Autophagy in Ischemic Livers: A Critical Role of Sirtuin 1/Mitofusin 2 Axis in Autophagy Induction

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No-flow ischemia occurs during cardiac arrest, hemorrhagic shock, liver resection and transplantation. Recovery of blood flow and normal physiological pH, however, irreversibly injures the liver and other tissues. Although the liver has the powerful machinery for mitochondrial quality control, a process called mitophagy, mitochondrial dysfunction and subsequent cell death occur after reperfusion. Growing evidence indicates that reperfusion impairs mitophagy, leading to mitochondrial dysfunction, defective oxidative phosphorylation, accumulation of toxic metabolites, energy loss and ultimately cell death. The importance of acetylation/deacetylation cycle in the mitochondria and mitophagy has recently gained attention. Emerging data suggest that sirtuins, enzymes deacetylating a variety of target proteins in cellular metabolism, survival and longevity, may also act as an autophagy modulator. This review highlights recent advances of our understanding of a mechanistic correlation between sirtuin 1, mitophagy and ischemic liver injury.

Key words: Autophagy, Mitochondria, Liver, Ischemia/Reperfusion, Acetylation

INTRODUCTION

Timely removal of unnecessary cellular constituents and abnormal organelles is essential to sustain cell viability. Intracellular protein degradation and protein synthesis are tightly balanced to maintain cell survival. Two catabolic pathways account for protein degradation: 1) the ubiquitin-proteasome pathway for degradation of short-lived proteins, and 2) the autophagy or “self-eating” pathway for degradation of long-lived proteins and abnormal organelles (1). In the liver, long-lived proteins constitute more than 99% of cell proteins and thus, autophagic degradation is the primary catabolic process for proteins (2). Three major forms of autophagy have been described in mammalian cells: microautophagy, macroautophagy and chaperone-mediated autophagy (1). Among the three forms, macroautophagy is of particular importance in the liver as this form of autophagy not only clears unneeded intracellular proteins but also digests injured or dysfunctional organelles such as abnormal mitochondria. Although macroautophagy is generally considered a random process, growing evidence shows the existence of selective macroautophagy, especially for clearance of the mitochondria, termed mitophagy (3).

The liver is vulnerable to hypoxic and anoxic stresses. Although prolonged interruption of blood flow (ischemia) eventually causes hepatocyte death, extensive injury paradoxically occurs mostly after the restoration of blood flow and oxygen, a phenomenon called reperfusion injury (4). Ischemia/reperfusion (I/R) injury is a major pathological event in low flow disease states, including hemorrhagic shock, and cardiac arrest, and intentionally during surgical procedures such as liver resection and transplantation. Mitochondrial dysfunction is a causal mechanism attributing to I/R injury (4,5). Injured mitochondria hamper energy production and precipitate further injury to neighboring mitochondria through release of cytotoxic compounds (5). Therefore, elimination of damaged mitochondria in a timely manner is critical to sustain viability in ischemic hepatocytes. As mitophagy is the only known cellular mechanism to dispose abnormal mitochondria, active recruitment of mitophagy has a therapeutic potential for mitochondria-related diseases (4).

THE LIVER

The liver, the largest internal organ located in the upper right quadrant of the abdomen, performs multiple functions
in the body, including synthesis of essential proteins and cofactors; regulation of glycogen synthesis and degradation; storage of vitamins and minerals; production and secretion of hormones and bile; and clearance of toxic metabolites. To accomplish these varied functions, distinct types of hepatic cells - hepatocytes, Kupffer cells, stellate cells, sinusoidal endothelial cells, cholangiocytes, lymphocytes and dendrite cells - require high amounts of cellular energy in order to synthesize and eliminate a plethora of complex molecules. Such a high demand for cellular energy in the liver can likewise be inferred from its unique dual-blood supply system: 1) the portal venous supply from the gut, pancreas, and spleen, and 2) arterial supply from the heart (6). Using these two sources of blood supply, the liver is continuously nourished with oxygen, energy substrates and nutrients. Hepatic blood vessels encompass about 22% of the liver mass/volume and the liver contains about 12% of the total blood volume (7). Blood flow through the liver amounts to 1,500–2,000 mL/min (8). The hepatic artery and the portal vein furnish approximately 25% and 75% of the resting cardiac output, respectively, and complete mixture of both portal and arterial blood occurs in the hepatic sinuoids (6). Hence, the liver is a highly aerobic organ and innately vulnerable to hypoxic and ischemic stresses. Sinusoidal blood flow to the hepatic lobules and subsequent addition of synthetic products and metabolic wastes to the blood create gradients of oxygen and metabolites between perportal and pericentral regions of the liver lobule (9). As a consequence, the liver exhibits a distinct zonal dependence on specific biochemical reactions. For instance, while ureagenesis, gluconeogenesis, beta-oxidation of fatty acids, and cholesterol synthesis are enriched in the periportal hepatocytes, lipogenesis, glycolysis and drug detoxification occurs mainly in the pericentral hepatocytes (9). Hepatic disease likewise demonstrates zonal dependency. Hypoxic injury is observed first in the pericentral region due to the intralobular oxygen gradient (10). Hemosiderin accumulation in hemochromatosis and drug-induced hepatotoxicity frequently exist in perportal and pericentral area, respectively (7).

LIVER SURGERY AND I/R INJURY

Chronic liver disease is the fifth leading cause of death worldwide (11). Total deaths worldwide from liver cirrhosis and cancer have increased from 676,000 in 1980 to over 1 million in 2010 (12). Liver resection refers to the surgical removal of portions of the liver that contain cancer, benign tumors or cystic disease. A common technique employed in liver resection in order to reduce intraoperative blood loss is the Pringle maneuver, which involves the clamping of the portal triad (hepatic artery, portal vein, and bile duct) (6). When this technique is employed, the blood flow to the liver is interrupted, resulting in oxygen and nutrient depletion and subsequently impeding mitochondrial oxidative phosphorylation and ATP generation (4). At the same time, stored glycogen hydrolyzes into glucose that prompts anaerobic glycolysis (13). Although this cytosolic glucose utilization pathway replenishes some ATP to the liver, the end product, lactic acid, accumulates in the cell resulting in tissue acidosis. Moreover, hydrogen ions released from ATP hydrolysis and acidic organelles during ischemia further enhance tissue acidosis (14,15). Restoration of blood flow upon release of vascular clamping results in a return to physiologic pH, an event that worsens ischemic tissue injury (4,16). Another setting from which I/R injury can occur to the liver is liver transplantation. Approximately 6,700 liver transplantsations are performed annually in the United States (17). Donor livers exposed to ischemia during harvest, storage and transport undergo subsequent reperfusion injury once they are anastomosed to recipient vasculature, and blood flow is restored.

MECHANISMS OF I/R INJURY

The mechanisms underlying hepatic I/R injury are multifactorial and include Ca\(^{2+}\) deregulation, mitochondrial dysfunction, generation of reactive oxygen and nitrogen species, loss of cellular antioxidants, stimulation of catabolic enzymes, and loss of autophagy (4). Progression of I/R injury can be viewed as three different stages: Within the first few minutes of reperfusion, sequential events of calcium overload and reactive oxygen species (ROS) accumulation in the mitochondria cause mitochondrial dysfunction and the onset of mitochondrial permeability transition (MPT), leading to ATP depletion and necrotic death of hepatocytes. (4,5) Increased ROS also activate Kupffer cells to promote even greater ROS production. During the next 6 hrs of reperfusion, activated Kupffer cells continue to release ROS, cytokines and chemokines to recruit neutrophils. Finally, at the late phase of reperfusion, neutrophil infiltration becomes uncontrollable and incites irreversible systemic inflammation. Although tissue inflammation is an important pathology in hepatic I/R injury, it should be noted that the damage to hepatocytes after reperfusion is the earliest event that conduces to permanent I/R injury to the liver (4,5).

Mitochondrial dysfunction is the main causative mechanism of hepatocyte death after I/R (4,16). The mitochondrion, a power plant in the cell, contains both inner and outer membranes. In contrast to the mitochondrial outer membrane, the mitochondrial inner membrane is virtually impermeable to all solutes except for those having specific carriers or exchangers. While acidic pH during ischemia prevents mitochondrial permeabilization, the opening of MPT pores upon reperfusion disrupts the inner membrane barrier in the mitochondria, which allows an unregulated influx of solutes up to 1,500 Da into the mitochondrial matrix (5,16). Free diffusion of solutes successively induces mitochondrial
swelling, uncoupling of oxidative phosphorylation, and depolarization of the mitochondrial membrane potential. As the proton motive force in the mitochondria is governed equally by mitochondrial membrane potential and pH gradient, the onset of MPT after reperfusion collapses the proton motive force, causing ATP depletion and cellular necrosis. Thus, MPT onset and subsequent mitochondrial dysfunction are the key mechanisms contributing to hepatocyte death after I/R (5,16). Besides necrosis, the onset of MPT can also provoke apoptotic cell death. The loss of mitochondrial membrane integrity releases cytochrome c that is normally sequestered in the intermembrane space in the mitochondria. Once released, cytochrome c binds to the apoptosis-inducing factor-1 and pro-caspases to form a protein complex, the apoptosome, which, in turn, activates downstream effector caspases and develops apoptosis (16,18). Thus, MPT onset is a common pathway to both necrotic and apoptotic hepatocyte death after reperfusion. In contrast to necrosis, apoptosis requires ATP. The availability of glycolytic ATP is a critical determinant of cell death fate (18): When hepatocytes are depleted of ATP after MPT onset, necrosis is a predominant cell death fate. However, when ATP is available, hepatocytes undergo apoptotic death instead (16).

Molecular composition of the MPT pores remains incompletely understood; however, three major components, the adenine nucleotide translocator (ANT) on the inner mitochondrial membrane, voltage-dependent anion channel (VDAC) on the outer mitochondrial membrane, and cyclophilin D in the mitochondrial matrix have been identified (16,19). Multiple studies have proposed that the conformational change from trans to cis ANT by cyclophilin D results in MPT onset (16,19-21). Other proteins might also play a role, including hexokinase and Bcl-2 family members (16). Low pH and cyclosporine A (CsA), an immunosuppressive agent, suppress the MPT by inhibiting cyclophilin D (VDAC) on the outer mitochondrial membrane, voltage-dependent anion channel (ANT) by cyclophilin D, while concomitantly serving protective functions against unexhanced mitochondrial overloading of Ca$^{2+}$ and overproduction of ROS, events upstream to the MPT, still prevail even after the MPT is blocked (10). Therefore, it is unlikely that significant and persistent levels of cytoprotection could be achieved with current therapeutic strategies. As mitophagy selectively targets and removes abnormal mitochondria, this endogenous mitochondrial quality control machinery could have therapeutic potentials for I/R injury and other mitochondrial diseases.

**AUTOPHAGY**

Autophagy is an evolutionarily conserved catabolic process that eliminates protein aggregates and surplus or damaged intracellular organelles. Autophagy was first described by Christian de Duve and is defined as a cell’s “self-eating” event (29). As macroautophagy clears both cellular constituents and dysfunctional mitochondria, this review focuses on macroautophagy and refers to it as autophagy hereafter.

Autophagy is a sequential process that begins with the initiation and formation of an autophagosome, a double membrane structure that sequesters and delivers cellular cargo to the lysosome. Canonical autophagy relies on the recruitment of multiple autophagy-related proteins (ATG) onto a cup-shaped double membrane complex termed a phagophore. Non-canonical autophagy is incompletely understood but can occur in the absence of some key ATG proteins where the expansion of phagophore membrane is dependent on vesicular transport vesicles originated from the Golgi and endosomes (30). Autophagy can selectively or non-selectively enclose cargo material. Selective autophagy includes the removal of specific cellular constituents and intracellular organelles: peroxisomes (pexophagy) (31), mitochondria (mitophagy) (3), ribosomes (ribophagy) (32), endoplasmic reticulum (reticulophagy) (33), lipids (lipophagy) (34), and iron (ferritintrinophagy) (35).

Autophagy is slow under basal conditions but becomes stimulated by certain conditions such as nutrient depletion or starvation (1,29). Under nutrient and amino acid-rich conditions, UNC-51 like kinase 1 (ULK1), a mammalian ortholog of yeast ATG1, is phosphorylated at the residue of Ser757 by mammalian or mechanistic target of rapamycin (mTORC1) and dissociated from adenosine monophosphate-activated protein kinase (AMPK). This process promotes cell growth and proliferation but prevents autophagy initiation (36). However, under nutrient insufficiency, AMPK senses the changes in energy contents and initiates autophagy by phosphorylating the residues of Ser317, 555, 777 of ULK1 (37,38). Activated ULK1 serves in recruiting and phosphorylating both ATG13 and focal adhesion kinase family interacting protein of 200 kDa (FIP200), generating the ULK1-ATG13-FIP200 complex on the surface of phagophores (39,40). Moreover, ULK1 subdues the kinase activity of mTORC1 through its binding with raptor (41). Thus, the orchestrated coordination between mTORC1, AMPK, and ULK1 is an integral part of autophagy initiation. Another important event in autophagy initiation is the phosphorylation and activation of Beclin-1 (BECN1). When
nutrients are abundant, anti-apoptotic protein, B cell lymphoma-2 (Bcl-2), binds to BECN1 and inhibits autophagy. In contrast, under nutrient insufficiency, Bcl-2 is phosphorylated by stress-responsive c-Jun N-terminal protein kinase 1 (JNK1). The change in phosphorylation status of Bcl-2 dissociates BECN1 from Bcl-2, and liberated BECN1 then stimulates autophagy (42,43). BECN1 is also a key component in the BECN1-ATG14-VPS34-VPS15 class III PI3K core complexes (44). Vacuolar sorting protein 34 (VPS34), a class III lipid kinase, modulates vesicle trafficking and the formation of autophagosomal membranes (45,46). The initiation of autophagy is further regulated by other factors, including ultraviolet irradiation resistance-associated gene (UVRA) (47), BIF-1 (48), ATG14L (49) or RUN domain Beclin-1-interacting cysteine-rich-containing protein (RUBICON) (50).

ATG12 and microtubule-associated protein 1 light chain 3 (LC3) play a major role in autophagosome maturation. Upon the initial activation by ATG7, an E1-ubiquitin like enzyme, ATG12 is covalently connected to ATG5 by ATG10, an E2-like ubiquitin carrier protein. Conjugated ATG12-ATG5 complexes then interact with Atg16L1 later. Other proteins including ATG4B, ATG7, ATG3, are also involved in the maturation of autophagosomes (51). The conjugation of LC3-I to phosphatidylethanolamine by ATG7 and ATG3 generates LC3-II that localizes to autophagosomal membranes. On the contrary, unconjugated LC3-I resides in the cytosol. Due to its distinct location and unique chemical structure, LC3-II is commonly used to monitor autophagy (52,53). The conversion of LC3-I to LC3-II requires ATG4B, a cysteine protease. Importantly, ATG4B can also act on LC3-II to release LC3 from phosphatidylethanolamine (54). The removal of phospholipid not only relocates LC3 from the autophagosomal membrane, but also facilitates its subsequent fusion with the endosome/lysosome.

The mature autophagosome fuses with the lysosome to produce the autolysosome. It has been shown that Ras-like GTPases are involved in this tethering process (55). Specifically, overexpression of Rab7 promotes autophagy, whereas its silencing prevents autophagy (56). The interaction of UVRAG-BECN1-PI3KIII complex with the class C vacuolar protein sorting complex further facilitates Rab7-mediated fusion (57). The formation of autolysosomes is also fine-tuned by soluble N-ethylmaleimide-sensitive fusion factors (SNARES) (57,58), showing the complexity of autophagy network. Once the formation of autolysosomes is completed, its luminal contents are rapidly degraded by acidic proteases, lipases, nucleases, and glycosidases. The final end products following this process are later recycled back to the cell for other metabolic purposes (37).

**MITOPHAGY**

Mitophagy mediates mitochondrial turnover, which occurs every 15 to 25 days (59). Therefore, functional mitophagy not only prevents the accumulation of abnormal or damaged mitochondria, but also is essential to maintain a stable number of healthy mitochondria. Lemasters’ group has proposed that three different types of mitophagy exist in the cell (3). The mechanisms of type I mitophagy are similar to those in canonical autophagy described above. This mitophagy requires PI3KIII signaling and can occur at the phagophore assembly. In contrast, mitochondrial depolarization instigates the onset of type II mitophagy. 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. Overall, both type I and II mitophagy engulf an entire mitochondrion for removal, while type III selectively eliminates damaged and oxidized mitochondrial components.

Several proteins have been proposed to induce mitophagy. Under normal conditions, the mitochondrial serine protease, presenilin-associated rhomboid like protein (PARKL) cleaves tension homolog-induced putative kinase protein 1 (PINK1) in the mitochondria (60). When the mitochondria depolarize under stress conditions, a decrease in PARL activity and following inhibition of PINK cleavage translocate a full length PINK1 to the outer mitochondrial membrane. Soon after, PINK1 recruits PARKIN, an E3 ubiquitin ligase, to the mitochondria where PARKIN directs the ubiquitination of target proteins such as p62 and VDAC of damaged mitochondria (61-63). Transcription factor p62 is known to act as a linker protein between autophagic cargo and autophagosomes (64). The mitochondrial accumulation of PARKIN appears to be voltage-dependent, and does not require changes in pH or ATP levels (65). Mitochondrial receptors Bcl2/adenovirus EB 19-kDA interacting protein 3 (BNIP3) or FUN14 domain-containing protein-1 (FUND1C) likely also plays a role in mitophagy (66). BNIP3, also called Bnip3L or NIX, shares homology with Bcl-2 in the BH3 domains. FUND1C-mediated mitophagy requires ULK1, wherein activated ULK1 phosphorylates FUND1C upon mitochondrial depolarization (67,68). Multiple studies have posited that BNIP3 and FUND1C trigger mitophagy by binding to LC3 through a WXXL motif (66,69,70).

**MITOPHAGY IN LIVER I/R INJURY**

Autophagy is a highly energy-dependent process. Hence, ATP depletion during hepatic I/R adversely impacts the autophagic machinery. Anoxia during ischemia impedes the formation of autophagic vesicles, as evidenced by lack of LC3-II increase in the presence of lysosomal inhibitors such as bafilomycin or chloroquine (71,72). Although a transient repolarization of the mitochondrial membrane potential during the early stage of reperfusion can provide some ATP to cells and operate autophagy temporarily, the demand for mitoph-
agy to remove swollen and injured mitochondria exceeds the autophagic capacity in reperfused hepatocytes. A few minutes after reperfusion, hepatocytes thus encounter accumulation of abnormal or dysfunctional mitochondria, uncontrolled Ca\(^{2+}\) and ROS overloading, activation of injurious enzymes, the onset of MPT and eventually cell death (5,71,72). To make things worse, key autophagy proteins such as ATG7 and BECN1 become hydrolyzed by calpains as a consequence of Ca\(^{2+}\) overloading (5,71,72). Hence, loss of key autophagy proteins and depletion of ATP synergistically impair mitophagy after I/R. The importance of mitophagy in ischemic livers is substantiated by findings that both pharmacological and genetic approaches that stimulate mitophagy confer cytoprotection against hepatic I/R injury (5,71,72). Of note, observations that initial MPT onset occurs in a subset of mitochondria prior to widespread MPT in the cell suggest that some mitochondria are more prone to I/R stress. Toxic metabolites and byproducts from injured mitochondria can propagate to neighboring healthy mitochondria, culminating in widespread mitochondrial dysfunction (73). Since mitophagy enhancement blocks the onset of MPT and cell death after reperfusion, timely clearance of these stress-prone mitochondria appears to be indispensable for sustaining functional bioenergetics and cell survival.

### Sirtuins in the Liver

Acetylation is a post-translational modification of proteins by covalent addition of an acetyl group to lysine residues. In general, removal of positively-charged lysine neutralizes the total charge balance of their active sites. Acetylation or deacetylation can impact a variety of cellular functions such as DNA binding affinity, catalytic activity, stability and localization of target proteins (74). Protein acetylation is a highly dynamic process that is governed by balanced action between lysine acetyltransferases (KATs, formerly known as histone acetyltransferases, HATs) and deacetylases (KDACs, formerly termed as histone deacetylases, HDACs). KATs are categorized into three major groups: 1) KAT2/GCN5-related N-acetyltransferases (GNAT family), 2) E1A binding protein p300 (EP300/CREBBP family), and 3) MYST family (75). KDACs are further subdivided into 4 classes, based on their sequence homology to the original yeast enzymes and domain organization. Designated as Class III KDACs, sirtuins have some distinctive features from other classes. They are mammalian ortholog of yeast silent information regulator 2 (Sir2) and utilize oxidized nicotinamide adenine dinucleotide (NAD\(^{+}\)) as a cofactor for their enzyme activity (76-80). In mammals, seven different isoforms of sirtuins (SIRT1-7) have been identified. Although individual isoforms contain a uniquely conserved NAD\(^{+}\) deacetylase domain, deacetylation activity varies among the isoforms. The conserved catalytic domain of sirtuins contains up to 270 amino acid residues and forms a characteristic reverse Rossmann-fold, and zinc ribbon (81,82). Recent reports that acetyl-coenzyme A (AcCoA), a major component of the Krebs cycle and \(\beta\)-oxidation in the mitochondria as well as glycolysis and catabolism of branched amino acids in the cytosol, donates its acetyl moiety to the target lysine residues (83,84) demonstrate the involvement of deacetylation reactions in cellular energy metabolism. Expectedly therefore, studies confirm that deacetylation reactions by sirtuins are distinctly coupled to transcription, mitochondrial biogenesis, oxidative phosphorylation, and autophagy (85,86).

Sirtuin 1 (SIRT1) localizes in the cytosol and nucleus (87) and is known to regulate circadian rhythms (88-90), autophagy (91-95), gluconeogenesis (96,97), fatty acid oxidation (96,98), mitochondrial biogenesis (96,99,100), cell proliferation (101,102) and antioxidant defense (92). Embryonic lethality in SIRT1-null transgenic mice implicates its essential role in tissue viability (103). In the liver, SIRT1 can deacetylate a myriad of non-histone targets including peroxisome proliferator-activated receptor gamma-coactivator-1 alpha (PGC-1\(\alpha\)) (97), CREB regulated transcription coactivator 2 (104), Forkhead transcription factors (FOXO) (105), fibroblast growth factor 21 (FGF21) (106), and signal transducer and activator of transcription 3 (STAT3) (107), all of which are closely associated with hepatic energy homeostasis and metabolism.

The roles of other sirtuin isoforms in physiology and pathophysiology are beginning to be elucidated as well. The mitochondria-localized SIRT3, for instance, modulates intramitochondrial metabolic activities and ROS formation by deacetylating the NDUFA9 subunit of Complex I in the mitochondrial electron transfer chain (108-112). Another connection of SIRT3 with mitochondrial energy homeostasis comes from experiments demonstrating that SIRT3 can directly deacetylate mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2, a late limiting enzyme in the synthesis of ketone body (112-114). SIRT5 is another mitochondrial isoform of sirtuins encompassing deacetylation (115), desuccinylation (116), demalonylation (116), and deglutarylation (117). This mitochondrial matrix enzyme has long been known to regulate carbamoyl phosphate synthase 1 (CPS1), a rate limiting enzyme for the urea cycle and ammonia clearance (115-117). Taken together, both mitochondrial and extramitochondrial sirtuins affect cellular energy metabolism and homeostasis.

### ROLE OF ACETYLATION/DEACETYLATION IN AUTOPHAGY

Although sirtuins are not an essential component of the autophagic machinery, evidence is accumulating to indicate that autophagy is regulated by the cycle of acetylation/deacetylation. Table 1 summarizes autophagy-related pro-
tein targets that are regulated by acetylation status. Acetyla-
tion/deacetylation-dependent modulation of autophagy occurs
transcriptionally and translationally. For example, spermi-
dine, a natural inhibitor of KATs, enhances autophagy
through hypoacetylation of ATG7 promoter (118). Studies
have shown that changes in acetylation status significantly
impact the activity of FOXO1 or FOXO3, transcription fac-
tors associated with autophagy induction (119). More direct
evidences come from the studies which demonstrate that
p300 can acetylate ATG5, ATG7, LC3 and ATG12 (120),
whereas SIRT1 can deacetylate ATG5, ATG7 and LC3 under
nutrient insufficiency (91). SIRT1-dependent deacetylation
also determines intracellular distribution of autophagy com-
ponents. Huang et al. recently reported that deacetylation of
LC3 by SIRT1 redistributes LC3 from the nucleus to the
cytoplasm, and that deacetylated cytosolic LC3 produces
more stable autophagosomes (121). Thus, acetylation/de-
acetylation cycle not only regulates the activity of autoph-
agy but also ensures an effective redistribution of autophagy
elements between intracellular compartments. BECN1 appears
to be another autophagy target that is regulated by acety-
lation/deacetylation since acetylated BECN1 inhibits auto-
phagosome maturation and endocytic trafficking (122).
Studies also suggest that acetylated ULK1 can enhance auto-
phagy process by stimulating its kinase activity (123,124). Thus,
changes in acetylation status highly affect both initia-
tion and elongation stage of autophagy.

### SIRT1, AUTOPHAGY AND I/R INJURY

We recently demonstrated in human liver biopsies that hepatic inflow occlusion during liver resection decreases
SIRT1 expression to 30% of basal levels (125). Such a
reduction was also evident in the mouse livers and hepato-
cytes after in vivo and in vitro I/R, respectively. Calpain
activation due to Ca\(^{2+}\) overloading during I/R appears to be,
at least in part, responsible for SIRT1 loss. Although reper-
fusion after prolonged ischemia leads to near-complete
depletion of SIRT1 in both the cytosol and nucleus, subcel-
larular fractionation assays revealed that cytosolic SIRT1 loss
precedes nuclear SIRT1 loss, implying that cytosolic SIRT1
is more susceptible to hepatocellular I/R injury. Adenoviral
overexpression or pharmacological activation of SIRT1 by
resveratrol or SRT1720 enhanced cytosolic levels of SIRT1
and mitophagy, and sustained mitochondrial structural
integrity after reperfusion, substantiating the importance of
cytosolic SIRT1 in mitochondrial quality. The mechanism
by which SIRT1 elevates mitophagy is likely associated
with ATG7 because SIRT1 overexpression significantly
increases ATG7 expression, whereas levels of other auto-
phagy-related proteins such as ATG3, ATG4B, ATG12,5,
ATG14L, BECN1, RUBICON, LAMP2A, and Cathepsin D
remain unaltered by this treatment. Importantly, both treat-
ments conferred cytoprotection against global MPT onset
and necrosis, which was not observed in hepatocytes from
SIRT1 conditional knockout mice. In contrast to wild-type
hepatocytes, the cells from SIRT1-deficient mice exhibited
a rapid onset of the MPT and increased cell death even after
short ischemia. Taken together, these results indicate that
SIRT1 depletion contributes to I/R injury in hepatocytes
and cytosolic SIRT1 is required for mitochondrial integrity
and function (Fig. 1).

Many mitochondrial proteins exist in an acetylated form.
About 35% of all mitochondrial proteins are endogenously
acetylated, 24% of which are mechanistically linked to
energy homeostasis (126). Hyperacetylation or defective

### Table 1. Summary of autophagy related proteins regulated by acetylation/deacetylation

| Protein  | Description                                           | References          |
|----------|-------------------------------------------------------|---------------------|
| ULK1     | Enhancing autophagy upon acetylation by TIP60         | (123,124)           |
| ATG3, ATG5, ATG7 | Decreasing autophagy upon acetylation by p300 | (91,120)           |
|          | Enhancing autophagy upon deacetylation by SIRT1       |                     |
| ATG12    | Decreasing autophagy upon acetylation by p300         | (120)               |
| LC3      | Decreasing autophagy upon acetylation by p300         | (91,120,121)        |
|          | Enhancing autophagy upon deacetylation by SIRT1       |                     |
| BECN1    | Decreasing autophagy upon acetylation by p300         | (122)               |
|          | Enhancing autophagy upon deacetylation by SIRT1       |                     |
| FOXO1, FOXO3 | Decreasing autophagy upon acetylation by p300   | (119)               |
|          | Enhancing autophagy upon deacetylation by sirtuins    |                     |
|          | (SIRT1, 2 and 3)                                      |                     |
| MFN2     | Enhancing autophagy upon deacetylation by SIRT1       | (125)               |
| Tubulin  | Enhancing autophagy upon acetylation by α-TAT1/MEC17  | (128,129)           |
|          | Decreasing autophagy upon deacetylation by HDAC6      |                     |
| Hsp70    | Decreasing autophagy upon acetylation by p300         | (130)               |
|          | Enhancing autophagy upon deacetylation by HDAC6       |                     |
deacetylation of the mitochondrial proteins has been shown to account for liver steatosis and obesity (114,127). The mechanisms underlying SIRT1-mediated cytoprotection against I/R injury are likely linked to deacetylation of mitofusin 2 (MFN2), a mitochondrial outer membrane protein, by SIRT1 and subsequent augmentation of mitophagy. Immunoprecipitation and immunoblotting approaches unveiled that while cytosolic SIRT1 physically interacts with both MFN1 and MFN2, it deacetylates only MFN2. The importance of SIRT1/MFN2 interaction in hepatic I/R injury was further supported by the result that knock-down of MFN2 with a small hairpin RNA abolishes a series of beneficial effects by SIRT1, including SIRT1-mediated mitophagy induction, cytoprotection against mitochondrial dysfunction, and cell death after reperfusion. Though the acetylated residues of mouse MFN2 are currently unknown, bioinformatic analysis conforming to either X6-K-[Y,W,F]-X5 or X6-KX5-[Y,W,F] motif predicts at least five different SIRT1 target sites of MFN2: K37, K215, K357, K655, and K662 (125). Noticeably, K215 localizes at the GTPase domain, a critical site for catalytic activity of MFN2. Both K655 and K662 reside in the C-terminal flanking domain that directs mitochondrial localization of MFN2. Consistent with this prediction, deletion of N-terminal regions of MFN2 blunted SIRT1-mediated autophagy induction. One interesting observation is that while hepatocytes are relatively tolerant of a large loss in SIRT1, a similar reduction of MFN2 causes greater cell death after reperfusion, implying a central role of MFN2 in I/R injury to the liver. It has been reported that MFN2-null animals are embryonically lethal, whereas SIRT1 knockout mice are born alive (125). Hence, it is speculated that MFN2-deficient cells may be more prone to I/R injury than SIRT1-null counterpart. Although the minimal levels of MFN2 needed for adequate responses to I/R and other stresses remain to be determined, the interaction of MFN2 and its subsequent deacetylation by SIRT1 are likely pivotal events in autophagy regulation and cell survival after I/R (Fig. 2).

**CONCLUSION AND FUTURE PERSPECTIVES**

I/R injury has a profound impact on the burden of liver diseases. Efforts to improve liver function after I/R, however, have not been successful largely due to an incomplete understanding of I/R injury. The mechanisms behind hepatic I/R injury are multifactorial, including defective mitophagy, the onset of MPT and mitochondrial dysfunction. Such a complexity of reperfusion injury is further underscored by the recent study which shows that the SIRT1/MFN2 axis controls mitophagy and mitochondrial function in ischemic livers. Although enhancing mitophagy has emerged as a new potential strategy against reperfusion injury, there exist
a few unanswered questions, such as the effects of mitophagy on non-parenchymal cells after I/R, roles of different types of mitophagy in ischemic livers, similarities and differences in mitophagy signaling pathways between normal and ischemic livers, and impact of other mitochondrial sirtuins and their potential interactions with SIRT1 before and after ischemia. It should also be noted that current strategies including treatment with autophagy inducers prior to ischemia and the viral delivery of specific autophagy genes lack of specific autophagy enhancers without compromising the immune system all limit their clinical applications. Better understanding of the pathological complexity of reperfusion injury and its mechanistic insights into mitophagy could lead to the development of promising treatment strategies for hepatic I/R injury and mitochondrial diseases.

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REFERENCES

1. Mizushima, N., Levine, B., Cuervo, A.M. and Klionsky, D.J. (2008) Autophagy fights disease through cellular self-digestion. Nature, 451, 1069-1075.

2. Mizushima, N. and Klionsky, D.J. (2007) Protein turnover via autophagy: implications for metabolism. Annu. Rev. Nutr., 27, 19-40.

3. Lemasters, J.J. (2014) Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). Redox Biol., 2, 749-754.

4. Cursio, R., Colosetti, P. and Gugenheim, J. (2015) Autophagy and liver ischemia-reperfusion injury. Biomed Res Int., 2015, 417590.

5. Kim, J.S., Nitta, T., Mohuczy, D., O’Malley, K.A., Moldawer, L.L., Dunn, W.A. Jr. and Behrens, K.E. (2008) Impaired autophagy: A mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. Hepatology, 47, 1725-1736.

6. Blumgart, L.H. and Hann, L.E. (2012) Surgical and Radiological Anatomy of the Liver, Biliary Tract and Pancreas, Blumgart’s Surgery of the Liver, Biliary tract and Pancreas (5th Edition). Elsevier, Philadelphia, pp. 31.

7. Arias, I.M., Alter, H.J., Boyer, J.L., Cohen, D.E., Fausto, N., Shafritz, D.A. and Wolkoff, A.W. (2009) The liver: Biology and pathobiology (5th Edition). Wiley-Blackwell, New Jersey.

8. Bradley, S.E., Ingelfinger, F.J., Bradley, G.P. and Curry, J.J. (1945) The estimation of hepatic blood flow in man. J. Clin. Invest., 24, 890-897.

9. Bradford, B.U., Marotto, M., Lemasters, J.J. and Thurman, R.G. (1986) New, simple models to evaluate zone-specific damage due to hypoxia in the perfused rat liver: time course and effect of nutritional state. J. Pharmacol. Exp. Ther., 236, 263-268.

10. Malhi, H., Gores, G.J. and Lemasters, J.J. (2006) Apoptosis and necrosis in the liver: a tale of two deaths? Hepatology, 43, S31-44.

11. World Health Organization (WHO). (2013) Study of liver disease mortality.

12. Mokdad, A.A., Lopez, A.D., Shrank, S., Lozano, R., Mokdad, A.H., Stanaway, J., Murray, C.J. and Naghavi, M. (2014) Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med., 12, 145.

13. Tang, L., Tian, F., Tao, W. and Cui, J. (2007) Hepatocellular glycogen in alleviation of liver ischemia-reperfusion injury during partial hepatectomy. World J. Surg., 31, 2039-2043.

14. Lemasters, J.J., Caldwell-Kenkel, J.C., Gao, W., Nieminen, A.L., Herman, B. and Thurman, R.G. (1992) Hypoxic, ischemic and reperfusion injury in the liver in Pathophysiology of Reperfusion Injury (Das, D.K. edition). CRC, Florida, pp. 101-135.

15. Bronk, S.F. and Gores, G.J. (1991) Efflux of protons from acidic vesicles contributes to cytosolic acidification of hepatocytes during ATP depletion. Hepatology, 14, 626-633.

16. Kim, J.S., He, L. and Lemasters, J.J. (2003) Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem. Biophys. Res. Commun., 304, 463-470.

17. United Network for Organ Sharing (UNOS). (2014).

18. Kim, J.S., Qian, T. and Lemasters, J.J. (2003) Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. Gastroenterology, 124, 494-503.

19. Leung, A.W. and Halestrap, A.P. (2008) Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore. Biochim. Biophys. Acta, 1777, 946-952.

20. Hauensloy, D., Wynne, A., Duchen, M. and Yellon, D. (2004) Transient mitochondrial permeability transition pore opening mediates preconditioning-induced protection. Circulation, 109, 1714-1717.

21. He, L. and Lemasters, J.J. (2002) Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function? FEBS Lett., 512, 1-7.

22. Akhtar, M.Z., Henderson, T., Sutherland, A., Vogel, T. and Friend, P.J. (2013) Novel approaches to preventing ischemia-reperfusion injury during liver transplantation. Transplant. Proc., 45, 2083-2092.

23. Yamashita, Y., Shimada, M., Hamatsu, T., Rikimaru, T., Tanaka, S., Shirabe, K. and Sugimachi, K. (2001) Effects of preoperative steroid administration on surgical stress in hepatic resection: prospective randomized trial. Arch. Surg., 136, 328-333.

24. Pan, L.J., Zhang, Z.C., Zhang, Z.Y., Wang, W.J., Xu, Y. and Zhang, Z.M. (2012) Effects and mechanisms of store-operated calcium channel blockade on hepatic ischemia-reperfusion injury in rats. World J. Gastroenterol., 18, 356-367.

25. Gurusamy, K.S., Gonzalez, H.D. and Davidson, B.R. (2010) Current protective strategies in liver surgery. World J. Gastroenterol., 16, 6098-6103.

26. Uchida, M., Takemoto, Y., Nagasue, N., Dhar, D.K., Kohno, H. and Nakamura, T. (1994) Effect of verapamil on hepatic reperfusion injury after prolonged ischemia in pigs. J. Hepatol., 21, 217-223.
27. Kon, K., Kim, J.S., Jaeschke, H. and Lemasters, J.J. (2004) Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. *Hepatology*, **40**, 1170-1179.

28. Kim, J.S., Jin, Y. and Lemasters, J.J. (2006) Reactive oxygen species, but not Ca\(^{2+}\) overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. *Am. J. Physiol. Heart Circ. Physiol.*, **290**, H2024-H2034.

29. Klionsky, D.J. (2007) Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat. Rev. Mol. Cell Biol.*, **8**, 931-937.

30. Codogno, P., Mehrpour, M. and Prickas-Cezanne, T. (2011) Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat. Rev. Mol. Cell Biol.*, **13**, 7-12.

31. Dunn, W.A. Jr., Cregg, J.M., Kiel, J.A., van der Klei, I.J., Oku, M., Sakai, Y., Sibiry, A.A., Stasyk, O.V. and Veenhuis, M. (2005) Pexophagy: the selective autophagy of peroxisomes. *Autophagy*, **1**, 75-83.

32. Kraft, C., Deplazes, A., Sohrmann, M. and Peter, M. (2008) Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat. Cell Biol.*, **10**, 610-621.

33. Hamasaki, M., Noda, T., Baba, M. and Ohsumi, Y. (2005) Starvation triggers the delivery of the endoplasmic reticulum to the vacuole via autophagy in yeast. *Traffic*, **6**, 56-65.

34. Singh, R., Kauhnik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M. and Czaja, M.J. (2009) Autophagy regulates lipid metabolism. *Nature*, **458**, 1131-1135.

35. Mancias, J.D., Wang, X., Gigli, S.P., Harper, J.W. and Kimmelman, A.C. (2014) Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature*, **509**, 105-109.

36. Settembre, C., Di Malta, C., Polito, V.A., Garcia Arencibia, M., Vetrini, F., Erdin, S., Erdin, S.U., Huynh, T., Medina, D., Colella, P., Sardiello, M., Rubinsztein, D.C. and Ballabio, A. (2011) TFEB links autophagy to lysosomal biogenesis. *Science*, **332**, 1429-1433.

37. Rabinowitz, J.D. and White, E. (2010) Autophagy and metabolism. *Science*, **330**, 1344-1348.

38. Kim, J., Kundu, M., Violett, B. and Guan, K.L. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.*, **13**, 132-141.

39. Hosokawa, N., Har a, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., Guan, J.L., Oshiro, N. and Mizushima, N. (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell*, **20**, 1981-1991.

40. Har a, T., Takamura, A., Kishi, C., Iemura, S., Natsume, T., Guan, J.L. and Mizushima, N. (2008) FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J. Cell Biol.*, **181**, 497-510.

41. Jung, C.H., Seo, M., Otto, N.M. and Kim, D.H. (2011) ULK1 inhibits the kinase activity of mTORC1 and cell proliferation. *Autophagy*, **7**, 1212-1221.

42. Dai, D.F., Johnson, S.C., Villar in, J.J., Chin, M.T., Nieves-Cintrón, M., Chen, T., Marcinek, D.J., Dorn, G.W., Kang, Y.J., Prolla, T.A., Santana, L.F. and Rabinovitch, P.S. (2011) Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphq overexpression-induced heart failure. *Circ. Res.*, **108**, 837-846.

43. Wei, Y., Pattingre, S., Sinha, S., Bassik, M. and Levine, B. (2008) JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol. Cell*, **30**, 678-688.

44. Russell, R.C., Tian, Y., Yuan, H., Park, H.W., Chang, Y.Y., Kim, J., Kim, H., Neufeld, T.P., Dillain, A. and Guan, K.L. (2013) ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.*, **15**, 741-750.

45. Backer, J.M. (2008) The regulation and function of Class III PI3Ks: novel roles for Vps34. *Biochem. J.*, **410**, 1-17.

46. Obara, K. and Ohsumi, Y. (2011) Atg14: a key player in orchestrating autophagy. *Int. J. Cell Biol.*, **2011**, 713435.

47. Liang, C., Feng, P., Ku, B., Dotan, I., Canaani, D., Oh, B.H. and Jung, J.U. (2006) Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat. Cell Biol.*, **8**, 688-699.

48. Takahashi, Y., Coppola, D., Matsushita, N., Cualing, H.D., Sun, M., Sato, Y., Liang, C., Jung, J.U., Cheng, J.Q., Mulé, J.J., Pledger, W.J. and Wang, H.G. (2007) Bi1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat. Cell Biol.*, **9**, 1142-1151.

49. Itakura, E., Kishi, C., Inoue, K. and Mizushima, N. (2008) Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol. Biol. Cell*, **19**, 5360-5372.

50. Matsu naga, K., Saitoh, T., Tabata, K., Omori, H., Sato, T., Kurot ori, N., Maejima, I., Shirahama-Noda, K., Ichimura, T., Isobe, T., Akira, S., Noda, T. and Yoshimori, T. (2009) Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat. Cell Biol.*, **11**, 385-396.

51. Mizushima, N., Yoshimori, T. and Ohsumi, Y. (2011) The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell Dev. Biol.*, **27**, 107-132.

52. Ravikumar, B., Sarkar, S., Davies, J.E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z.W., Jimenez-Sanchez, M., Korolchuk, V.I., Lichtenberg, M., Luo, S., Massey, D.C., Menzies, F.M., Moreau, K., Narayanan, U., Renna, M., Siddiqi, F.H., Underwood, B.R., Winslow, A.R. and Rubinsztein, D.C. (2010) Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol. Rev.*, **90**, 1383-1435.

53. Mizushima, N., Yoshimori, T. and Levine, B. (2010) Methods in mammalian autophagy research. *Cell*, **140**, 313-326.

54. Levine, B. and Klionsky, D.J. (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev. Cell*, **6**, 463-477.

55. Chua, C.E., Gan, B.Q. and Tang, B.L. (2011) Involvement of members of the Rab family and related small GTPases in autophagosome formation and maturation. *Cell. Mol. Life Sci.*, **68**, 3349-3358.

56. Ao, X., Zou, L. and Wu, Y. (2014) Regulation of autophagy by the Rab GTPase network. *Cell Death Differ.*, **21**, 348-358.

57. Moreau, K., Renna, M. and Rubinsztein, D.C. (2013) Connections between SNAREs and autophagy. *Trends Biochem. Sci.*, **38**, 57-63.

58. Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B.,
Gmachl, M., Parlati, F., Stöllner, T.H. and Rothman, J.E. (1998) SNAREpins: minimal machinery for membrane fusion. Cell, 92, 759-772.

Menzies, R.A. and Gold, P.H. (1971) The turnover of mitochondria in a variety of tissues of young adult and aged rats. J. Biol. Chem., 246, 2425-2429.

Deas, E., Plum-Favreau, H., Gandhi, S., Desmond, H., Kjaer, S., Loh, S.H., Renton, A.E., Harvey, R.J., Whitworth, A.J., Martins, L.M., Abramov, A.Y. and Wood, N.W. (2011) PINK1 cleavage at position A103 by the mitochondrial protease PARL. Hum. Mol. Genet., 20, 867-879.

Geisler, S., Holmström, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J. and Springer, W. (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat. Cell Biol., 12, 119-131.

Michiorri, S., Gelmetti, V., Giarda, E., Lombardi, F., Romano, F., Marongiu, R., Nerini-Molteni, S., Sale, P., Vago, R., Arena, G., Torosantucci, L., Cassina, L., Russo, M.A., Dallapiccola, B., Valente, E.M. and Casari, G. (2010) The Parkinson-associated protein PINK1 interacts with Beclin1 and promotes autophagy. Cell Death Differ., 17, 962-974.

Okatsu, K., Oka, T., Iguchi, M., Imamura, K., Kosako, H., Tani, N., Kimura, M., Go, E., Koyano, F., Funayama, M., Shiiba-Fukushima, K., Sato, S., Shimizu, H., Fukunaga, Y., Taniguchi, H., Komatsu, M., Hattori, N., Mihara, K., Tanaka, K. and Matsuda, N. (2012) PINK1 autophosphorylation upon mitochondrial potential dissipation is essential for Parkin recruitment to damaged mitochondria. Nat. Commun., 3, 1016.

Kirkin, V., McEwan, D.G., Novak, I. and Dikic, I. (2009) A role for ubiquitin in selective autophagy. Mol. Cell, 34, 259-269.

Narendra, D., Tanaka, A., Suen, D.F. and Youle, R.J. (2009) Parkin-induced mitophagy in the pathogenesis of Parkinson disease. Autophagy, 5, 706-708.

Feng, D., Liu, L., Zhu, Y. and Chen, Q. (2013) Molecular signaling toward mitophagy and its physiological significance. Exp. Cell Res., 319, 1697-1705.

Liu, L., Feng, D., Chen, G., Chen, M., Zheng, Q., Song, P., Ma, Q., Zhu, C., Wang, R., Qi, W., Huang, L., Xue, P., Li, B., Wang, X., Jin, H., Wang, J., Yang, F., Liu, P., Zhu, Y., Sui, S. and Chen, Q. (2012) Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. Nat. Cell Biol., 14, 177-185.

Su, W., Tian, W., Hu, Z., Chen, G., Huang, L., Li, W., Zhang, X., Xue, P., Zhou, C., Liu, L., Zhu, Y., Zhang, X., Li, L., Zhang, L., Sui, S., Zhao, B. and Feng, D. (2014)ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. EMBO Rep., 15, 566-575.

Chen, M., Sandoval, H. and Wang, J. (2008) Selective mitochondrial autophagy during erythroid maturation. Autophagy, 4, 926-928.

Sandoval, H., Thiagarajan, P., Dasgupta, S.K., Schumacher, A., Prehal, J.T., Chen, M. and Wang, J. (2008) Essential role for Nix in autophagic maturation of erythrocyt cells. Nature, 454, 232-235.

Wang, J.H., Ahn, I.S., Fischer, T.D., Byeon, J.L., Dunn, W.A., Jr., Behrens, K.E., Leeuwenburgh, C. and Kim, J.S. (2011) Autophagy suppresses age-dependent ischemia and reperfusion injury in livers of mice. Gastroenterology, 141, 2188-2199.

Kim, J.S., Wang, J.H., Biel, T.G., Kim, D.S., Flores-Toro, J.A., Vijayvargiya, R., Zendejas, I. and Behrns, K.E. (2013) Carbamazepine suppresses calpain-mediated autophagy impairment after ischemia/reperfusion in mouse livers. Toxicol. Appl. Pharmacol., 273, 600-610.

Pacher, P. and Hajnoczky, G. (2001) Propagation of the apoptotic signal by mitochondrial waves. EMBO J., 20, 4107-4121.

Choudhary, C., Weinert, B.T., Nishida, Y., Verdin, E. and Mann, M. (2014) The growing landscape of lysine acetylation links metabolism and cell signalling. Nat. Rev. Mol. Cell Biol., 15, 536-550.

Lee, K.K. and Workman, J.L. (2007) Histone acetyltransferase complexes: one size doesn’t fit all. Nat. Rev. Mol. Cell Biol., 8, 284-295.

Haigis, M.C. and Guarente, L.P. (2006) Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. Genes Dev., 20, 2913-2921.

North, B.J. and Verdin, E. (2004) Sirtuins: Sir2-related NAD-dependent protein deacetylases. Genome Biol., 5, 224.

Michan, S. and Sinclair, D. (2007) Sirtuins in mammals: insights into their biological function. Biochem. J., 404, 1-13.

Houtkooper, R.H., Pirinen, E. and Auwerx, J. (2012) Sirtuins as regulators of metabolism and healthspan. Nat. Rev. Mol. Cell Biol., 13, 225-238.

Anderson, K.A., Green, M.F., Huyhn, F.K., Wagner, G.R. and Hirschi, M.D. (2014) SnapShot: Mammalian Sirtuins. Cell, 159, 956.

Finnin, M.S., Donigian, J.R. and Pavletich, N.P. (2001) Structure of the histone deacetylase SRT2. Nat. Struct. Biol., 8, 621-625.

Min, J., Landry, J., Strenglanz, R. and Xu, R.M. (2001) Crystal structure of a SIR2 homolog-NAD complex. Cell, 105, 269-279.

Pietrocola, F., Galluzzi, L., Bravo-San Pedro, J.M., Madeo, F. and Kroemer, G. (2015) Acetyl coenzyme A: a central metabolite and second messenger. Cell Metab., 21, 805-821.

Maríño, G., Pietrocola, F., Eisenberg, T., Kong, Y., Malik, S.A., Andryushkova, A., Schroeder, S., Pendl, T., Harger, A., Niso-Santano, M., Zamzami, N., Scoazec, M., Durand, S., Enot, D.P., Fernández, Á.F., Martins, I., Kepp, O., Senovilla, L., Bauvy, C., Morselli, E., Vacchelli, E., Bennett, M., Magnes, C., Sinner, F., Pieber, T., López-Otin, C., Maiuri, M.C., Codogno, P., Andersen, J.S., Hill, J.A., Madeo, F. and Kroemer, G. (2014) Regulation of autophagy by cytosolic acetyl-coenzyme A. Mol. Cell, 53, 710-725.

Lombard, D.B., Tishkoff, D.X. and Bao, J. (2011) Mitochondrial sirtuins in the regulation of mitochondrial activity and metabolic adaptation in Histone Deacetylases: the Biology and Clinical Implication (Yao, T.P. and Seto, E. Edition). Springer, Heidelberg, pp. 163-188.

Satoh, A., Stein, L. and Imai, S. (2011) The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity in Histone Deacetylases: the Biology and Clinical Implication (Yao, T.P. and Seto, E. Edition). Springer, Heidelberg, pp. 126-163.

Tanno, M., Sakamoto, J., Miura, T., Shimamoto, K. and Horiyo, Y. (2007) Nucleocytoplasmic shuttling of the NAD-dependent histone deacetylase SRT1. J. Biol. Chem., 282, 6823-6832.
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88. Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., Mostoslavsky, R., Alt, F.W. and Schibler, U. (2008) SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell*, **134**, 317-328.

89. Belding, W.J. and Dunlap, J.C. (2008) SIRT1 is a circadian deacetylase for core clock components. *Cell*, **134**, 212-214.

90. Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P. and Sassone-Corsi, P. (2008) The NAD⁺-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell*, **134**, 329-340.

91. Lee, I.H., Cao, L., Mostoslavsky, R., Lombard, D.B., Liu, J., Bruns, N.E., Tsokos, M., Alt, F.W. and Finkel, T. (2008) A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 3374-3379.

92. Hariharan, N., Maejima, Y., Nakae, J., Paik, J., Depinho, R.A. and Sadoshima, J. (2010) Deacetylation of FoxO by Sirt1 Plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. *Circ. Res.*, **107**, 1470-1482.

93. Fang, E.F., Schelbye-Knudsen, M., Brace, L.E., Kassahun, H., SenGupta, T., Nilsen, H., Mitchell, J.R., Croteau, D.L. and Bohr, V.A. (2012) Defective mitophagy in XPA via PARP-1 hyperactivation and NAD⁺/SIRT1 reduction. *Cell*, **157**, 882-896.

94. Jiang, S.Y., Kang, H.T. and Hwang, E.S. (2012) Nicotinamide-induced mitophagy: event mediated by high NAD ⁄ NADH ratio and SIRT1 protein activation. *J. Biol. Chem.*, **287**, 19304-19314.

95. Rickenbacher, A., Jang, J.H., Limani, P., Ungethum, U., Lehmann, K., Oberkofler, C.E., Weber, A., Graf, R., Humar, B. and Clavien, P.A. (2014) Fasting promotes liver from ischemic injury through Sirt1-mediated downregulation of circulating HMGB1 in mice. *J. Hepatol.*, **61**, 301-308.

96. Gerhart-Hines, Z., Rodgers, J.T., Bare, O., Lerin, C., Kim, S.H., Mostoslavsky, R., Alt, F.W., Wu, Z. and Puigserver, P. (2007) Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO J.*, **26**, 1913-1923.

97. Rodgers, J.T., Lerin, C., Haas, W., Gygi, S.P., Spiegelman, B.M. and Puigserver, P. (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature*, **434**, 113-118.

98. Purushotham, A., Schug, T.T., Xu, Q., Surapureddi, S., Guo, X. and Li, X. (2009) Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.*, **9**, 327-338.

99. Aquilano, K., Vigilananza, P., Baldelli, S., Paglioni, B., Rotilio, G. and Ciriolo, M.R. (2010) Peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. *J. Biol. Chem.*, **285**, 21590-21599.

100. Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P. and Auwerx, J. (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell*, **127**, 1109-1122.

101. García-Rodríguez, J.L., Barbier-Torres, L., Fernández-Alvarez, S., Gutierrez-de Juan, V., Monte, M.J., Halibasic, E., Herranz, D., Alvarez, L., Aspichueta, P., Marín, J.J., Trauner, M., Mato, J.M., Serrano, M., Beraza, N. and Martínez-Chantar, M.L. (2010) SIRT1 controls liver regeneration by regulating bile acid metabolism through farnesoid X receptor and mammalian target of rapamycin signaling. *Hepatology*, **59**, 1972-1983.

102. Kabra, N., Li, Z., Chen, L., Li, B., Zhang, X., Wang, C., Yeatman, T., Coppola, D. and Chen, J. (2009) Sirt1 is an inhibitor of proliferation and tumor formation in colon cancer. *J. Biol. Chem.*, **284**, 18210-18217.

103. Cheng, H.L., Mostoslavsky, R., Saito, S., Manis, J.P., Gu, Y., Patel, P., Bronson, R., Appella, E., Alt, F.W. and Chua, K.F. (2003) Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 10794-10799.

104. Liu, Y., Dentin, R., Chen, D., Hedrick, S., Ravnkjaer, K., Schen, S., Milne, J., Meyers, D.J., Cole, P., Yates, J. 3rd., Olefsky, J., Guarente, L. and Montminy, M. (2008) A fasting-inducible switch modulates gluconeogenesis via activator/coactivator exchange. *Nature*, **456**, 269-273.

105. Park, J.M., Kim, T.H., Bae, J.S., Kim, M.Y., Kim, K.S. and Ahn, Y.H. (2010) Role of resveratrol in FOXO1-mediated gluconeogenic gene expression in the liver. *Biochem. Biophys. Res. Commun.*, **403**, 329-334.

106. Li, Y., Wong, K., Giles, A., Jiang, J., Lee, J.W., Adams, A.C., Khartonovenok, A., Yang, Q., Gao, B., Guarente, L. and Zhang, M. (2014) Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. *Gastroenterology*, **146**, 539-549.

107. Nie, Y., Erion, D.M., Yuan, Z., Dietrich, M., Shulman, G.L., Horvath, T.L. and Gao, Q. (2009) STAT3 inhibition of gluconeogenesis is downregulated by Sirt1. *Nat. Cell Biol.*, **11**, 492-500.

108. Schwcr, B., Eckersdorff, M., Li, Y., Silva, J.C., Fermin, D., Kurtev, M.V., Chua, K.F., Bronson, R., Appella, E., Alt, F.W. and Lombard, D.B. (2009) Calorie restriction alters mitochondrial protein acetylation. *Aging Cell*, **8**, 604-606.

109. Scher, M.B., Vaquero, A. and Reinberg, D. (2007) SirT3 inhibition of gluconeogenesis is downregulated by Sirt1. *Nat. Cell Biol.*, **21**, 920-928.

110. Lombard, D.B., Alt, F.W., Cheng, H.L., Bunkenborg, J., Streepner, R.S., Mostoslavsky, R., Kim, J., Yanopoulos, G., Valenzuela, D., Murphy, A., Yang, Y., Chen, Y., Hirschey, M.D., Bronson, R.T., Haigis, M., Guarente, L.P., Farese, R.V., Weissman, S., Verdin, E. and Schwcr, B. (2007) Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol. Cell Biol.*, **27**, 8807-8814.

111. Peek, C.B., Affinati, A.H., Ramsey, K.M., Kuo, H.Y., Yu, W., Sena, L.A., Ikayeva, O., Marcheva, B., Kobayashi, Y., Omura, C., Levine, D.C., Bacsik, D.J., Gius, D., Newgard, C.B., Goetzman, E., Chandel, N.S., Denu, J.M., Mrksich, M. and Bass, J. (2011) Circadian clock NAD⁺ cycle drives mitochondrial oxidative metabolism in mice. *Science*, **342**, 1243417.

112. Ahn, B.H., Kim, H.S., Song, S., Lee, I.H., Liu, J., Vassilopoulos, A., Deng, C.X. and Finkel, T. (2008) A role for the
mitochondrial deacetylase Sirt3 in regulating energy homeostasis. *Proc. Natl. Acad. Sci. U.S.A.*, 105, 14447-14452.

113. Shimazu, T., Hirschey, M.D., Hua, L., Dittenhafer-Reed, K.E., Schwert, B., Lombard, D.B., Li, Y., Bunkenborg, J., Alt, F.W., Denu, J.M., Jacobson, M.P. and Verdin, E. (2010) SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production. *Cell Metab.*, 12, 654-661.

114. Kendrick, A.A., Choudhury, M., Rahman, S.M., McCurdy, C.E., Friederich, M., Van Hove, J.J., Watson, P.A., Birdsey, N., Bao, J., Gius, D., Sack, M.N., Jing, E., Kahn, C.R., Friedman, J.E. and Jonscher, K.R. (2011) Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation. *Biochem. J.*, 433, 505-514.

115. Nakagawa, T., Lomb, D.J., Haigis, M.C. and Guarente, L. (2009) SIRT5 Deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle. *Cell*, 137, 560-570.

116. Du, J., Zhou, Y., Su, X., Yu, J.J., Khan, S., Jiang, H., Kim, J., Woo, J., Kim, J.H., Choi, B.H., He, B., Chen, W., Zhang, S., Cerione, R.A., Auwerx, J., Hao, Q. and Lin, H. (2011) Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science*, 334, 806-809.

117. Tan, M., Peng, C., Anderson, K.A., Chhoy, P., Xie, Z., Dai, L., Park, J., Chen, Y., Huang, H., Zhang, Y., Ro, J., Wagner, G.R., Green, M.F., Madsen, A.S., Schmiesing, J., Peterson, B.S., Xu, G., Ilkayeva, O.R., Muehlbauer, M.J., Braulke, T., Mühlenhaupt, C., Backos, D.S., Olsen, C.A., McGuire, P.J., Pletcher, S.D., Lombard, D.B., Hirschey, M.D. and Zhao, Y. (2014) Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. *Cell Metab.*, 19, 605-617.

118. Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstein, C., Carmona-Gutierrez, D., Ring, J., Schroeder, S., Magnes, C., Antonucci, L., Fussi, H., Desceiz, L., Hartl, R., Schraml, E., Criollo, A., Megalou, E., Weiskopf, D., Laun, P., Heeren, G., Breitenbach, M., Grubeck-Loebenstein, B., Herker, E., Fahrenkrog, B., Fröhlich, K.U., Sinner, F., Tavemarakis, N., Minois, N., Kroemer, G and Madeo, F. (2009) Induction of autophagy by spermidine promotes longevity. *Nat. Cell Biol.*, 11, 1304-1314.

119. Daitoku, H., Sakamaki, J. and Fukamizu, A. (2011) Regulation of FoxO transcription factors by acetylation and protein-protein interactions. *Biochim. Biophys. Acta*, 1813, 1954-1960.

120. Lee, J.H. and Finkel, T. (2009) Regulation of autophagy by the p300 acetyltransferase. *J. Biol. Chem.*, 284, 6322-6328.

121. Huang, R., Xu, Y., Wan, W., Shou, X., Qian, J., You, Z., Liu, B., Chang, C., Zhou, T., Lippincott-Schwartz, J. and Liu, W. (2015) Deacetylation of Nuclear LC3 Drives Autophagy Initiation under Starvation. *Mol. Cell*, 57, 456-466.

122. Sun, T., Li, X., Zhang, P., Chen, W.D., Zhang, H.L., Li, D.D., Deng, R., Qian, X.J., Jiao, L., Ji, J., Li, Y.T., Wu, R.Y., Yu, Y., Feng, G.K. and Zhu, X.F. (2015) Acetylation of Beclin 1 inhibits autophagosome maturation and promotes tumour growth. *Nat. Commun.*, 6, 7215.

123. Yi, C., Ma, M., Ran, L., Zheng, J., Tong, J., Zhu, J., Ma, C., Sun, Y., Zhang, S., Feng, W., Zhu, L., Le, Y., Gong, X., Yan, X., Hong, B., Jiang, F.J., Xie, Z., Miao, D., Deng, H. and Yu, L. (2012) Function and molecular mechanism of acetylation in autophagy regulation. *Science*, 336, 474-477.

124. Lin, S.Y., Li, T.Y., Liu, Q., Zhang, C., Li, X., Chen, Y., Zhang, S.M., Lian, G, Liu, Q., Ruan, K., Wang, Z., Zhang, C.S., Chien, K.Y., Wu, J., Li, Q., Han, J. and Lin, S.C. (2012) GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. *Science*, 336, 477-481.

125. Biel, T.G., Lee, S., Flores-Toro, J.A., Dean, J.W., Go, K.L., Lee, M.H., Law, B.K., Law, M.E., Dunn, W.A. Jr., Zendejas, I., Behrens, K.E. and Kim, J.S. (2016) Sirtuin 1 suppresses mitochondrial dysfunction of ischemic mouse livers in a mitofusin 2-dependent manner. *Cell Death Differ.*, 23, 279-290.

126. Anderson, K.A. and Hirschey, M.D. (2012) Mitochondrial protein acetylation regulates metabolism. *Essays Biochem.*, 52, 23-35.

127. Hirschey, M.D., Shimazu, T., Jing, E., Grueter, C.A., Collins, A.M., Auouizerat, B., Stancakova, A., Goetzen, E., Lam, M.M., Schwert, B., Stevens, R.D., Muehlbauer, M.J., Kakar, S., Bass, N.M., Kuusisto, J., Laakso, M., Alt, F.W., Newgard, C.B., Farese, R.V. Jr., Kahn, C.R. and Verdin, E. (2011) SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol. Cell*, 44, 177-190.

128. Mackeh, R., Lorin, S., Ratier, A., Mejidoubi-Charef, N., Baillet, A., Bruneel, A., Hamai, A., Codogno, P., Pois, C. and Perdiz, D. (2014) Reactive oxygen species, AMP-activated protein kinase, and the transcription cofactor p300 regulate α-tubulin acetyltransferase-1 (αTAT1-MEC-17) dependent microtubule hyperacetylation during cell stress. *J. Biol. Chem.*, 289, 11816-11828.

129. McLeod, P.M., Ferguson, B.S., Osinski, H., Bhuiyan, M.S., James, J., McKinsey, T.A. and Robbins, J. (2014) Tubulin hyperacetylation is adaptive in cardiac proteotoxicity by promoting autophagy. *Proc. Natl. Acad. Sci. U.S.A.*, 111, E5178-E5186.

130. Yang, Y., Fiskus, W., Yong, B., Atadja, P., Takahashi, Y., Pandita, T.K., Wang, H.G. and Bhalla, K.N. (2013) Acetylated hsp70 and KAP1-mediated Vps34 SUMOylation is required for autophagosome creation in autophagy. *Proc. Natl. Acad. Sci. U.S.A.*, 110, 6841-6846.