

# UNIVERSITY OF BIRMINGHAM

## Research at Birmingham

### Mechanisms of autophagy activation in endothelial cell and their targeting during normothermic machine liver perfusion

Longatto Boteon, Yuri; Laing, Richard; Mergental, Hynek; Reynolds, Gary; Mirza, Darius; Afford, Simon; Bhogal, Ricky

*License:*

Creative Commons: Attribution-NonCommercial (CC BY-NC)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Longatto Boteon, Y, Laing, R, Mergental, H, Reynolds, G, Mirza, D, Afford, S & Bhogal, R 2017, 'Mechanisms of autophagy activation in endothelial cell and their targeting during normothermic machine liver perfusion', *World Journal of Gastroenterology*, vol. 23, no. 48, pp. 8443-8451.

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**

Checked for eligibility: 08/01/2018

**General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

**Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2017 December 28; 23(48): 8439-8678





### EDITORIAL

- 8439 Serum levels of angiotensin converting enzyme as a biomarker of liver fibrosis  
*Miranda AS, Simões e Silva AC*

### MINIREVIEWS

- 8443 Mechanisms of autophagy activation in endothelial cell and their targeting during normothermic machine liver perfusion  
*Boteon YL, Laing R, Mergental H, Reynolds GM, Mirza DF, Afford SC, Bhogal RH*

### ORIGINAL ARTICLE

#### Basic Study

- 8452 Human small intestine is capable of restoring barrier function after short ischemic periods  
*Schellekens DH, Hundscheid IH, Leenarts CA, Grootjans J, Lenaerts K, Buurman WA, Dejong CH, Derikx JP*
- 8465 Stable gastric pentadecapeptide BPC 157 in treatment of colitis and ischemia and reperfusion in rats: New insights  
*Duzel A, Vlainic J, Antunovic M, Malekinusic D, Vrdoljak B, Samara M, Gojkovic S, Krezic I, Vidovic T, Bilic Z, Knezevic M, Sever M, Lojo N, Kokot A, Kolovrat M, Drmic D, Vukojevic J, Kralj T, Kasnik K, Siroglavic M, Seiwerth S, Sikiric P*
- 8489 Exploring pathogenesis of primary biliary cholangitis by proteomics: A pilot study  
*Deng CW, Wang L, Fei YY, Hu CJ, Yang YJ, Peng LY, Zeng XF, Zhang FC, Li YZ*
- 8500 Influence of TBX21 T-1993C variant on autoimmune hepatitis development by Yin-Yang 1 binding  
*Sun W, Wu HY, Chen S*
- 8512 Astragaloside IV inhibits pathological functions of gastric cancer-associated fibroblasts  
*Wang ZF, Ma DG, Zhu Z, Mu YP, Yang YY, Feng L, Yang H, Liang JQ, Liu YY, Liu L, Lu HW*

#### Retrospective Cohort Study

- 8526 Prevalence and outcomes of pancreatic cystic neoplasms in liver transplant recipients  
*Liu K, Joshi V, van Camp L, Yang QW, Baars JE, Strasser SI, McCaughan GW, Majumdar A, Saxena P, Kaffes AJ*

#### Retrospective Study

- 8533 Analysis of 12 variants in the development of gastric and colorectal cancers  
*Cavalcante GC, Amador MA, Ribeiro dos Santos AM, Carvalho DC, Andrade RB, Pereira EE, Fernandes MR, Costa DF, Santos NP, Assumpção PP, Ribeiro dos Santos Á, Santos S*

- 8544** Childhood-onset inflammatory bowel diseases associated with mutation of Wiskott-Aldrich syndrome protein gene

*Ohya T, Yanagimachi M, Iwasawa K, Umetsu S, Sogo T, Inui A, Fujisawa T, Ito S*

- 8553** Comparison of totally laparoscopic total gastrectomy using an endoscopic linear stapler with laparoscopic-assisted total gastrectomy using a circular stapler in patients with gastric cancer: A single-center experience

*Gong CS, Kim BS, Kim HS*

- 8562** Prognostic significance of pretreatment serum carcinoembryonic antigen levels in gastric cancer with pathological lymph node-negative: A large sample single-center retrospective study

*Xiao J, Ye ZS, Wei SH, Zeng Y, Lin ZM, Wang Y, Teng WH, Chen LC*

- 8570** Fecal microbiota transplantation induces remission of infantile allergic colitis through gut microbiota re-establishment

*Liu SX, Li YH, Dai WK, Li XS, Qiu CZ, Ruan ML, Zou B, Dong C, Liu YH, He JY, Huang ZH, Shu SN*

- 8582** Prognostic value of lymph node metastasis in patients with T1-stage colorectal cancer from multiple centers in China

*Sun ZQ, Ma S, Zhou QB, Yang SX, Chang Y, Zeng XY, Ren WG, Han FH, Xie X, Zeng FY, Sun XT, Wang GX, Li Z, Zhang ZY, Song JM, Liu JB, Yuan WT*

**Clinical Trial Study**

- 8591** Association between acute pancreatitis and small intestinal bacterial overgrowth assessed by hydrogen breath test

*Zhang M, Zhu HM, He F, Li BY, Li XC*

**Observational Study**

- 8597** Endoscopic papillary large balloon dilatation with sphincterotomy is safe and effective for biliary stone removal independent of timing and size of sphincterotomy

*Aujla UI, Ladep N, Dwyer L, Hood S, Stern N, Sturgess R*

- 8605** Person-centered endoscopy safety checklist: Development, implementation, and evaluation

*Dubois H, Schmidt PT, Creutzfeldt J, Bergenmar M*

**Randomized Clinical Trials**

- 8615** Multicenter, randomized study to optimize bowel preparation for colon capsule endoscopy

*Kastenber D, Burch WC, Romeo DP, Kashyap PK, Pound DC, Papageorgiou N, Fernández-Urien Sainz I, Sokach CE, Rex DK*

**SYSTEMATIC REVIEWS**

- 8626** *Fusobacterium's* link to colorectal neoplasia sequenced: A systematic review and future insights

*Hussan H, Clinton SK, Roberts K, Bailey MT*

- 8651** Psychiatric morbidity after surgery for inflammatory bowel disease: A systematic review

*Zangenberg MS, El-Hussuna A*

**CASE REPORT**

- 8660** Stricturing Crohn's disease-like colitis in a patient treated with belatacept

*Bozon A, Jeantet G, Rivière B, Funakoshi N, Dufour G, Combes R, Valats JC, Delmas S, Serre JE, Bismuth M, Ramos J, Le Quintrec M, Blanc P, Pineton de Chambrun G*

- 8666** Emphysematous pancreatitis associated with penetrating duodenal ulcer

*Tana C, Silingardi M, Giamberardino MA, Cipollone F, Meschi T, Schiavone C*

- 8671** Infiltrative xanthogranulomatous cholecystitis mimicking aggressive gallbladder carcinoma: A diagnostic and therapeutic dilemma

*Nacif LS, Hessheimer AJ, Rodríguez Gómez S, Montironi C, Fondevila C*



**ABOUT COVER**

Editorial board member of *World Journal of Gastroenterology*, Paola Iovino, MD, Associate Professor, Lecturer, Department of Medicine and Surgery, AOU San Giovanni di Dio e Ruggi di Aragona, Salerno 84131, Italy

**AIMS AND SCOPE**

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup> each month. The *WJG* Editorial Board consists of 1375 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

**INDEXING/ABSTRACTING**

*World Journal of Gastroenterology* (*WJG*) is now indexed in Current Contents<sup>®</sup>/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch<sup>®</sup>), Journal Citation Reports<sup>®</sup>, Index Medicus, MEDLINE, PubMed, PubMed Central and Directory of Open Access Journals. The 2017 edition of Journal Citation Reports<sup>®</sup> cites the 2016 impact factor for *WJG* as 3.365 (5-year impact factor: 3.176), ranking *WJG* as 29<sup>th</sup> among 79 journals in gastroenterology and hepatology (quartile in category Q2).

**FLYLEAF**

**I-IX** Editorial Board

**EDITORS FOR THIS ISSUE**

**Responsible Assistant Editor:** *Xiang Li*  
**Responsible Electronic Editor:** *Yu-Jie Ma*  
**Proofing Editor-in-Chief:** *Lian-Sheng Ma*

**Responsible Science Editor:** *Ze-Mao Gong*  
**Proofing Editorial Office Director:** *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Gastroenterology*

**ISSN**  
 ISSN 1007-9327 (print)  
 ISSN 2219-2840 (online)

**LAUNCH DATE**  
 October 1, 1995

**FREQUENCY**  
 Weekly

**EDITORS-IN-CHIEF**  
**Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon**, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28040, Spain

**Stephen C Strom, PhD, Professor**, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

**Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology**, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach,

CA 90822, United States

**EDITORIAL BOARD MEMBERS**  
 All editorial board members resources online at <http://www.wjgnet.com/1007-9327/editorialboard.htm>

**EDITORIAL OFFICE**  
 Jin-Lei Wang, Director  
 Ze-Mao Gong, Vice Director  
*World Journal of Gastroenterology*  
 Baishideng Publishing Group Inc  
 7901 Stoneridge Drive, Suite 501,  
 Pleasanton, CA 94588, USA  
 Telephone: +1-925-2238242  
 Fax: +1-925-2238243  
 E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
 Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

**PUBLISHER**  
 Baishideng Publishing Group Inc  
 7901 Stoneridge Drive, Suite 501,  
 Pleasanton, CA 94588, USA  
 Telephone: +1-925-2238242  
 Fax: +1-925-2238243  
 E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
 Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
 December 28, 2017

**COPYRIGHT**  
 © 2017 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
 All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
 Full instructions are available online at <http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.f6publishing.com>

## Mechanisms of autophagy activation in endothelial cell and their targeting during normothermic machine liver perfusion

Yuri L Boteon, Richard Laing, Hynek Mergental, Gary M Reynolds, Darius F Mirza, Simon C Afford, Ricky H Bhogal

Yuri L Boteon, Richard Laing, Hynek Mergental, Darius F Mirza, Ricky H Bhogal, The Liver Unit, University Hospitals of Birmingham, Mindelsohn Way, Edgbaston, Birmingham B15 2TT, United Kingdom

Yuri L Boteon, Richard Laing, Hynek Mergental, Gary M Reynolds, Darius F Mirza, Simon C Afford, Ricky H Bhogal, The Centre for Liver Research, Institute for Biomedical Research, University of Birmingham, Birmingham B15 2TT, United Kingdom

**Author contributions:** Boteon YL and Bhogal RH wrote the manuscript; Mergental H, Mirza DF and Afford SC critically appraised the manuscript; Reynolds GM performed the immunohistochemistry; Bhogal RH formatted and edited the final manuscript.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Dr. Ricky H Bhogal, the Centre for Liver Research, Institute for Biomedical Research, University of Birmingham, Birmingham B15 2TT, United Kingdom. [ricky.bhogal@uhb.nhs.uk](mailto:ricky.bhogal@uhb.nhs.uk)  
Telephone: +44-121-4155739

Received: May 1, 2017

Peer-review started: May 5, 2017

First decision: June 7, 2017

Revised: June 19, 2017

Accepted: July 12, 2017

Article in press: July 12, 2017

Published online: December 28, 2017

### Abstract

Ischaemia-reperfusion injury (IRI) is the leading cause of injury seen in the liver following transplantation. IRI also causes injury following liver surgery and haemodynamic shock. The first cells within the liver to be injured by IRI are the liver sinusoidal endothelial cells (LSEC). Recent evidence suggests that LSEC coordinate and regulates the liver's response to a variety of injuries. It is becoming increasingly apparent that the cyto-protective cellular process of autophagy is a key regulator of IRI. In particular LSEC autophagy may be an essential gatekeeper to the development of IRI. The recent availability of liver perfusion devices has allowed for the therapeutic targeting of autophagy to reduce IRI. In particular normothermic machine liver perfusion (NMP-L) allow the delivery of pharmacological agents to donor livers whilst maintaining physiological temperature and hepatic flow rates. In this review we summarise the current understanding of endothelial autophagy and how this may be manipulated during NMP-L to reduce liver IRI.

**Key words:** Autophagy; Liver transplant; Ischaemia-reperfusion injury; Normothermic machine liver perfusion

© **The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Liver sinusoidal endothelial cells autophagy regulates liver ischaemia reperfusion injury and this process can be targeted for therapeutic benefit using normothermic machine liver perfusion.

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(48): 8443-8451 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i48/8443.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i48.8443>

## INTRODUCTION

The term autophagy is derived from the Greek meaning "eating of self" and the precise cellular role of autophagy has been controversial<sup>[1]</sup>. However research over the past decade has demonstrated that the evolutionarily conserved process of autophagy is primarily a cell survival mechanism allowing cells and tissues to maintain homeostasis during periods of stress such as starvation and ischaemia<sup>[2]</sup>. Specifically autophagy eliminates damaged organelles, long-lived proteins or intracellular pathogens through the co-ordinated engulfment of the targeted cargo in a double membrane cytoplasmic structure known as an autophagosome<sup>[3,4]</sup>. Autophagosomes then fuse with lysosomes to form autophagolysosomes that degrade the engulfed cargo allowing their reuse and thus potentially negating periods of cellular stress<sup>[2,4-6]</sup>. Hence unsurprisingly autophagy is involved in number of cellular processes such as metabolism, protein synthesis and cellular transportation<sup>[4,7]</sup>. Dysregulated or uncoordinated autophagy is linked to cell injury and a number of disease processes such as neurodegenerative diseases and cancer<sup>[2,4,6,7]</sup>.

Three distinct types of autophagy have been characterised. The mostly widely studied is macroautophagy (referred to as autophagy hereafter) and is the primary focus of this review<sup>[4,5,8]</sup>. Microautophagy is characterized by the invagination of the target by the lysosomal membrane itself<sup>[5,9]</sup>. Chaperone-mediated autophagy targets proteins with the KFERQ motif to the lysosome via interaction with lysosomal-associated membrane protein (LAMP)<sup>[9,10]</sup>. More recently distinct macroautophagy signalling pathways have been characterised that are activated to specifically eliminate portions of the cell and/or cytoplasm leading to the characterisation of mitophagy (mitochondria), ERphagy (endoplasmic reticulum), xenophagy (microorganisms), lipophagy (lipids) and ribophagy (ribosomes). It is becoming increasingly apparent that specific forms of autophagy are important in the development and pathophysiology of different disease processes. The precise regulation of each type of autophagy is beyond the scope of this review but the reader is referred to recent excellent reviews on the subject<sup>[2,4,6,7]</sup>.

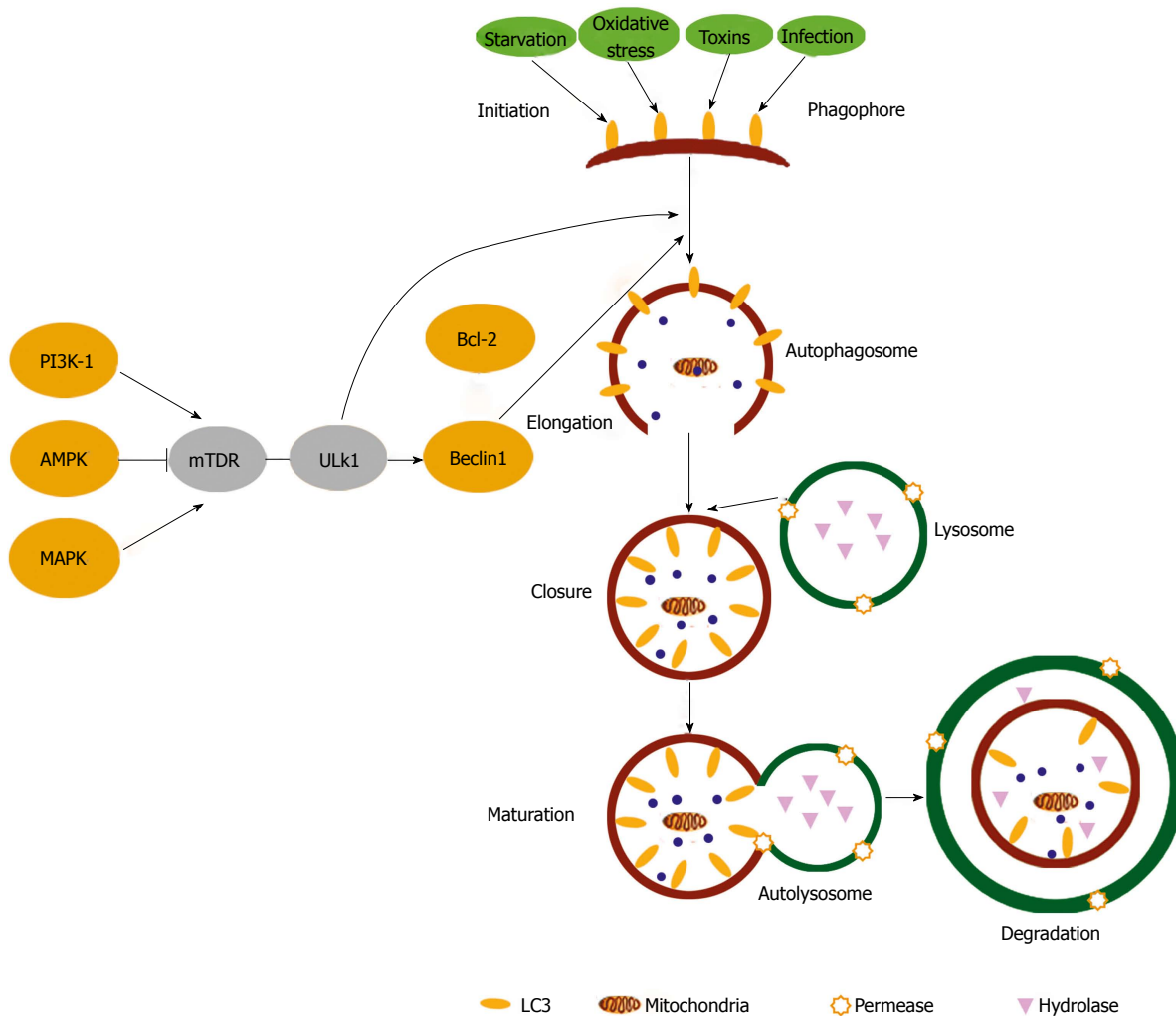
The therapeutic targeting of autophagy has gathered momentum since the publication of the first clinical trials using autophagy inhibitors in treatment of

cancers<sup>[11-13]</sup>. For instance autophagy inhibitors (*e.g.*, hydroxychloroquine) were used in combination with chemotherapy in patients with advanced pancreatic cancer and in those patients with increased levels of the autophagy marker LC3 in peripheral blood mononuclear cells there was an improvement in disease free and patient survival<sup>[11]</sup>. However in other trials using hydroxychloroquine in patients with glioblastoma the optimal therapeutic dose was not found due to the marked side effects with the investigators concluding that drugs with less toxicity should be awaited<sup>[12]</sup>. Although these studies have provided the impetus to manipulate autophagy it still remains to be established whether autophagy should be activated or inhibited to derive therapeutic benefit in many disease processes. Moreover many groups working on the therapeutic manipulation of autophagy are now suggesting that the targeting of autophagy during pathophysiological processes needs to be tissue and possibly even cell specific<sup>[14]</sup>.

The use of autophagy as a target in treating liver diseases have been the focus of intense recent research<sup>[15]</sup>. Indeed the manipulation of autophagy may be useful in treating many liver diseases. For instance induction of autophagy/lipophagy may reduce steatosis in fatty liver disease<sup>[16]</sup> and inhibiting autophagy in hepatobiliary cancers may promote cancer cell death<sup>[11,17]</sup>. The manipulation of the autophagy signalling pathway also holds significant promise in attempting to reduce the liver ischaemia-reperfusion injury (IRI). IRI is an antigen independent pro-inflammatory process that mediates liver injury following transplantation, liver surgery and haemorrhagic shock<sup>[18]</sup>. The injury occurs in two distinct phases. In the ischaemia phase blood flow to the liver is interrupted leading to tissue hypoxia and the generation of reactive oxygen species (ROS). During the reperfusion phase although blood flow to the liver is restored there is a concomitant increase in pro-inflammatory mediators, ROS and inflammatory cells that amplifies the liver injury<sup>[18]</sup>. It is well established that IRI targets and injures the liver parenchymal cells such as hepatocytes and liver sinusoidal endothelial cells (LSEC)<sup>[19]</sup>. However early IRI is characterised by LSEC injury and dysfunction<sup>[19]</sup>. Indeed recent studies demonstrate the key role of LSEC in co-ordinating the liver's response to injury whilst also mediating the recovery from liver injury<sup>[20]</sup>. Thus the targeting of autophagy in the LSEC during IRI is an attractive method with which to reduce liver IRI.

The emerging technology of normothermic machine perfusion of the liver (NMP-L) provides an exciting modality with which to target autophagy in LSEC whilst simultaneously allowing an assessment of liver function<sup>[21]</sup>. There are different NMP-L devices available allowing either selective hepatic artery perfusion or dual hepatic perfusion of donor livers. Using primarily blood based perfusion fluids, oxygen is delivered to donor livers whilst maintaining a normal





**Figure 1 Autophagy signalling pathways.** Upstream autophagy activation is regulated by the integration of signalling from a number of pathways including AMPK, PI-3K and the Mitogen-Activated Protein Kinases. Phagophore initiation is directly regulated by the serine/threonine protein kinases ULK1 that forms a complex with Beclin 1. Upon initiation, cytoplasmic constituents are enclosed in a double membraned isolation structure known as an autophagosome that is elongated mainly through the action of two ubiquitin-like conjugation systems. Autophagosomes fuse with lysosomes to form autophagolysosomes, where breakdown of the vesicle contents/cargo takes place along with the autophagosome inner membrane. Autophagy can be activated by many stimuli including starvation, toxins, oxidative stress and infections. (Taken from Shan NN *et al* Targeting autophagy as a potential therapeutic approach for immune thrombocytopenia therapy. *Crit Rev Oncol Hematol* 2016; **100**: 11-15. DOI: 10.1016/j.critrevonc.2016.01.011).

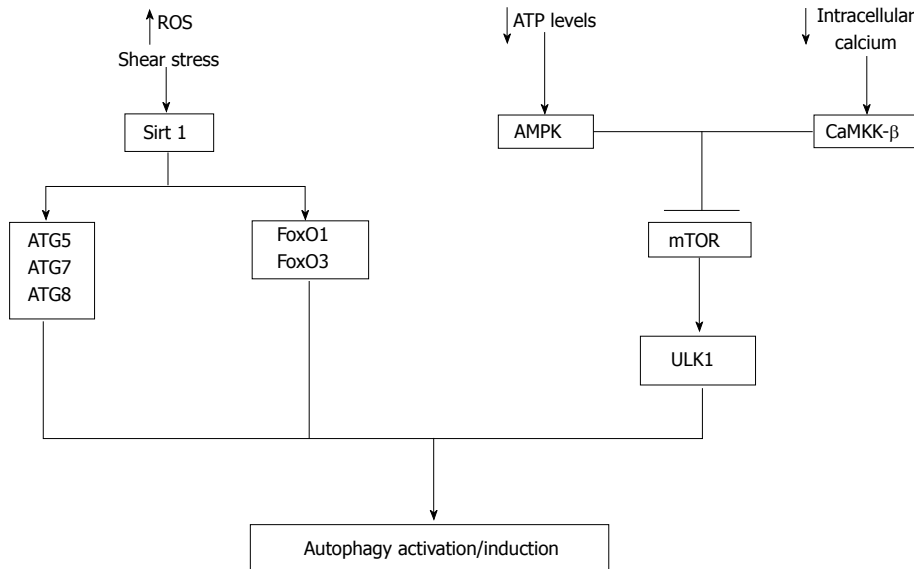
temperature (37 °C). NMP-L is typically performed for 6 h and the sequential sampling of perfusates and liver tissue allows a dynamic assessment of liver function. One of the many potential benefits of NMP-L is the reduction in the liver IRI. Therefore, the manipulation of autophagy in LSEC during NMP-L is an attractive therapeutic target with which to improve donor liver organ function prior to transplantation.

## AUTOPHAGY SIGNALLING PATHWAY

The autophagy signalling pathway is regulated by specific and dedicated cellular machinery. These proteins were first isolated in yeast two-hybrid screens and are now known as the Autophagy-related proteins (ATGs). To date 30 ATGs have been characterised that are essential for autophagy induction. Recent reviews by Stork *et al*<sup>[21]</sup> provide an in-depth review

of the pathway whilst a brief overview is given here. In general the autophagy signalling pathway can be divided into distinct phases including the initiation, elongation, autophagosome formation, fusion and autophagolysosomal formation (Figure 1).

The formation of autophagosomes commences upon omegasomes and is known as the initiation phase<sup>[22]</sup>. This process is regulated by the ATG proteins unc-51 like autophagy activation kinase (ULK1) and WIPI1-4/ATG18<sup>[22,23]</sup>. Starvation is a classical activator of autophagy and in nutrient rich conditions the activation of autophagy is inhibited by Mammalian Target of Rapamycin (mTOR)<sup>[24]</sup>. However when autophagy is activated, the inhibitory effect of mTOR is lost allowing for the activation of ULK1 kinase complex during the initiation phase<sup>[9]</sup>. In addition autophagy can also be initiated by AMP-activated protein kinase (AMPK)<sup>[25]</sup> (Figure 1). Subsequently there is



**Figure 2 Autophagy activation in endothelial cells.** A number of mechanisms potentially regulate autophagy activation in endothelial cells. A decrease in cellular ATP or reduction in growth factors availability leads to the activation of AMP-activated protein kinase (AMPK). Once activated AMPK can inhibit mTOR leading to the activation of ULK1 and hence autophagy activation. In addition decreases in intracellular calcium can activate CaMKK- $\beta$  leading to mTOR inhibition and autophagy activation. Moreover, Sirt1 can activate autophagy via deacetylation of ATG5, ATG7, ATG8 and increased transcription of FoxO1 and FoxO3 that then regulate the expression ATGs via deacetylation of Akt. Reactive oxygen species (ROS) and shear stress are important regulators of Sirt1 activity.

recruitment of class III phosphatidylinositol 3-kinase (PI3-K), Vps34 and Beclin-1/ATG6 to the developing autophagosome<sup>[5,9]</sup>. ULK1 is then able to activate the PI3-K complex leading to the activation of Beclin-1 and the later phases of autophagy. This pathway details the canonical autophagy signalling pathway but recent evidence suggests that autophagy can be activated by Beclin-1 independent mechanisms/non-canonical pathways too<sup>[9,26]</sup>.

The elongation phase of autophagy involves two separate ATG conjugation systems. Firstly ATG12 conjugates ATG5 via ATG7/ATG10. The ATG12-ATG5 complex is then able to interact with ATG16 resulting in the formation of the ATG12-ATG5-ATG16L complex<sup>[5,27]</sup>. The second conjugation system involves LC3-I/ATG8 that is cleaved by ATG4 in a process requiring ATG7 and ATG3. This culminates in the formation of LC3-II<sup>[5,28]</sup>. Both these conjugation systems are integral to autophagosome membrane expansion<sup>[4,7,9]</sup>. At the point at which appropriate membrane expansion has been reached there is closure of double layered membrane that is regulated by amongst other proteins the docking protein p62 which is located on the outer membrane of the autophagosome and is responsible for docking with the lysosome via a dynein-dependent mechanism<sup>[5]</sup>. Thereafter, lysosomal acid hydrolases degrade the engulfed content/cargo and lysosomal efflux permeases release the final products to the cytosol for anabolic processes (Figure 1).

## AUTOPHAGY AND ENDOTHELIAL CELLS

### Regulation of autophagy activation in endothelial cells

Much of our understanding of the role of autophagy in endothelial cells, such as LSEC, has come from the study of endothelial cell biology in cardiovascular disease. Recent studies have demonstrated how autophagy dysfunction in endothelial cells contributes to the pathogenesis of atherosclerosis by regulating angiogenesis, haemostasis and nitric oxide production<sup>[9]</sup>.

In endothelial cells upstream activation of the autophagy is regulated by the integration of signalling from the AMPK and mTOR-ULK1 pathways<sup>[9]</sup> (Figure 1). AMPK can detect decreases in cellular ATP levels and activate autophagy primarily to provide substrates for energy used for essential cellular processes<sup>[9,29]</sup> (Figure 2). Specifically AMPK phosphorylates the Tuberous Sclerosis 2 (TSC2) protein that inhibits mTOR and activates autophagy in an analogous mechanism to that seen during starvation<sup>[9,29-33]</sup>. In HeLa cells starvation increases intracellular calcium levels and can activate AMPK through Calmodulin-dependent protein kinase kinase- $\beta$  (CaMKK- $\beta$ ) that then subsequently activates autophagy<sup>[9,34,35]</sup>. In endothelial cells CaMKK- $\beta$  mediated AMPK activation inhibits mTOR leading to ULK1 upregulation and autophagy activation<sup>[34]</sup>. Thus in endothelial cells the activation of autophagy is a dynamic interplay between AMPK, intracellular calcium, mTOR and ULK-1.

The deacetylation protein Sirt1 has attracted recent attention as an important regulator of autophagy activation. Specifically Sirt1 can deacetylate ATG5, ATG7, and ATG8<sup>[9,36]</sup> whilst activating the transcription factors Forkhead box O (FoxO) FoxO1 and FoxO3. Both FoxO1 and FoxO3 can regulate the expression of ATGs *via* deacetylation of Akt<sup>[9,35,37-39]</sup>. Indeed during liver IRI ROS can regulate the activation of Sirt1, which may have important implications for endothelial cell autophagy during NMP-L<sup>[9,40-43]</sup>. Specifically perfusion fluid used during NMP-L induces tangential force across the endothelium causing shear stress that is associated with autophagy activation *via* the Sirt1-FoxO pathway<sup>[43]</sup>. Low shear stress is associated with reduced levels of autophagy suggesting an important relationship between shear stress and autophagy<sup>[44]</sup>. Therefore autophagy activation in endothelial cell can be regulated by multiple signalling pathways and at present it remains to be established which of these pathways is important during NMP-L.

#### **Effects of autophagy on endothelial cell function**

Autophagy activation has recently been demonstrated to have a number of important effects upon endothelial cell function. Following endothelial injury exposure of the sub-intimal layer promotes glycoproteins, expressed on platelets, to bind to subendothelial von Willebrand Factors (vWF) leading to the formation of a platelet thrombus and production of thromboxane A<sub>2</sub>, serotonin and adenosine diphosphate<sup>[34]</sup>. Recent studies demonstrate that vWF is found in close proximity to autophagosomes in endothelial cells<sup>[35]</sup>. The inhibition of autophagy impairs the secretion of vWF from endothelial cells suggesting that endothelial cell autophagy may have an anti-thrombotic function<sup>[45]</sup>.

In endothelial cells, endothelial nitric oxide synthase (eNOS) regulates nitric oxide (NO) production that in turn regulates vascular tone, platelet aggregation and leukocyte adhesion<sup>[46]</sup>. Indeed this may be a central function of endothelial cells during liver IRI and a potential protective mechanism induced within the liver by NMP-L. Furthermore eNOS can regulate autophagy induction<sup>[47,48]</sup>. The exact mechanisms involved in eNOS/NO-autophagy axis are not yet understood although impaired autophagy does result in reduced eNOS function reduced NO production<sup>[49]</sup>.

As eluded to earlier blood flow within the liver generates shear stress across the endothelium that is associated with induction of eNOS and NO production<sup>[50]</sup>. This is in turn associated with a reduction in oxidative stress and inflammation in endothelial cells. Disturbed shear stress generated by non-laminar flow is associated with endothelial dysfunction that can be implicated in disease processes such as atherosclerosis<sup>[50]</sup>. Hence low shear induces autophagy dysfunction<sup>[48]</sup> which is associated with insufficient autophagy activation in endothelial cells and thus endothelial dysfunction<sup>[43]</sup>. NMP-L may aid endothelial

cell autophagy by ensuring laminar flow within the hepatic vasculature thereby prompting autophagy leading to increased eNOS transcription and NO production. In summary, physiologic shear stress is an essential mechanism for the maintenance of endothelial cells function and is in part mediated by autophagy activation<sup>[47]</sup>.

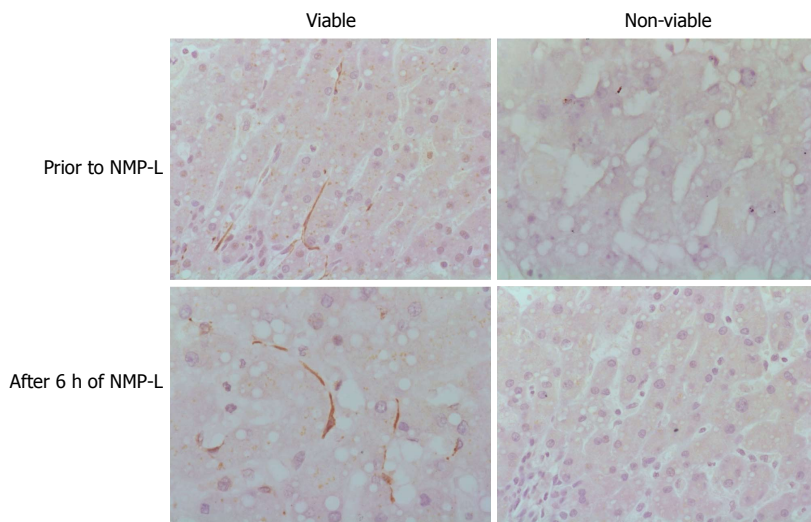
Autophagy activation is also associated with angiogenesis in endothelial cells particularly within ischaemic microenvironments<sup>[51]</sup>. The relationship between redox signalling and autophagy is complex and out of the scope of this review although it has been recently reviewed<sup>[42]</sup>. Autophagy regulation of angiogenesis appears to be dependent upon the activation of the Akt signalling pathway in endothelial cells although the precise mechanism remains to be determined<sup>[9,51,52]</sup>.

#### **Therapeutic targeting of autophagy in endothelial cells**

The evolving understanding of the autophagy signalling pathway has led to the targeting of the pathway for potential therapeutic benefit in many clinical scenarios. However what is becoming evident from these studies is that for the successful therapeutic targeting of autophagy the timing of the intervention, the part of the autophagy pathway targeted, the cell type targeted and whether autophagy should be inhibited or activated are all critical factors. The targeting of autophagy within endothelial cells remains a nascent field with few studies investigating this area.

Epigallocatechin gallate, found in green tea, can induce the specific form of autophagy known as lipophagy in endothelial cells. Lipophagy causes the specific elimination of lipid droplets. In atherosclerosis the degradation of lipid droplets in vascular endothelial cells can potentially modulate the disease process by allowing endothelial cells to resist the effects of lipotoxicity<sup>[35,53]</sup>. In human umbilical vein endothelial cells (HUVEC) exposed to oxidative stress, vitamin D dependent autophagy activation prevents cell death by activating Beclin1 that prevents mitochondrial depolarisation and caspase activation<sup>[54]</sup>. Pterostilbene can activate AMPK to stimulate autophagy in HUVEC promoting the elimination of excess lipids and thus reducing apoptosis<sup>[53]</sup> whilst other groups have suggest that Advanced glycation end-products (AGEs) may regulate the activation of autophagy in this scenario<sup>[55]</sup>. Furthermore the autophagy activator rapamycin can increase the viability of HUVEC during starvation<sup>[56]</sup>. These limited studies do suggest that selective activation of autophagy in cells of endothelial lineage promotes cell survival.

Very few studies have addressed the role of autophagy in endothelial cells *in vivo*. Torisu *et al*<sup>[45]</sup> reported, using a Cre-lox conditional ATG7 endothelial knockdown mice, that inhibition of autophagy leads a reduction in the secretion of vWF suggesting that autophagy may have a role in preventing thrombosis.



**Figure 3 Autophagy and liver viability following normothermic machine liver perfusion.** Immunohistochemical analysis was performed for the autophagy protein LC3B in liver tissue prior to normothermic machine liver perfusion (NMP-L) and after 6 h of NMP-L. Livers deemed viable after NMP-L demonstrated LC3B immunostaining in the hepatic sinusoids prior to commencing NMP-L and at the end of perfusion. Donor livers not fulfilling viability criteria demonstrated no LC3B immunostaining.

However the role of endothelial cell autophagy *in vivo* need much more investigation.

## THERAPEUTIC MANIPULATION OF ENDOTHELIAL CELL AUTOPHAGY DURING NMP-L

NMP-L is a novel technique that can be employed to assess and recondition donor livers prior to transplantation. One of the potential benefits of NMP-L is the potential reduction in liver IRI especially when compared to traditional static cold storage<sup>[57,58]</sup>. Recent studies have established haemodynamic and biochemical parameters that allow donor livers to be classified as viable and non-viable where viable denotes a liver that can be transplanted following NMP-L. Indeed donor livers represent conditions where autophagy is expected to be activated; relative tissue ischaemia and reduced availability of nutrients. Recent data from our laboratory has shown that donor livers that demonstrate these viability criteria shows increased levels of autophagy within hepatic sinusoids as assessed by immunohistochemical analysis of the specific autophagy marker LC3B (Figure 3). This suggests a relationship between autophagy induction and liver viability although further work is needed to fully establish this relationship.

As discussed above targeting of autophagy needs to be considered in terms of the timing of intervention, the cell type targeted and the part of the autophagy pathway targeted. During NMP-L there are two forms of intervention that could modulate autophagy in endothelial cells; mechanical or pharmacological.

Livers exposed to NMP-L are associated with increased cellular ATP levels in comparison to cold static stored livers. For instance in a porcine model of

donation after cardiac death (DCD) livers exposed to 1 h warm ischaemia, followed by 2 h of cold ischaemia and then 4 h of NMP-L increased cellular ATP levels by 80% in comparison with livers maintained in cold static storage for 2 h<sup>[59]</sup>. Higher ATP levels within liver grafts prior to liver transplantation are associated with better patient outcomes in some series<sup>[60,61]</sup>. Although it must be remembered that these studies have assessed global liver ATP levels and not specifically LSEC ATP levels. Additionally, NMP-L is associated with preservation of liver architecture and integrity of the mitochondria<sup>[59]</sup>. The regulation of mitochondrial function and number is regulated in large part by the specific autophagy process termed mitophagy<sup>[62]</sup>. The regulation of mitophagy during NMP-L and liver IRI is now being actively investigated as a potential method to reduce liver injury following transplantation<sup>[62]</sup>. The manipulation of mitophagy in LSEC is emerging as a method to reduce liver injury. However despite this it remains to be established whether LSEC autophagy contributes to increased ATP levels during NMP-L and whether autophagy, through promoting survival of LSEC, improves the survival of neighbouring liver parenchymal cells.

NMP-L may also regulate autophagy activation through calcium signalling. During NMP-L the perfusion fluid is supplemented with calcium to maintain physiological extracellular levels of the ion ensuring that the electrochemical gradient of calcium is maintained across cell membranes<sup>[58]</sup>. Low intracellular levels of calcium are reported to induce CaMK- $\beta$  activation followed by mTOR inhibition and therefore ULK1/autophagy activation<sup>[34]</sup>. Physiological calcium levels during NMP-L potentially promote normal autophagy activity within LSEC and other liver cells ensuring homeostasis is maintained.

The mechanical manipulation of autophagy during



NMP-L is dependent upon shear stress. NMP-L has the advantage of providing an adjustable vascular/laminar flow rate to livers. In turn these flow rate provides a near physiological shear stress known to promote autophagy induction<sup>[43]</sup>. Activated autophagy in endothelial cells is fundamental for eNOS transcription, NO production and maintenance of vascular tone maintenance<sup>[47]</sup>. Increased NO may also reduce platelet aggregation and leukocyte adhesion in endothelial cells thus reducing IRI and hepatic microcirculatory disturbance<sup>[63]</sup>.

Pharmacological modulation of autophagy during NMP-L may also allow the targeting of endothelial cells dysfunction in donor liver grafts prior to transplantation. Extended criteria liver donors (*e.g.*, DCD grafts and steatotic grafts) tend to be associated with lower cellular ATP content and increased ROS production increasing their susceptibility to IRI<sup>[57,59]</sup>. Pharmacological agents promoting activation of autophagy during NMP-L may promote elimination of damaged organelles and toxins prior to graft implantation. In particular aiding mitophagy function may be crucial to maintaining LSEC function during NMP-L and thus aiding the survival of other liver cells. As described above, many drugs are already known to regulate autophagy activation and the use of these drugs in experimental NMP-L perfusion offers an important method to evaluate these interventions. This manipulation of autophagy in LSEC using NMP-L is an exciting area of interest for research groups working on liver IRI particularly as this technology will become more widespread in addition to the development of new autophagy modulating drugs.

## CONCLUSION

In summary, autophagy is a complex metabolic process that is essential for the cell survival; it promotes clearance of harmful substances and provides energy to the cell during cellular stress. The role of autophagy in endothelial cells and its manipulation using NMP-L offers significant promise in reducing liver IRI thereby improving the quality of donor liver organs used for transplantation.

## REFERENCES

- 1 Swart C, Du Toit A, Loos B. Autophagy and the invisible line between life and death. *Eur J Cell Biol* 2016; **95**: 598-610 [PMID: 28340912 DOI: 10.1016/j.ejcb.2016.10.005]
- 2 Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. *J Pathol* 2010; **221**: 3-12 [PMID: 20225336 DOI: 10.1002/path.2697]
- 3 Goldman SJ, Zhang Y, Jin S. Autophagic degradation of mitochondria in white adipose tissue differentiation. *Antioxid Redox Signal* 2011; **14**: 1971-1978 [PMID: 21126221 DOI: 10.1089/ars.2010.3777]
- 4 Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* 2010; **22**: 124-131 [PMID: 20034776 DOI: 10.1016/j.ceb.2009.11.014]
- 5 Bhogal RH, Weston CJ, Curbishley SM, Adams DH, Afford SC. Autophagy: a cyto-protective mechanism which prevents primary human hepatocyte apoptosis during oxidative stress. *Autophagy* 2012; **8**: 545-558 [PMID: 22302008 DOI: 10.4161/auto.19012]
- 6 Jing K, Lim K. Why is autophagy important in human diseases? *Exp Mol Med* 2012; **44**: 69-72 [PMID: 22257881 DOI: 10.3858/emmm.2012.44.2.028]
- 7 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]
- 8 Klionsky DJ. Autophagy. *Curr Biol* 2005; **15**: R282-R283 [PMID: 15854889 DOI: 10.1016/j.cub.2005.04.013]
- 9 Jiang F. Autophagy in vascular endothelial cells. *Clin Exp Pharmacol Physiol* 2016; **43**: 1021-1028 [PMID: 27558982 DOI: 10.1111/1440-1681.12649]
- 10 Saftig P, Beertsen W, Eskelinen EL. LAMP-2: a control step for phagosome and autophagosome maturation. *Autophagy* 2008; **4**: 510-512 [PMID: 18376150 DOI: 10.4161/auto.5724]
- 11 Boone BA, Bahary N, Zureikat AH, Moser AJ, Normolle DP, Wu WC, Singhi AD, Bao P, Bartlett DL, Liotta LA, Espina V, Loughran P, Lotze MT, Zeh HJ 3rd. Safety and Biologic Response of Pre-operative Autophagy Inhibition in Combination with Gemcitabine in Patients with Pancreatic Adenocarcinoma. *Ann Surg Oncol* 2015; **22**: 4402-4410 [PMID: 25905586 DOI: 10.1245/s10434-015-4566-4]
- 12 Rosenfeld MR, Ye X, Supko JG, Desideri S, Grossman SA, Brem S, Mikkelsen T, Wang D, Chang YC, Hu J, McAfee Q, Fisher J, Troxel AB, Piao S, Heitjan DF, Tan KS, Pontiggia L, O'Dwyer PJ, Davis LE, Amaravadi RK. A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy* 2014; **10**: 1359-1368 [PMID: 24991840 DOI: 10.4161/auto.28984]
- 13 Wang C, Hu Q, Shen HM. Pharmacological inhibitors of autophagy as novel cancer therapeutic agents. *Pharmacol Res* 2016; **105**: 164-175 [PMID: 26826398 DOI: 10.1016/j.phrs.2016.01.028]
- 14 Towers CG, Thorburn A. Therapeutic Targeting of Autophagy. *EBioMedicine* 2016; **14**: 15-23 [PMID: 28029600 DOI: 10.1016/j.ebiom.2016.10.034]
- 15 Netea-Maier RT, Plantinga TS, van de Veerdonk FL, Smit JW, Netea MG. Modulation of inflammation by autophagy: Consequences for human disease. *Autophagy* 2016; **12**: 245-260 [PMID: 26222012 DOI: 10.1080/15548627.2015.1071759]
- 16 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]
- 17 Nitta T, Sato Y, Ren XS, Harada K, Sasaki M, Hirano S, Nakanuma Y. Autophagy may promote carcinoma cell invasion and correlate with poor prognosis in cholangiocarcinoma. *Int J Clin Exp Pathol* 2014; **7**: 4913-4921 [PMID: 25197362]
- 18 Cannistrà M, Ruggiero M, Zullo A, Gallelli G, Serafini S, Maria M, Naso A, Grande R, Serra R, Nardo B. Hepatic ischemia reperfusion injury: A systematic review of literature and the role of current drugs and biomarkers. *Int J Surg* 2016; **33** Suppl 1: S57-S70 [PMID: 27255130 DOI: 10.1016/j.ijsu.2016.05.050]
- 19 Miyashita T, Nakanuma S, Ahmed AK, Makino I, Hayashi H, Oyama K, Nakagawara H, Tajima H, Takamura H, Ninomiya I, Fushida S, Harmon JW, Ohta T. Ischemia reperfusion-facilitated sinusoidal endothelial cell injury in liver transplantation and the resulting impact of extravasated platelet aggregation. *Eur Surg* 2016; **48**: 92-98 [PMID: 27110233 DOI: 10.1007/s10353-015-0363-3]
- 20 DeLeve LD. Liver sinusoidal endothelial cells and liver regeneration. *J Clin Invest* 2013; **123**: 1861-1866 [PMID: 23635783 DOI: 10.1172/JCI66025]
- 21 Ravikumar R, Jassem W, Mergental H, Heaton N, Mirza D, Perera MT, Quaglia A, Holroyd D, Vogel T, Coussios CC, Friend PJ. Liver Transplantation After Ex Vivo Normothermic Machine Preservation: A Phase 1 (First-in-Man) Clinical Trial. *Am J*



- Transplant* 2016; **16**: 1779-1787 [PMID: 26752191 DOI: 10.1111/ajt.13708]
- 22 **Wesselborg S**, Stork B. Autophagy signal transduction by ATG proteins: from hierarchies to networks. *Cell Mol Life Sci* 2015; **72**: 4721-4757 [PMID: 26390974 DOI: 10.1007/s00018-015-2034-8]
- 23 **Lamb CA**, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* 2013; **14**: 759-774 [PMID: 24201109 DOI: 10.1038/nrm3696]
- 24 **Ruf S**, Heberle AM, Langelaar-Makkinje M, Gelino S, Wilkinson D, Gerbeth C, Schwarz JJ, Holzwarth B, Warscheid B, Meisinger C, van Vugt MA, Baumeister R, Hansen M, Thedieck K. PLK1 (polo like kinase 1) inhibits MTOR complex 1 and promotes autophagy. *Autophagy* 2017; **13**: 486-505 [PMID: 28102733 DOI: 10.1080/1548627.2016.1263781]
- 25 **Song YM**, Lee YH, Kim JW, Ham DS, Kang ES, Cha BS, Lee HC, Lee BW. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. *Autophagy* 2015; **11**: 46-59 [PMID: 25484077 DOI: 10.4161/15548627.2014.984271]
- 26 **Spencer B**, Potkar R, Trejo M, Rockenstein E, Patrick C, Gindi R, Adame A, Wyss-Coray T, Masliah E. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson's and Lewy body diseases. *J Neurosci* 2009; **29**: 13578-13588 [PMID: 19864570 DOI: 10.1523/JNEUROSCI.4390-09.2009]
- 27 **Mizushima N**, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, Ohsumi Y. A protein conjugation system essential for autophagy. *Nature* 1998; **395**: 395-398 [PMID: 9759731 DOI: 10.1038/26506]
- 28 **Ichimura Y**, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M, Noda T, Ohsumi Y. A ubiquitin-like system mediates protein lipidation. *Nature* 2000; **408**: 488-492 [PMID: 11100732 DOI: 10.1038/35044114]
- 29 **Altman BJ**, Rathmell JC. Metabolic stress in autophagy and cell death pathways. *Cold Spring Harb Perspect Biol* 2012; **4**: a008763 [PMID: 22952396 DOI: 10.1101/cshperspect.a008763]
- 30 **Cantó C**, Auwerx J. AMP-activated protein kinase and its downstream transcriptional pathways. *Cell Mol Life Sci* 2010; **67**: 3407-3423 [PMID: 20640476 DOI: 10.1007/s00018-010-0454-z]
- 31 **Hoyer-Hansen M**, Jäättelä M. AMP-activated protein kinase: a universal regulator of autophagy? *Autophagy* 2007; **3**: 381-383 [PMID: 17457036 DOI: 10.4161/aut.4240]
- 32 **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]
- 33 **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]
- 34 **Ghislat G**, Patron M, Rizzuto R, Knecht E. Withdrawal of essential amino acids increases autophagy by a pathway involving Ca<sup>2+</sup>/calmodulin-dependent kinase kinase-β (CaMKK-β). *J Biol Chem* 2012; **287**: 38625-38636 [PMID: 23027865 DOI: 10.1074/jbc.M112.365767]
- 35 **Kim HS**, Montana V, Jang HJ, Parpura V, Kim JA. Epigallocatechin gallate (EGCG) stimulates autophagy in vascular endothelial cells: a potential role for reducing lipid accumulation. *J Biol Chem* 2013; **288**: 22693-22705 [PMID: 23754277 DOI: 10.1074/jbc.M113.477505]
- 36 **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]
- 37 **Sengupta A**, Molkentin JD, Yutzey KE. FoxO transcription factors promote autophagy in cardiomyocytes. *J Biol Chem* 2009; **284**: 28319-28331 [PMID: 19696026 DOI: 10.1074/jbc.M109.024406]
- 38 **Huang H**, Tindall DJ. Dynamic FoxO transcription factors. *J Cell Sci* 2007; **120**: 2479-2487 [PMID: 17646672 DOI: 10.1242/jcs.001222]
- 39 **Qiang L**, Banks AS, Accili D. Uncoupling of acetylation from phosphorylation regulates FoxO1 function independent of its subcellular localization. *J Biol Chem* 2010; **285**: 27396-27401 [PMID: 20519497 DOI: 10.1074/jbc.M110.140228]
- 40 **Shen W**, Tian C, Chen H, Yang Y, Zhu D, Gao P, Liu J. Oxidative stress mediates chemerin-induced autophagy in endothelial cells. *Free Radic Biol Med* 2013; **55**: 73-82 [PMID: 23195684 DOI: 10.1016/j.freeradbiomed.2012.11.011]
- 41 **Zeng M**, Wei X, Wu Z, Li W, Li B, Fei Y, He Y, Chen J, Wang P, Liu X. Reactive oxygen species contribute to simulated ischemia/reperfusion-induced autophagic cell death in human umbilical vein endothelial cells. *Med Sci Monit* 2014; **20**: 1017-1023 [PMID: 24943908 DOI: 10.12659/MSM.890897]
- 42 **Navarro-Yepes J**, Burns M, Anandhan A, Khalimonchuk O, del Razo LM, Quintanilla-Vega B, Pappa A, Panayiotidis MI, Franco R. Oxidative stress, redox signaling, and autophagy: cell death versus survival. *Antioxid Redox Signal* 2014; **21**: 66-85 [PMID: 24483238 DOI: 10.1089/ars.2014.5837]
- 43 **Liu J**, Bi X, Chen T, Zhang Q, Wang SX, Chiu JJ, Liu GS, Zhang Y, Bu P, Jiang F. Shear stress regulates endothelial cell autophagy via redox regulation and Sirt1 expression. *Cell Death Dis* 2015; **6**: e1827 [PMID: 26181207 DOI: 10.1038/cddis.2015.193]
- 44 **Li R**, Jen N, Wu L, Lee J, Fang K, Quigley K, Lee K, Wang S, Zhou B, Vergnes L, Chen YR, Li Z, Reue K, Ann DK, Hsiai TK. Disturbed Flow Induces Autophagy, but Impairs Autophagic Flux to Perturb Mitochondrial Homeostasis. *Antioxid Redox Signal* 2015; **23**: 1207-1219 [PMID: 26120766 DOI: 10.1089/ars.2014.5896]
- 45 **Torisu T**, Torisu K, Lee IH, Liu J, Malide D, Combs CA, Wu XS, Rovira II, Fergusson MM, Weigert R, Connelly PS, Daniels MP, Komatsu M, Cao L, Finkel T. Autophagy regulates endothelial cell processing, maturation and secretion of von Willebrand factor. *Nat Med* 2013; **19**: 1281-1287 [PMID: 24056772 DOI: 10.1038/nm.3288]
- 46 **Shu X**, Keller TC 4th, Begandt D, Butcher JT, Biwer L, Keller AS, Columbus L, Isakson BE. Endothelial nitric oxide synthase in the microcirculation. *Cell Mol Life Sci* 2015; **72**: 4561-4575 [PMID: 26390975 DOI: 10.1007/s00018-015-2021-0]
- 47 **Guo F**, Li X, Peng J, Tang Y, Yang Q, Liu L, Wang Z, Jiang Z, Xiao M, Ni C, Chen R, Wei D, Wang GX. Autophagy regulates vascular endothelial cell eNOS and ET-1 expression induced by laminar shear stress in an ex vivo perfused system. *Ann Biomed Eng* 2014; **42**: 1978-1988 [PMID: 24838486 DOI: 10.1007/s10439-014-1033-5]
- 48 **Yang Q**, Li X, Li R, Peng J, Wang Z, Jiang Z, Tang X, Peng Z, Wang Y, Wei D. Low Shear Stress Inhibited Endothelial Cell Autophagy Through TET2 Downregulation. *Ann Biomed Eng* 2016; **44**: 2218-2227 [PMID: 26493943 DOI: 10.1007/s10439-015-1491-4]
- 49 **Zhang L**, Wang X, Miao Y, Chen Z, Qiang P, Cui L, Jing H, Guo Y. Magnetic ferroferric oxide nanoparticles induce vascular endothelial cell dysfunction and inflammation by disturbing autophagy. *J Hazard Mater* 2016; **304**: 186-195 [PMID: 26551222 DOI: 10.1016/j.jhazmat.2015.10.041]
- 50 **Davies PF**. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat Clin Pract Cardiovasc Med* 2009; **6**: 16-26 [PMID: 19029993 DOI: 10.1038/nccardio1397]
- 51 **Du J**, Teng RJ, Guan T, Eis A, Kaul S, Konduri GG, Shi Y. Role of autophagy in angiogenesis in aortic endothelial cells. *Am J Physiol Cell Physiol* 2012; **302**: C383-C391 [PMID: 22031599 DOI: 10.1152/ajpcell.00164.2011]
- 52 **Jiang BH**, Liu LZ. AKT signaling in regulating angiogenesis. *Current Cancer Drug Targets* 2008; **8**: 19-26 [DOI: 10.2174/15680908783497122]
- 53 **Zhang L**, Cui L, Zhou G, Jing H, Guo Y, Sun W. Pterostilbene, a natural small-molecular compound, promotes cytoprotective macroautophagy in vascular endothelial cells. *J Nutr*

- Biochem* 2013; **24**: 903-911 [PMID: 22898568 DOI: 10.1016/j.jnutbio.2012.06.008]
- 54 **Uberti F**, Lattuada D, Morsanuto V, Nava U, Bolis G, Vacca G, Squarzanti DF, Cisari C, Molinari C. Vitamin D protects human endothelial cells from oxidative stress through the autophagic and survival pathways. *J Clin Endocrinol Metab* 2014; **99**: 1367-1374 [PMID: 24285680 DOI: 10.1210/jc.2013-2103]
- 55 **Xie Y**, You SJ, Zhang YL, Han Q, Cao YJ, Xu XS, Yang YP, Li J, Liu CF. Protective role of autophagy in AGE-induced early injury of human vascular endothelial cells. *Mol Med Rep* 2011; **4**: 459-464 [PMID: 21468592]
- 56 **Urbanek T**, Kuczmik W, Basta-Kaim A, Gabryel B. Rapamycin induces protective autophagy in vascular endothelial cells exposed to oxygen-glucose deprivation. *Brain Res* 2014; **1553**: 1-11 [PMID: 24462935 DOI: 10.1016/j.brainres.2014.01.017]
- 57 **Imber CJ**, St Peter SD, Lopez de Cenarruzabeitia I, Pigott D, James T, Taylor R, McGuire J, Hughes D, Butler A, Rees M, Friend PJ. Advantages of normothermic perfusion over cold storage in liver preservation. *Transplantation* 2002; **73**: 701-709 [PMID: 11907414 DOI: 10.1097/00007890-200203150-00008]
- 58 **op den Dries S**, Karimian N, Sutton ME, Westerkamp AC, Nijsten MW, Gouw AS, Wiersema-Buist J, Lisman T, Leuvenink HG, Porte RJ. Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. *Am J Transplant* 2013; **13**: 1327-1335 [PMID: 23463950 DOI: 10.1111/ajt.12187]
- 59 **Xu H**, Berendsen T, Kim K, Soto-Gutiérrez A, Bertheim F, Yarmush ML, Hertl M. Excorporeal normothermic machine perfusion resuscitates pig DCD livers with extended warm ischemia. *J Surg Res* 2012; **173**: e83-e88 [PMID: 22099594 DOI: 10.1016/j.jss.2011.09.057]
- 60 **Sumimoto K**, Inagaki K, Yamada K, Kawasaki T, Dohi K. Reliable indices for the determination of viability of grafted liver immediately after orthotopic transplantation. Bile flow rate and cellular adenosine triphosphate level. *Transplantation* 1988; **46**: 506-509 [PMID: 3051557 DOI: 10.1097/00007890-198810000-00007]
- 61 **Kamiike W**, Burdelski M, Steinhoff G, Ringe B, Lauchart W, Pichlmayr R. Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. *Transplantation* 1988; **45**: 138-143 [PMID: 3276042 DOI: 10.1097/00007890-198801000-00030]
- 62 **Go KL**, Lee S, Zendejas I, Behrns KE, Kim JS. Mitochondrial Dysfunction and Autophagy in Hepatic Ischemia/Reperfusion Injury. *Biomed Res Int* 2015; **2015**: 183469 [PMID: 26770970 DOI: 10.1155/2015/183469]
- 63 **Rockey DC**. Endothelial dysfunction in advanced liver disease. *Am J Med Sci* 2015; **349**: 6-16 [PMID: 25559279 DOI: 10.1097/MAJ.0000000000000403]

P- Reviewer: Yan LN S- Editor: Qi Y L- Editor: A  
E- Editor: Ma YJ





Published by **Baishideng Publishing Group Inc**  
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgooffice@wjgnet.com](mailto:bpgooffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>



ISSN 1007-9327

