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Autophagy in the liver: Cell's cannibalism and beyond

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Abstract

Chronic liver disease and its progression to liver failure are induced by various etiologies including viral infection, alcoholic and nonalcoholic hepatosteatosis. It is anticipated that the prevalence of fatty liver disease will continue to rise due to the growing incidence of obesity and metabolic disorder. Evidence is accumulating to indicate that the onset of fatty liver disease is causatively linked to mitochondrial dysfunction and abnormal lipid accumulation. Current treatment options for this disease are limited. Autophagy is an integral catabolic pathway that maintains cellular homeostasis both selectively and nonselectively. As mitophagy and lipophagy selectively remove dysfunctional mitochondria and excess lipids, respectively, stimulation of autophagy could have therapeutic potential to ameliorate liver function in steatotic patients. This review highlights our up-to-date knowledge on mechanistic roles of autophagy in the pathogenesis of fatty liver disease and its vulnerability to surgical stress, with an emphasis on mitophagy and lipophagy.

Keywords

autophagy; mitophagy; lipophagy; liver

Introduction

The liver is a central organ with multiple functions including the clearance of toxins, production of various hormones and proteins, and storage of vitamins. This largest internal organ also plays a pivotal role in the regulation of blood sugar, production and secretion of bile, production of ketone bodies, and regulation of lipid storage. Chronic liver disease stems from many etiologies such as viral infection, ethanol exposure, congenital disorders, pharmacological toxicities, and metabolic disorders. Its presentation may range from steatosis and steatohepatitis to fibrosis. Currently, chronic liver disease is the fifth leading cause of death worldwide, and its prevalence globally continues to rise. This disease is also a leading risk factor for hepatocellular carcinoma (HCC). In its latest annual report to the

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Nation on the Status of Cancer, the National Cancer Institute stated that HCC deaths increased 2.8% per year in men and 2.2% per year in women from 2003 to 2012, while all other cancer deaths and incidence rates for both men and women of all major racial and ethnic populations decreased substantially (Ryerson et al. 2016). Thus, chronic liver disease and its progression to HCC have become a major socio-economic burden. While viral hepatitis and alcohol-related liver disease (ALD) are among the most common causes of chronic liver disease, the increasing elderly population and growing rates of obesity and metabolic syndrome suggest that the prevalence of nonalcoholic fatty liver disease (NAFLD) will continue to escalate. Current therapeutic options for NAFLD, chronic ALD and their progression to HCC are limited, and the only strategy for long-term cure in these patients is surgical resection and liver transplantation. Due to its regenerative nature, the liver can recover after major hepatic resection. Risk of hepatic insufficiency or failure, however, continues to increase as patients approach end stages of liver disease. In the clinical setting, livers with metabolic dysfunction or genetic predisposition are substantially more prone to surgical stress than normal counterparts, although the mechanisms remain to be elucidated.

Understanding of the precise mechanisms behind the onset of chronic liver disease and enhanced vulnerability to stress is a key component to successful development of therapeutic strategies. Crucial mechanisms underlying this disease include mitochondrial dysfunction and abnormal lipid metabolism. Autophagy is a quality control process by degrading surplus or unnecessary cytoplasmic contents and abnormal intracellular organelles within the lysosome. Mitophagy and lipophagy are two specific autophagic pathways that regulate mitochondrial homeostasis and cellular levels of lipids, respectively. This review will discuss; 1) the importance of mitochondria in hepatic steatosis, 2) roles of selective autophagy in the pathogenesis of alcohol and nonalcoholic fatty liver disease, 3) signaling transduction in mitophagy and lipophagy, and 4) surgical challenges in steatotic livers.

Fatty liver disease and mitochondria

Hepatosteatosis, the accumulation of lipids greater than 5% of the wet weight of the liver, is one of the most rapidly growing diseases globally (Hornboll et al. 1982; Berson et al. 1998) and can occur through multiple etiologies including chronic or binge alcohol consumption, metabolic derangements and excess lipid intake (Marchesini et al. 2001; Hamaguchi et al. 2005). NAFLD describes the spectrum of disease that ranges from aberrant accumulation of triglycerides (TG) to nonalcoholic steatohepatitis (NASH), the development of fibrosis, cirrhosis and HCC. Approximately 4–29% of NASH patients exhibiting severe inflammation, hepatocyte ballooning and death, and fibrosis will develop liver cirrhosis and HCC within 10 years (Argo et al. 2009). Day et al. have posited a “2-hit” hypothesis to describe the progression of NAFLD to NASH (Day et al. 1998). The first hit of excess TG and cholesterol accumulation primes the liver for sensitization to the second hit, which is hallmarked by increased oxidative stress, inflammation, and induction of insulin resistance (Matsuzawa-Nagata et al. 2008). Insights into hepatic lipid transport and mitochondrial function have substantiated this 2-hit hypothesis.

Hepatocytes orchestrate with adipocytes for the proper storage and transport of lipids. In healthy individuals, lipids are primarily taken up and stored in adipose tissues; however

under aberrant conditions such as excess lipid intake, hepatocytes – the primary parenchymal cells of the liver - may uptake, store and metabolize lipids. Although too much lipid in hepatocytes can result in lipotoxicity and cell death, surplus hepatic free fatty acids (FFA) can undergo β -oxidation in the mitochondria or become esterified into TG. While normal levels of TG are naturally secreted from the liver in the form of very low density lipoproteins (VLDL), overloaded TG can be converted to lipid droplets (LDs) in hepatocytes. During the early phase of NAFLD, mitochondrial function increases to counteract hepatocellular fat overloading (Jiang et al. 2008; Hodson et al. 2010). However, sustained fat deposition progressively impairs mitochondrial function.

As a primary organelle responsible for ATP production, β -oxidation of fatty acids, and ketone body synthesis, the mitochondria rely on their proton motive force, comprising of both mitochondrial membrane potential and pH gradient across the mitochondrial inner membrane. Changes in either component, thus, significantly alter hepatic lipid metabolism and contribute to the development of liver disease. Though high levels of fat increase β -oxidation in the mitochondria to maximize fatty acid utilization, they can modify both flavin adenine dinucleotide (FADH₂)-mediated oxygen consumption (Feige et al. 2008) and proton leak across the mitochondrial inner membrane (Rolfe et al. 1994), resulting in increased formation of reactive oxygen species (ROS). Reduced electron transfer capacity, increased oxidative stress, and changes in mitochondrial redox potential have also been observed in rat models of fatty livers (Vial et al. 2011). Aside from altering mitochondrial function, excess fat consumption can adversely affect mitochondrial morphology and membrane structure. Lionetti et al. recently reported that a high fat diet decreases the expression of mitofusin 2 (MFN2), a key protein involved in the mitochondrial fusion process, and increases the levels of dynamin-related protein 1 (DRP1) and fission 1 protein (FIS1), proteins favoring mitochondrial fission (Lionetti et al. 2014). These alterations in expression suggest that fat overnutrition shifts mitochondrial dynamics toward the fission process. In a model of leptin knockout mice, the increase in mitochondrial fission proteins was likewise reported in skeletal muscles (Jheng et al. 2012). Aoun et al. have demonstrated in rats that dietary fat markedly remodels the content of cardiolipin as well as its fatty acid composition in liver mitochondrial membranes, which is associated with the development of overt hepatic steatosis (Aoun et al. 2012). Cardiolipin is a unique phospholipid that is almost exclusively localized in the mitochondrial inner membrane. This mitochondrial phospholipid is critical to maintain membrane fluidity (Unsay et al. 2013), ion trafficking (Subramani et al. 2016), protein translocation (Gebert et al. 2009), mitochondrial bioenergetics (Beyer et al. 1996), and mitochondrial-dependent apoptosis (Lutter et al. 2001).

ALD and mitochondria

The mechanisms underlying the pathogenesis of ALD are complex and multifactorial (Knecht et al. 1990; Knecht et al. 1995; Cederbaum et al. 2009). Although ALD shares similar pathological spectra with NAFLD, some disease characteristics are distinct between ALD and NAFLD (Tannapfel et al. 2011). For instance, while fat accumulation in the liver is more pronounced in NAFLD than in ALD, hepatic inflammation occurs to a greater degree in ALD. In addition, adverse modifications in blood vessels such as atherosclerosis are more common in ALD, suggesting that the mechanisms of ALD differ from those of NAFLD.

Like NAFLD, excess alcohol consumption, whether binge or chronic overuse, damages the mitochondria in hepatocytes (Williams et al. 2015). Binge consumption depolarizes the mitochondria since aldehyde, a toxic byproduct of alcohol metabolism, compromises the electron transport chain at complexes I and III, resulting in electron leak and over production of ROS (Bailey et al. 1998). After ethanol consumption, alcohol dehydrogenase (ADH), catalase and the cytochrome P450 system cooperatively metabolize ethanol to acetaldehyde. While this highly reactive metabolite of alcohol can rapidly be detoxified by mitochondrial aldehyde dehydrogenase (ALDH) in a healthy liver, mutations in dehydrogenases or mitochondrial dysfunction inhibit alcohol detoxification, leading to accumulation of acetaldehyde that can form abnormal proteins and DNA adducts (Zakhari 2006). As most mitochondrial genes originate from the nucleus, disturbances in these genes significantly diminish mitochondrial function. In addition, alcohol itself impacts the mitochondria. Patients with ALD show enlarged mitochondria (Bruguera et al. 1977), lowered oxidative phosphorylation (Bailey et al. 1999; Young et al. 2006), and defective FFA oxidation (Baraona et al. 1975). Alcohol is also known to increase mitochondrial ROS that further potentiate deletions and mutations in mitochondrial DNA (Knecht et al. 1990; Knecht et al. 1995). Thus, regardless of etiology, the mitochondria play a key role in the pathogenesis of alcohol-induced fatty liver disease. Strategies to mitigate mitochondrial dysfunction or sequester damaged mitochondria may limit these disease processes.

Autophagy

Autophagy is an evolutionarily conserved lysosomal pathway tasked with degrading long-lived, unnecessary, or damaged proteins and organelles to maintain intracellular homeostasis (He et al. 2009). This catabolic process also responds to various stresses including nutrient depletion/starvation, oxidative stress, and infection by viral and bacterial pathogens to confer cytoprotection (Kroemer et al. 2010). Autophagy can be subdivided into three classes: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (Mizushima et al. 2008). CMA is a selective form of autophagy that recognizes and hydrolyzes soluble proteins containing the KFERQ motif. Translocation of proteins with this motif is mediated by 70 kDa molecular chaperone heat shock protein (HSC70), which interacts with lysosome-associated membrane protein 2A (LAMP2A) to deliver target cargo to the lysosome for degradation (Massey et al. 2006). Microautophagy is the least understood class of autophagy and involves the direct delivery of cytosolic substrates to the late endosomal or lysosomal lumen by invagination of the membrane (Santambrogio et al. 2011). Macroautophagy, the most common form of autophagy, is driven by the highly regulated interplay of more than 30 autophagy-related genes (ATG) (Fig. 1). ATG proteins orchestrate the formation of a double-membrane phagophore near cellular target constituents. The phagophore expands and surrounds these constituents, forming the autophagosome, which ultimately fuses with the late endosome or lysosome to hydrolyze its cargo (Mizushima et al. 2008). Details on autophagy can be found elsewhere (Parzych et al. 2014). This review will focus on macroautophagy (referred to as autophagy hereafter) as this class not only plays a significant role in energy homeostasis and stress response in the liver, but also clears dysfunctional or abnormal mitochondria and excess lipid. Autophagy can be further subdivided into nonselective, or bulk autophagy, and selective autophagy that specifically targets organelles

or LDs for degradation (Singh et al. 2011). Types of selective autophagy include: ribosomal (ribophagy) (Kraft et al. 2008), peroxisomal (pexophagy) (Dunn, Jr. et al. 2005), iron-based (ferritinophagy) (Mancias et al. 2014), endoplasmic reticular (reticulophagy) (Nakatogawa et al. 2015), mitochondrial (mitophagy) (Lee et al. 2014), and lipid-based (lipophagy) (Singh et al. 2009). Among these selective forms of autophagy, mitophagy and lipophagy are of particular importance in regulation and onset of fatty liver disease.

Mitophagy

Functional mitophagy is essential not only for preventing the accumulation of abnormal or damaged mitochondria, but also for maintaining a stable number of healthy mitochondria (Fig. 2). At least three different types of mitophagy exist in the cell (Lemasters 2014). While type I mitophagy largely resembles canonical autophagy in that it requires phosphatidylinositol-3-kinase class III (PI3K-III) and phagophore assembly, the onset of type II mitophagy is instigated by mitochondrial depolarization and subsequent activation of PTEN-induced putative kinase 1 (PINK1) and Parkin, expression of Bcl2/adenovirus E1B 19kDa interacting protein 3 (BNIP3), and/or activation of FUN14 domain containing 1 (FUNDC1). The loss of mitochondrial membrane potential by mitochondrial uncoupling agents initiates this unique mitophagy pathway by preventing the translocation of PINK1 into the intermembrane space where presenilin-associated rhomboid-like protein (PARL), a protease that cleaves the transmembrane domain of PINK1, is located. As PINK1 translocation is dependent on mitochondrial membrane potential, mitochondrial depolarization inhibits the interaction of PINK1 with PARL, resulting in PINK1 accumulation on the mitochondrial outer membrane (Jin et al. 2010). Accumulated PINK1 subsequently phosphorylates both MFN2 (Chen et al. 2013) and ubiquitin (Koyano et al. 2014; Okatsu et al. 2015) and recruits Parkin, an E3 ubiquitin ligase (Sarraf et al. 2013). After ubiquitination of outer mitochondrial membrane proteins by Parkin, target proteins localize to the phagophore through their interaction with p62/SQSTM1 (Pankiv et al. 2007).

BNIP3 also plays a role in the clearance of depolarized mitochondria. During hypoxia, hypoxia inducible factor 1- α (HIF1- α) enhances the expression of BNIP3 and its translocation to the mitochondrial outer membrane where it inhibits B-cell lymphoma 2 (Bcl-2) (Chen et al. 1997; Bruick 2000; Bellot et al. 2009). Inhibition of Bcl-2 leads to the release of Beclin1 (BECN1) from the PI3K-III complex and initiates autophagy afterwards (Ray et al. 2000). It has been reported that BNIP3 has a microtubule-associated protein light chain 3 (LC3) interacting region (LIR) that facilitates the formation of the autophagosome that sequesters dysfunctional mitochondria (Zhu et al. 2013).

Hypoxia or mitochondrial uncoupling can also initiate mitophagy through the FUNDC1 pathway. Under normal conditions where the mitochondria remain polarized, mitophagy is suppressed since casein kinase 2 (CK2) and Src kinase phosphorylate FUNDC1 at Ser13 and Tyr18, respectively (Liu et al. 2012; Chen et al. 2014). However, when the mitochondria become depolarized by hypoxia or chemical uncouplers, PGAM family member 5 (PGAM5), a mitochondrial phosphatase, dephosphorylates FUNDC1 at Ser13 (Chen et al. 2014). At the same time, UNC51 like autophagy activating kinase 1 (ULK1) activates

FUNDC1's LIR domain by phosphorylating the Ser17 residue (Wu et al. 2014). Once activated, FUNDC1 recruits the phagophore to begin enwrapping depolarized mitochondria.

Type III mitophagy, termed "micromitophagy," depends on the formation of mitochondria-derived vesicles enriched in oxidized mitochondrial proteins that bud off and transit into multivesicular bodies, though a detailed mechanism remains to be elucidated. Overall, type I and II mitophagy engulf entire mitochondria for removal, whereas type III may selectively eliminate damaged regions of individual mitochondrion and oxidized mitochondrial components.

Mitophagy in ALD

Cytotoxic effects of acute and chronic ethanol consumption on the liver have been well demonstrated in cells, animals, and humans. As described earlier, ethanol alters the mitochondria through multiple mechanisms. Ni et al. have demonstrated that alcohol initially stimulates mitophagy by upregulating transcription and expression of PINK1/Parkin in a forkhead box O3a (FOXO3a)-dependent manner (Ni et al. 2013), suggesting the onset of mitophagy is an early adaptive strategy against alcohol toxicity. Cytoprotection by mitophagy has recently been confirmed in a rat model of binge alcohol exposure (Eid et al. 2016).

Alcoholic steatosis is characterized by macro- or micro-vesicular accumulation of LDs in the cytoplasm of hepatocytes. Although LDs have been proposed to have independent biological functions (Ohsaki et al. 2006), excess cytoplasmic LDs impede hepatocellular functions and heighten vulnerability of hepatocytes to stresses as well (Feldstein et al. 2011). Alcohol-induced hepatic steatosis can occur by enhanced de novo lipogenesis, increased mobilization of fatty acids from the gut and adipose tissue to the liver, or decreased fatty acid oxidation in the mitochondria. Studies have shown that hepatocytes attempt to counter these stresses through autophagy. Using a Lieber-DeCarli model, Eid et al. showed in rat hepatocytes that large LDs and damaged mitochondria constitute the main cargo in autophagic vesicles (Eid et al. 2013), accompanied with increased levels of PINK1 in the mitochondria and enhanced lysosomal activity. The importance of autophagy, particularly mitophagy and lipophagy, as a survival strategy against alcoholic steatosis is also supported by other studies where autophagy enhancement by rapamycin attenuates ROS production, lipid accumulation, and cellular injury by alcohol overdose (Wu et al. 2010; Lu et al. 2015).

Mitophagy in the fatty liver

Fat over-intake alters mitochondria both functionally and structurally. The mitochondria from a high-fat diet favor mitochondrial fission, a dynamic process that changes mitochondrial morphology to smaller, more discrete mitochondria (Lionetti et al. 2014; Wang et al. 2015). In addition, excess lipid intake adversely affects mitochondrial function, including fatty acid oxidation, expression of uncoupling protein 2 (Jiang et al. 2008) and proton leak across the inner mitochondrial membrane (Rolfe et al. 1994). Structurally, surplus fat consumption increases cardiolipin content in the mitochondrial inner membrane (Aoun et al. 2012). Relocation of cardiolipin from the inner to the outer membrane has been

proposed to mediate fusion between mitochondria and lysosomes, an integral part of mitophagy (Chu et al. 2013). Thus, it is intuitively expected that mitophagy may play a pivotal role in the pathogenesis of NAFLD. Wang *et al.* have recently reported that the hepatic expression of lysocardiolipin acyltransferase 1 (ALCAT1), an acyl CoA-dependent enzyme catalyzing the remodeling of cardiolipin in obesity, diabetes, and cardiovascular disease, is significantly up-regulated by high fat diet and leptin deficiency (Wang et al. 2015). Using ALCAT1-null mice, they demonstrated that elevated levels of ALCAT1 impair autophagy and mitochondrial function in hepatocytes. Thus, similar in ALD, mitophagy likely confers cytoprotection in NAFLD.

Lipophagy in the fatty liver

While mitophagy aids in the reduction of intrahepatic TG and lipid accumulation, lipophagy is specifically dedicated to removal of surplus lipids (Fig. 3). Lipophagy is likely to be cell type and context specific. For instance, fasting or nutrient depletion stimulates lipophagy in hepatocytes to meet the metabolic demand through β -oxidation (Singh et al. 2009). In contrast, neurons utilize lipophagy to increase levels of neuropeptide in the hypothalamus (Kaushik et al. 2011).

How lipophagy selectively targets LDs is largely unknown, although the core components in lipophagy activation are speculated to be conserved in various cells. Current research suggests that the interaction between ancient ubiquitous protein 1 (AUP1) and E2 ubiquitin conjugase G2 (UBE2G2) at the LD surface is an important step in lipophagy onset (Spandl et al. 2011). Like the canonical pathway of autophagy, ubiquitination may also be a pivotal factor in lipophagy, as evidenced by enhanced clustering of LDs by AUP1 ubiquitination (Lohmann et al. 2013). Interestingly, unlike other autophagic clearance mechanisms, removal of LDs may require both CMA and lipophagy (Schneider et al. 2014; Kaushik et al. 2015). The prerequisite role of CMA for lipophagy is supported by the observation that CMA-null mice are unable to degrade LDs. Furthermore, these animals exhibit pronounced hepatic steatosis as well as insulin resistance without affecting macroautophagy. During lipophagy, the autophagosome either engulfs small LDs or sequesters portions of large LDs through the conversion of LC3-I into LC3-II at the surface of LD in a RAB7 dependent manner (Singh et al. 2009; Schroeder et al. 2015). In macroautophagy, a small guanosine triphosphatase (GTPase) RAB7 and homotypic fusion and protein sorting complex (HOPS) are essential components in the formation of autolysosomes (Seals et al. 2000; Wurmser et al. 2000). RAB7 is likely indispensable for lipophagy onset because genetic depletion of this GTPase dampens the efficiency of lipophagy and changes the morphology of multivesicular bodies, lysosomes, and autophagosomes (Schroeder et al. 2015). Recently, pleckstrin homology domain containing protein family member 1 (PLEKM1) has been identified as a new RAB7 effector that functions at the late endocytosis and degradation stage of autophagy (McEwan et al. 2015).

Similar to alcohol administration, the duration of fatty acid uptake distinctly impacts lipophagy. While short-term incubation of hepatocytes with FFA activates autophagy, prolonged exposure suppresses autophagy and lipophagy, as indicated by decreased interaction between LDs and LC3 (Rodriguez et al. 2006). In addition, pharmacological and

genetic inhibition of autophagy using 3-methyladenine (3-MA), siRNA-guided knockdown of ATG5, and liver-specific knock out of ATG7, all cause a noticeable accumulation of LDs, underscoring the requirement of autophagy and lipophagy in hepatic lipid regulation. Of note, it has also been reported that chronic challenge with high fat diet reduces the fusion between autophagosomes and lysosomes to 30% of control values (Koga et al. 2010), indicating that overnutrition affects various stages of autophagy and lipophagy.

Autophagy in fatty liver inflammation

A hallmark of the progression of fatty liver disease is a sterile inflammation that occurs in the absence of pathogens. While invasion of pathogens induces inflammation through a cascade of pro-inflammatory factors such as tumor necrosis factor α (TNF- α) (Mizuhara et al. 1994), nuclear factor κ B (NF- κ B) (Dela et al. 2005), and toll-like receptors (TLR) (Zhai et al. 2004), sterile inflammation is triggered by endogenous damage-associated molecular patterns (DAMPs) that are often hidden from the extracellular environment (Kubes et al. 2012). More than 20 DAMPs have been identified, and they activate a wide range of immune responses including production of pro-inflammatory cytokines and localization of immune cells to the site of injury (Kono et al. 2008). Growing evidence indicates that many DAMPs such as mitochondrial DNA, formyl peptides, cytochrome c, and ATP, originate from the mitochondria (Zhang et al. 2010; Krysko et al. 2011; Shimada et al. 2012). This is, indeed, unsurprising as the mitochondria likely originate from obligate parasites, and as mitochondrial DNA retains similar molecular structures to bacteria (Yang et al. 1985). Moreover, the diversity of mitochondrial DAMPs can potentiate the extent of inflammation as one mitochondrion can generate a full range of signals from DNA to ATP that activate the inflammasome. In particular, the liver is prone to mitochondria-mediated inflammation due to the large number of mitochondria residing in the metabolically active hepatocyte.

Several lines of evidence suggest that autophagy inhibits the inflammasome. Immune cells with defective autophagy significantly increase the caspase-1-dependent release of interleukin-1b (IL-1 β) and interleukin-18 (IL-18) (Saitoh et al. 2008). Pharmacological or genetic inhibition of autophagy enhances interleukin-23 (IL-23) release from macrophages and dendritic cells through a TLR-dependent fashion (Peral de et al. 2012), indicative of the presence of dynamic cross talk between autophagy and inflammasomes. Importantly, autophagy directly regulates the processing and secretion of these inflammatory cytokines (Harris et al. 2011; Peral de et al. 2012). The integral role of autophagy in sterile inflammation is further supported by a recent study demonstrating that the autophagosome can sequester inflammasome components, including absent in melanoma 2 (AIM2), Nod like receptor protein 3 (NLRP3), and apoptosis-associated speck-like protein (ASC) for selective degradation (Shi et al. 2012). In a mouse model of NASH manifesting inflammation and hepatic fibrosis, autophagy augmentation by rapamycin reduces neutrophil infiltration, the pro-inflammatory cytokine monocyte chemoattractant protein 1 (MCP-1), IL-1 β , and TNF- α . In marked contrast, suppression of autophagy with chloroquine increases these factors, substantiating an inhibitory effect of autophagy on the activation of inflammasomes (Chen et al. 2016).

Ischemia/reperfusion (I/R) injury and fatty liver disease: clinical implications and mechanistic insights

For patients with advanced stage liver disease, surgical treatment is the only curative option. I/R injury is, however, unavoidable during surgery. During liver resection, occlusion of the portal triad (portal vein, hepatic artery, and common bile duct) is often utilized to reduce intraoperative blood loss. When this technique is employed, the liver is inevitably exposed to ischemia, leading to depletion of oxygen and nutrients, loss of mitochondrial ATP production, and tissue acidosis (Kim et al. 2003). Although prolonged ischemia ultimately results in irreversible damage, restoration of blood flow upon release of vascular clamping worsens tissue injury (Kim et al. 2008), a phenomenon called reperfusion injury. I/R injury likewise affects transplanted livers. During organ procurement and storage, donor livers are exposed to cold ischemia and warm reperfusion.

The adverse effects of steatosis on the outcome of liver surgery have been well documented (Soejima et al. 2003; Spitzer et al. 2010). Steatosis has also been linked to increased perioperative morbidity and mortality after liver resection (Behrns et al. 1998; Belghiti et al. 2000), and steatotic livers are more prone to oxidative stresses as compared to lean counterparts (Kathirvel et al. 2010). Severe steatosis likewise limits the pool of suitable transplant donors, exacerbating the existing shortage of donor livers (Dahm et al. 2005; Gehrau et al. 2015). Further, even mild steatosis is known to increase the incidence of primary non-function and graft failure after liver transplantation (Ploeg et al. 1993). Therefore, patients with fatty liver disease are at an increased risk of perioperative mortality due to preexisting liver dysfunction and ischemic stress during surgery.

Hepatic I/R injury is a multifactorial event involving Ca^{2+} deregulation (Kim et al. 2012), mitochondrial dysfunction (Kim et al. 2003), oxidative stress (Jaeschke et al. 1991), uncontrolled stimulation of catabolic enzymes (Kim et al. 2013), and loss of autophagy (Kim et al. 2008; Wang et al. 2012). During the initial phase of reperfusion, mitochondrial calcium overloading and ROS overproduction incite the onset of mitochondrial permeability transition (MPT) (Kim et al. 2012). As the mitochondrial inner membrane is impermeable to virtually all solutes under normal conditions, the opening of MPT pores after reperfusion disrupts the integrity of the mitochondrial inner membrane, allowing an uncontrolled influx of solutes up to 1,500 Da into the mitochondrial matrix. As a result, the mitochondria swell, oxidative phosphorylation in the electron transport chain becomes uncoupled, and mitochondrial membrane potential is lost. Furthermore, during this stage, mitochondrial ROS diffuse into the cytoplasm, and cytosolic ROS are generated from extra-mitochondrial compartments, all of which activate non-parenchymal cells in the liver. Mitochondrial dysfunction and ROS accumulation eventually deplete hepatocytes of ATP, leading to necrotic cell death. At the late stage of reperfusion, substances released from both dead hepatocytes and non-parenchymal cells further recruit neutrophils and provoke uncontrollable systemic inflammation (Bremer et al. 1994).

Besides mitochondria-dependent cell death, I/R also impairs hepatocellular autophagy (Kim et al. 2008). As autophagy is an energy-requiring process, ATP depletion during hepatic I/R halts the autophagic machinery. In addition, key autophagy proteins such as ATG7 and

human disease, and ultimately patient clinical trials are necessary to address this growing problem.

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Fig. 1. Diagram of autophagy process

Nutrient deprivation or rapamycin treatment initiates autophagy. Upon initiation, the preinitiation complex comprising of ULK1, ATG13, and FIP200, works in concert with the PI3K-III complex to induce membrane nucleation of a phagophore. LC3-I becomes lipidated and activated to LC3-II afterwards by a cascade of autophagy proteins, including ATG4B, ATG5, ATG7, ATG10, ATG12, and ATG16L1. This activated form of LC3 guides the phagophore to recruit intracellular cargo. During the maturation stage, LC3-II is inserted into both cytosolic and luminal plane of the growing phagophore. While LC3-II on the

luminal side interacts with adaptor proteins for autophagic cargo, the cytosolic side LC3-II interacts later with the endosomal/lysosomal compartment. During the degradation phase, PLEKHM1 localizes to both the endosome and lysosome in a HOPS- and RAB7-dependent manner. The PLEKHM1-RAB7-HOPS complex aids in the fusion between two compartments. This complex may also localize to the autophagosome. Finally, the late autophagosome fuses with the lysosome to generate the autolysosome, wherein lysosome-associated membrane protein (LAMP) promotes vesicular fusion events along microtubules. Finally, luminal contents in the autolysosome are degraded by lysosomal enzymes.

Fig. 2. Mitophagy pathway

There exist at least three distinct forms of mitophagy. (A) Type 1 mitophagy is initiated by nutrient starvation and it clears polarized mitochondria in a PI3K-III-dependent way. PI3K-III inhibitors such as wortmannin or 3-methyl-adenine (3-MA) block this mitophagic pathway. (B) In contrast, type 2 mitophagy eliminates depolarized mitochondria in a PINK1/Parkin, BNIP3, and/or FUNDC1-dependent fashion. During hypoxia, HIF1- α promotes BNIP3 expression and its translocation into the mitochondrial outer membrane. BNIP3 stimulates mitophagy through its interaction with Bcl-2. The onset of type 2 mitophagy is

also modulated by phosphorylation status of FUNDC1. Type 2 mitophagy occurs independently of PI3K-III. (C) Mild oxidative stress induces type 3 mitophagy by generating a multivesicular body with mitochondria-derived vesicles. PINK1 and Parkin might be involved in this process.

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Fig. 3. Lipophagy pathway

Following starvation or overnutrition, AUP1 at the LD becomes ubiquitinated by UBEG2, which recruits a phagophore. In addition, the interaction of RAB7 with HOPS is likely an important step in the onset of lipophagy.