdominal CT scan performed 23 mo after transplant showed a patent retrohepatic vena cava and adequate right lobe vascularization (Figure 5). The patient remains well on outpatient clinic follow-up 25 mo after the procedure, under an anticoagulation protocol with warfarin and without signs of HCC recurrence (alpha-fetoprotein 6.5 ng/mL).

DISCUSSION

Despite the numerous treatment modalities available for BCS, LT is performed in 10% to 20% of patients [1,2]. Nevertheless, it is a rare cause for LT, accounting for approximately 1%[10,11]. This a challenging

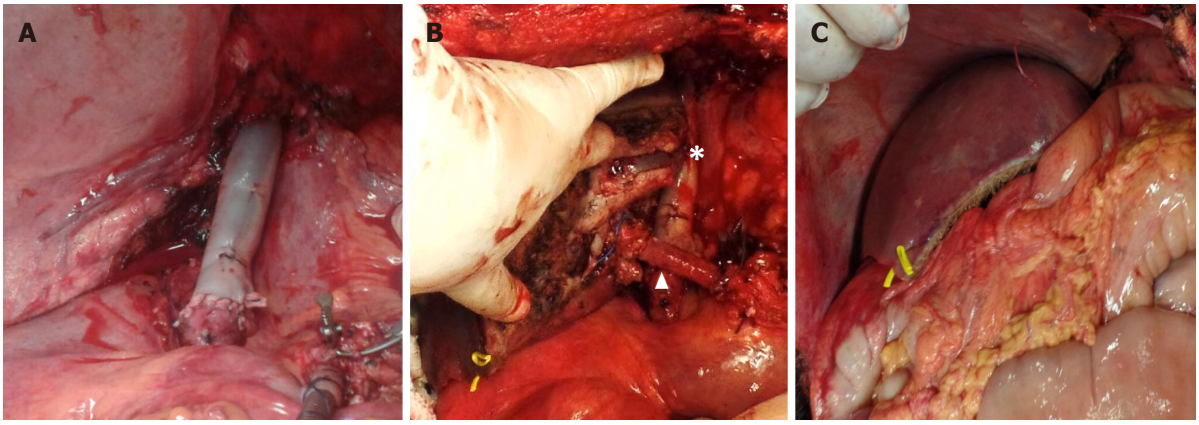

Figure 4 Intraoperative images. A: Reconstructed retrohepatic vena cava using an infrahepatic vena cava graft of a deceased donor; B: Revascularized graft showing the venous conduit anastomosed to the newly formed vena cava (asterisk) and the portal vein anastomosis (arrowhead); C: Graft final aspect after arterialization at the end of transplantation.

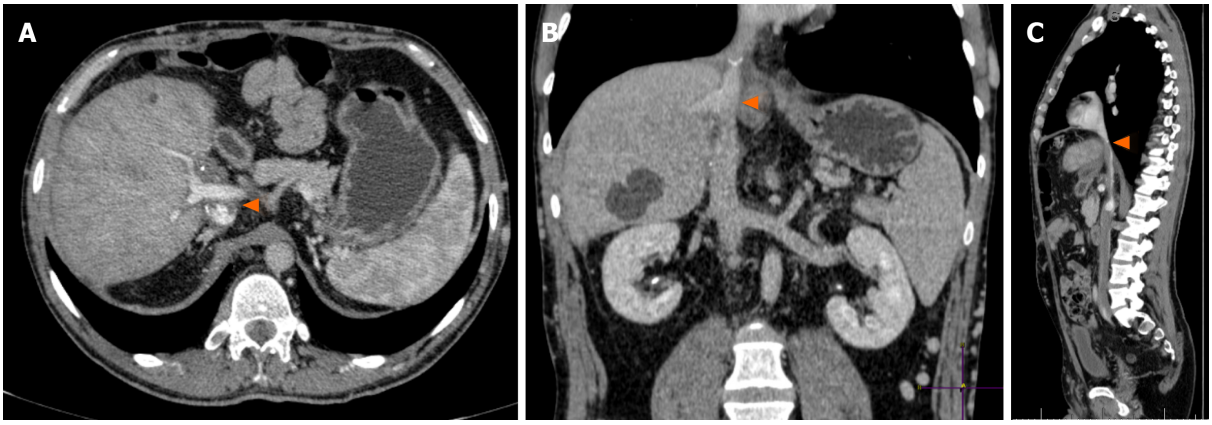

Figure 5 Late postoperative abdominal computed tomography scan, portal phase. A: Graft with adequate aspect and preserved portal inflow (arrowhead); B: Coronal view showing patent retrohepatic vena cava (arrowhead) and preserved graft outflow; C: Sagittal view of patent retrohepatic vena cava (arrowhead).

indication for LT due to a combination of massive liver and increased bleeding, caudate lobe enlargement, retroperitoneal diffuse fibrosis, firm retrohepatic IVC adhesions and frequently presents with stenosis and/or thrombosis of the IVC[3]. Especially in LDLT, in which the donor's IVC cannot be used, the retrohepatic IVC dissection performed during the piggyback technique and the venous outflow reconstruction are particularly problematic. Novel alternative techniques, aimed at eliminating stenosis or obstruction in the recipient IVC, are thus needed for LDLT in the context of BCS[6]. Some of them include cross-clamping the supra- and infrahepatic IVC and excising its thickened wall to create a wide orifice for graft implantation[7] or the V-Y plasty technique[12].

Nevertheless, when the IVC is completely occluded, which is known as obliterative hepatocavopathy (OHC), it is advisable to remove the IVC *en bloc* with the native liver[13], as the piggyback dissection becomes technically unfeasible due to dense inflammatory adhesions, enlarged collaterals and hypertrophied caudate lobe. If an LDLT is performed in this situation, it may be necessary to reconstruct the retrohepatic IVC. In 2006, Yan *et al*[14] reported the first LDLT for BCS with IVC reconstruction using an interposed cryopreserved cadaveric IVC graft[14]. Since then, many other studies have addressed IVC reconstruction with interposing autologous veins[15], cadaveric venous allografts[3,7,16-18], cadaveric aortic allografts[7,17-20], synthetic material[12,13,18] or a combination of synthetic material and autologous vein[21,22] or venous allografts[18,23]. Table 2 provides a review of all cases found in the literature of LDLT for BCS with IVC resection.

In the present report, we faced three ordeals in the preoperative period. First, the massive liver was associated with extensive IVC thrombosis starting close to the renal veins and progressing up to the transition between the IVC and the right atrium. Second, it was necessary to use a living donor right lobe with the potential risk of postoperative small-for-size syndrome, given the association of extensive thrombosis, portal hypertension and partial graft[12]. Finally, the LDLT was performed in a patient with HCC beyond the Milan criteria, which, according to Brazilian law, prevented the use of a deceased-donor graft in case of postoperative graft dysfunction.

Table 2 Summary of all reported cases of living-donor liver transplantation for Budd-Chiari syndrome with inferior vena cava resection

Ref.	Number of cases	Technique	Venovenous bypass use	Outcomes
Yan *et al*[14], 2006	*n* = 1	IVC replacement with cadaveric IVC allograft	Yes	Alive after 3 mo
Yamada *et al* [2], 2006	*n* = 1	IVC resection without replacement	No	Alive after 10 mo
Shimoda *et al* [15], 2007	*n* = 1	IVC replacement with autologous internal jugular vein, external iliac vein and suprarenal IVC	No	Alive after 17 mo
Sasaki *et al*[16], 2009	*n* = 1	IVC replacement with cadaveric IVC allograft	No	N/A
Kazimi *et al* [32], 2009	*n* = 1	IVC resection without replacement	No	Alive after 3 mo
Choi *et al*[3], 2010	*n* = 2	IVC replacement with cadaveric IVC allograft (*n* = 1) and RHV-atrial shunt using preexisting mesoatrial shunt (*n* = 1)	No	Both alive after a median follow-up of 18 mo
Ogura *et al*[21], 2011	*n* = 1	IVC replacement with an inverted composite graft (Gore-Tex stretch vascular graft and transposed IVC)	Yes	Alive after 24 mo
Sakçak *et al* [19], 2012	*n* = 1	IVC replacement with cadaveric aortic allografts	No	Alive after 4 mo
Fukuda *et al* [24], 2013	*n* = 1	IVC resection without replacement	No	Alive after 60 mo
Yagci *et al*[17], 2015	*n* = 4	IVC replacement with cadaveric IVC (*n* = 1), iliac vein (*n* = 1) and aorta allografts (*n* = 2)	No	2 patients died due to biliary complications after 5 mo of follow-up
Cetinkunar *et al* [20], 2015	*n* = 1	IVC replacement by cadaveric aortic allograft	No	Alive after 4 mo
Ara *et al*[7], 2016	*n* = 7	IVC replacement with cadaveric IVC (*n* = 4) and cadaveric aorta allografts (*n* = 2). No replacement in one case	No	2 patients died due to recent HAT after LT, and 2 patients died of sepsis during follow-up
Pahari *et al*[12], 2016	*n* = 2	IVC replacement with e-PTFE graft	No	Both alive after a median follow-up of 18 mo
Karaca *et al*[6], 2017	*n* = 3	IVC resection without replacement	No	N/A
Sabra *et al*[25], 2018	*n* = 1	IVC resection without replacement	No	Alive after 3 mo
Yagi *et al*[22], 2018	*n* = 1	IVC replacement with an inverted composite graft (e-PTFE graft and transposed IVC)	Yes	Alive after 36 mo
Ionescu *et al* [23], 2018	*n* = 2	IVC replacement with caval-dacron composite graft	No	Both alive (follow-up not available)
Yoon *et al*[13], 2019	*n* = 5	IVC replacement with synthetic material (ringed polyester)	Yes (*n*=3)	All alive after a median follow-up of 10.5 years
Gonultas *et al* [18], 2020	*n* = 12	IVC replacement with cadaveric IVC allograft (*n* = 6), cadaveric aorta allograft (*n* = 1), synthetic material (*n* = 3, Dacron) and caval-dacron composite graft (*n* = 2)	No	All alive after median follow-up of 15 mo
Present study	*n* = 1	IVC replacement with cadaveric IVC allograft	Yes	Alive after 25 mo

N/A: Not available; e-PTFE: Polytetrafluoroethylene; HAT: Hepatic artery thrombosis; IVC: Inferior vena cava; RHV: Right hepatic vein; LT: Liver transplantation.

Most authors describe a transdiaphragmatic access to the supradiaphragmatic IVC or even the right atrium, although a rarely performed lower median sternotomy may be helpful in some cases[13,24]. In the present report, through a standard *Makuuchi* incision, the recipient's liver was removed *en bloc* with the retrohepatic vena cava, from just above the renal veins to the beginning of the right atrium. This surgical approach, without thoracic access, was very useful as the patient had no major bleeding or hemodynamic instability. The interposition of a conduit replacing the retrohepatic IVC was necessary because we could observe considerable venous flow from the suprarenal vena cava. There is no consensus in the literature regarding the best material for IVC reconstruction[18]. The use of synthetic material raises concerns regarding the long-term patency of the anastomosis between the hepatic vein from the liver graft and the prosthesis, due to the possibility of thrombosis, deformity of the synthetic

orifice and anastomosis kinking consequent to growth of the liver graft[25]. Infection of prosthetic material is also an issue[26]. Many centers, including ours, therefore prefer autologous or allogeneic grafts, which present less thrombosis and infection risk[18,27]. Even cadaveric IVC recovered 25 h after the donor´s circulatory death has been successfully used[28]. As a high-volume center of DDLT, there is great availability of allografts in our institution biobank. Storage of such grafts is feasible and inexpensive, only requiring sterile Ringer Lactate solution and a laboratory freezer[29]. However, in countries with scarce deceased donor organ donation and in centers with a high volume of LDLT, access to these grafts may be difficult[18].

Given the complexity of such procedures, it is paramount to obtain a suitable amount of liver parenchyma[30]. Therefore, we used the right lobe, as in most reported cases; however, some authors have also used the right posterior segment[15], the left lateral segment (pediatric recipients)[7,17,19], the left lobe[2,22,24,25] and dual grafts[13]. Another concern is the possibly elevated portal inflow to the graft[31]. That is the reason why we routinely measure the portal venous pressure by a catheter inserted *via* a jejunal branch. As the portal pressure was below 14 mmHg in this case after graft implantation, we did not implement further strategies to decrease the portal inflow.

In most cases reported, venovenous bypass was not used (Table 2). Due to the chronicity of IVC obstruction, venous return is expected to occur *via* collaterals involving the azygos, hemiazygos, accessory hemiazygos and thoracolumbar veins[24]. In a large series addressing LDLT with IVC resection for various reasons in 29 patients by Gonultas *et al*[18], venovenous bypass was not used in any case, as there was no hemodynamic instability during IVC clamping. In our case, the patient presented a well-developed collateral circulation; however, we observed that it was mainly composed of a massive subcutaneous plexus in the abdominal and thoracic wall (Figures 1 and 2). Thus, we decided to use the extracorporeal venovenous bypass before the abdominal skin was incised. We feared that an abdominal incision could lead to hemodynamic instability, since it was necessary to ligate the collaterals forming this enormous subcutaneous plexus. Therefore, when we accessed the abdominal cavity and clamped the IVC, the patient was already on venovenous bypass.

Retrohepatic IVC resection without replacement in LDLT for BCS has also been reported[2,6,7,24,25,32], in which the liver graft is anastomosed directly to the right atrium[6,32], to the intrapericardical IVC [24,25] or to the rarely preserved supra-hepatic IVC[2,6,7]. In one patient, the graft was directly anastomosed to a previous mesoatrial shunt[3]. This raises the question of whether or not it necessary to reconstruct the IVC. As addressed by Gonultas *et al*[18], the venous continuity should be maintained in patients without a venous collateral circulation system or in those with insufficient venous drainage. For patients that have a well-developed venous collateral, on the other hand, the liver graft may be, in theory, anastomosed directly to the suprahepatic IVC without the need for reconstruction. In our case, as the collaterals forming the subcutaneous plexus were ligated during the skin incision, the IVC reconstruction was required. We also observed a significant blood flow in the infra-hepatic IVC after the native liver was removed, suggesting the necessity of venous continuity restoration with an IVC interposition graft.

Despite the complexity of cases, most studies describe successful outcomes after LDLT (Table 2). The literature review identified 2 deaths due to early hepatic arterial thrombosis and another 4 patients died during follow-up due to infectious and biliary complications occurring months after transplant. In the series by Gonultas *et al*[18], 4 patients experienced late thrombosis of the replaced IVC during follow-up that were successfully treated with percutaneous balloon dilatation and/or stenting. The early use of low-dose aspirin and low molecular weight heparin a few days after LDLT is important to prevent the recurrence of thrombosis[12,13,18,32].

CONCLUSION

We describe a novel surgical approach for LDLT in BCS with OHC and HCC beyond the Milan criteria that can be used in highly selected patients. Due to its complexity and rarity, LDLT in such situations is feasible using a meticulous surgical technique and tailored strategies.

FOOTNOTES

Author contributions: Rocha-Santos V, Carneiro-D'Alburquerque LA, Nacif LS and Waisberg DR were the patient's surgeons; Andraus W, Pinheiro RS and Ducatti L performed the donor operation; Rocha-Santos V, Waisberg DR, Pinheiro RS and Nacif LS drafted the manuscript; Arantes RM, Ducatti L, Martino RB and Haddad LB performed the literature review and contributed to manuscript drafting; Galvao FH, Andraus W and Carneiro-D'Alburquerque LA were responsible for revision of the manuscript and for important intellectual content; all authors issued final approval for the version to be submitted.

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REFERENCES

1 **Akamatsu N**, Sugawara Y, Kokudo N. Budd-Chiari syndrome and liver transplantation. *Intractable Rare Dis Res* 2015; **4**: 24-32 [PMID: 25674385 DOI: 10.5582/irdr.2014.01031]
2 **Yamada T**, Tanaka K, Ogura Y, Ko S, Nakajima Y, Takada Y, Uemoto S. Surgical techniques and long-term outcomes of living donor liver transplantation for Budd-Chiari syndrome. *Am J Transplant* 2006; **6**: 2463-2469 [PMID: 16939520 DOI: 10.1111/j.1600-6143.2006.01505.x]
3 **Choi GS**, Park JB, Jung GO, Chun JM, Kim JM, Moon JI, Kwon CH, Kim SJ, Joh JW, Lee SK. Living donor liver transplantation in Budd-Chiari syndrome: a single-center experience. *Transplant Proc* 2010; **42**: 839-842 [PMID: 20430186 DOI: 10.1016/j.transproceed.2010.02.045]
4 **Valla DC**. Budd-Chiari syndrome/hepatic venous outflow tract obstruction. *Hepatol Int* 2018; **12**: 168-180 [PMID: 28685257 DOI: 10.1007/s12072-017-9810-5]
5 **Parekh J**, Matei VM, Canas-Coto A, Friedman D, Lee WM; Acute Liver Failure Study Group. Budd-chiari syndrome causing acute liver failure: A multicenter case series. *Liver Transpl* 2017; **23**: 135-142 [PMID: 27656864 DOI: 10.1002/lt.24643]
6 **Karaca C**, Yilmaz C, Ferecov R, Iakobadze Z, Kilic K, Caglayan L, Aydogdu S, Kilic M. Living-Donor Liver Transplantation for Budd-Chiari Syndrome: Case Series. *Transplant Proc* 2017; **49**: 1841-1847 [PMID: 28923635 DOI: 10.1016/j.transproceed.2017.04.028]
7 **Ara C**, Akbulut S, Ince V, Karakas S, Baskiran A, Yilmaz S. Living donor liver transplantation for Budd-Chiari syndrome: Overcoming a troublesome situation. *Medicine (Baltimore)* 2016; **95**: e5136 [PMID: 27787368 DOI: 10.1097/MD.0000000000005136]
8 **Doğrul AB**, Yankol Y, Mecit N, Kanmaz T, Acarlı K, Kalayoğlu M. Orthotopic Liver Transplant for Budd-Chiari Syndrome: An Analysis of 14 Cases. *Exp Clin Transplant* 2016; **14**: 641-645 [PMID: 26669436 DOI: 10.6002/ect.2015.0026]
9 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
10 **Segev DL**, Nguyen GC, Locke JE, Simpkins CE, Montgomery RA, Maley WR, Thuluvath PJ. Twenty years of liver transplantation for Budd-Chiari syndrome: a national registry analysis. *Liver Transpl* 2007; **13**: 1285-1294 [PMID: 17763380 DOI: 10.1002/Lt.21220]
11 **Mackiewicz A**, Kotulski M, Zieniewicz K, Krawczyk M. Results of liver transplantation in the treatment of Budd-Chiari syndrome. *Ann Transplant* 2012; **17**: 5-10 [PMID: 22466903 DOI: 10.12659/aot.882630]
12 **Pahari H**, Chaudhary RJ, Thiagarajan S, Raut V, Babu R, Bhangui P, Goja S, Rastogi A, Vohra V, Soin AS. Hepatic Venous and Inferior Vena Cava Morphology No Longer a Barrier to Living Donor Liver Transplantation for Budd-Chiari Syndrome: Surgical Techniques and Outcomes. *Transplant Proc* 2016; **48**: 2732-2737 [PMID: 27788809 DOI: 10.1016/j.transproceed.2016.08.009]
13 **Yoon YI**, Lee SG, Moon DB, Ahn CS, Hwang S, Kim KH, Ha TY, Song GW, Jung DH, Park GC, Kim DS, Choo SJ. Surgical Techniques and Long-term Outcomes of Living-donor Liver Transplantation With Inferior Vena Cava Replacement Using Atriocaval Synthetic Interposition Graft for Budd-Chiari Syndrome. *Ann Surg* 2019; **269**: e43-e45 [PMID: 30080720 DOI: 10.1097/SLA.0000000000002847]
14 **Yan L**, Li B, Zeng Y, Wen T, Zhao J, Wang W, Xu M, Yang J, Ma Y, Chen Z, Wu H. Living donor liver transplantation for Budd-Chiari syndrome using cryopreserved vena cava graft in retrohepatic vena cava reconstruction. *Liver Transpl* 2006; **12**: 1017-1019 [PMID: 16721775 DOI: 10.1002/Lt.20773]

15 **Shimoda M**, Marubashi S, Dono K, Miyamoto A, Takeda Y, Nagano H, Umeshita K, Monden M. Utilization of autologous vein graft for replacement of the inferior vena cava in living-donor liver transplantation for obliterative hepatocavopathy. *Transpl Int* 2007; **20**: 804-807 [PMID: 17623051 DOI: 10.1111/j.1432-2277.2007.00519.x]

16 **Sasaki K**, Kasahara M, Fukuda A, Shigeta T, Tanaka H, Nakagawa S, Nakagawa A, Nakayasiro M. Living donor liver transplantation with vena cava reconstruction using a cryopreserved allograft for a pediatric patient with Budd-Chiari syndrome. *Transplantation* 2009; **87**: 304-305 [PMID: 19155990 DOI: 10.1097/TP.0b013e3181938b10]

17 **Yagci MA**, Tardu A, Karagul S, Ince V, Ertugrul I, Kirmizi S, Unal B, Aydin C, Kayaalp C, Yilmaz S. Living Donor Liver Transplantation With Vena Cava Replacement. *Transplant Proc* 2015; **47**: 1453-1457 [PMID: 26093741 DOI: 10.1016/j.transproceed.2015.04.019]

18 **Gonultas F**, Akbulut S, Barut B, Usta S, Kutluturk K, Kutlu R, Yilmaz S. Usability of Inferior Vena Cava Interposition Graft During Living Donor Liver Transplantation: Is This Approach Always Necessary? *J Gastrointest Surg* 2020; **24**: 1540-1551 [PMID: 31385171 DOI: 10.1007/s11605-019-04342-6]

19 **Sakçak I**, Eriş C, Ölmez A, Kayaalp C, Yılmaz S. Replacement of the vena cava with aortic graft for living donor liver transplantation in Budd-Chiari syndrome associated with hydatid cyst surgery: a case report. *Transplant Proc* 2012; **44**: 1757-1758 [PMID: 22841264 DOI: 10.1016/j.transproceed.2012.04.023]

20 **Cetinkunar S**, Ince V, Ozdemir F, Ersan V, Yaylak F, Unal B, Yilmaz S. Living-Donor Liver Transplantation for Budd-Chiari Syndrome--Resection and Reconstruction of the Suprahepatic Inferior Vena Cava With the Use of Cadaveric Aortic Allograft: Case Report. *Transplant Proc* 2015; **47**: 1537-1539 [PMID: 26093762 DOI: 10.1016/j.transproceed.2015.04.043]

21 **Ogura Y**, Kanazawa H, Yoshizawa A, Nitta T, Ikeda T, Uemoto S. Supradiaphragmatic approach for Budd-Chiari syndrome with transjugular intrahepatic portosystemic shunt stent in combination with inferior vena cava reconstruction during living donor liver transplantation: a case report. *Transplant Proc* 2011; **43**: 2093-2096 [PMID: 21693334 DOI: 10.1016/j.transproceed.2011.03.046]

22 **Yagi T**, Takagi K, Yoshida R, Umeda Y, Nobuoka D, Kuise T, Fujiwara T, Takaki A. New Left Lobe Transplantation Procedure with Caval Reconstruction Using an Inverted Composite Graft for Chronic Budd-Chiari Syndrome in Living-Donor Liver Transplantation-A Case Report. *Transplant Proc* 2018; **50**: 1192-1195 [PMID: 29731092 DOI: 10.1016/j.transproceed.2017.11.078]

23 **Ionescu MI**, de Usera MA, Muiesan P, Mirza D, Isaac JR. Donation after Circulatory Death Type 2 Liver Transplantation in a Large Referral Centre in the United Kingdom: A Feasibility Study. 2018 Congress of the International Liver Transplantation Society. May 23 - 26, 2018 in Lisbon, Portugal [DOI: 10.3252/pso.eu.ILTS2018.2018]

24 **Fukuda A**, Ogura Y, Kanazawa H, Mori A, Kawaguchi M, Takada Y, Uemoto S. Living donor liver transplantation for Budd-Chiari syndrome with hepatic inferior vena cava obstruction after open pericardial procedures. *Surg Today* 2013; **43**: 1180-1184 [PMID: 23188387 DOI: 10.1007/s00595-012-0440-1]

25 **Sabra TA**, Okajima H, Tajima T, Fukumitsu K, Hata K, Yasuchika K, Masui T, Taura K, Kaido T, Uemoto S. Living donor liver transplantation for adult Budd Chiari syndrome - Resection without replacement of retrohepatic IVC: A case report. *Int J Surg Case Rep* 2018; **42**: 50-54 [PMID: 29216531 DOI: 10.1016/j.ijscr.2017.11.050]

26 **Mancuso A**, Martinelli L, De Carlis L, Rampoldi AG, Magenta G, Cannata A, Belli LS. A caval homograft for Budd-Chiari syndrome due to inferior vena cava obstruction. *World J Hepatol* 2013; **5**: 292-295 [PMID: 23717741 DOI: 10.4254/wjh.v5.i5.292]

27 **Koc C**, Akbulut S, Ozdemir F, Kose A, Isik B, Yologlu S, Yilmaz S. Analysis of Risk Factors Affecting the Development of Infection in Artificial Vascular Grafts Used for Reconstruction of Middle Hepatic Vein Tributaries in Living Donor Liver Transplantation. *Transplantation* 2019; **103**: 1871-1876 [PMID: 30747841 DOI: 10.1097/TP.0000000000002583]

28 **Palma AF**, Oberkofler CE, Raptis DA, Eshmuminov D, de Rougemont O, Schnyder A, Dimitroulis D, Lesurtel M, Dutkowski P, Clavien PA. Novel rescue procedure for inferior vena cava reconstruction in living-donor liver transplantation using a vascular graft recovered 25 h after donors' circulatory death and systematic review. *Transpl Int* 2014; **27**: 204-210 [PMID: 24289717 DOI: 10.1111/tri.12238]

29 **Aydin C**, Ince V, Otan E, Akbulut S, Koc C, Kayaalp C, Yilmaz S. Storage of allogeneic vascular grafts: experience from a high-volume liver transplant institute. *Int Surg* 2013; **98**: 170-174 [PMID: 23701155 DOI: 10.9738/INTSURG-D-12-00035.1]

30 **Lee SG**. A complete treatment of adult living donor liver transplantation: a review of surgical technique and current challenges to expand indication of patients. *Am J Transplant* 2015; **15**: 17-38 [PMID: 25358749 DOI: 10.1111/ajt.12907]

31 **Soin AS**. Smoothing the path: reducing biliary complications, addressing small-for-size syndrome, and making other adaptations to decrease the risk for living donor liver transplant recipients. *Liver Transpl* 2012; **18** Suppl 2: S20-S24 [PMID: 22927168 DOI: 10.1002/lt.23541]

32 **Kazimi M**, Karaca C, Ozsoy M, Ozdemir M, Apaydin AZ, Ulukaya S, Zeytunlu M, Kilic M. Live donor liver transplantation for Budd-Chiari syndrome: anastomosis of the right hepatic vein to the right atrium. *Liver Transpl* 2009; **15**: 1374-1377 [PMID: 19790160 DOI: 10.1002/lt.21815]

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