**Autophagy and its Role in Metabolism**

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Macroautophagy (hereafter autophagy) is a cellular quality control mechanism that evolved to recycle cellular waste and maintain energy homeostasis under starvation. However, its implications in regulating metabolism has only recently begun to be uncovered. Recent studies have revealed new functions of autophagy such as sensing damaged tissue mitochondria and preventing cellular injury by removing redundant protein aggregates. Moreover, the observation that autophagy regulates lipid metabolism has widened our understanding of the delicate and indispensable role autophagy plays in cellular metabolism and holds promise of a relatively new and exciting strategy for treating metabolic disorders.

**Cellular Mechanics of Macroautophagy**

The molecular mechanism for autophagy has been mainly uncovered by studies in yeast *S. cerevisiae*. To date, genetic studies of *S.cerevisiae* have revealed over 30 genes involved in autophagy (ATG; Autophagy-related gene), with 18 genes that are essential for autophagosome formation. Importantly, most ATG genes are well conserved in mammals, and have similar functions as their corresponding proteins in yeast. During macroautophagy, intact organelles (such as mitochondria) and portions of the cytosol are sequestered into a double-membrane vesicle, termed an autophagosome, which subsequently fuses with an endosome and/or lysosome, to form an autolysosome. This latter step exposes the cargo to lysosomal hydrolases to allow its breakdown, and the resulting macromolecules are transported back into the cytosol through membrane permeases for reuse (Figure 1). Among these Atg proteins, one subset is essential for autophagosome formation, and is referred to as the "core" molecular machinery for autophagy. These core Atg proteins are composed of four subgroups: (a) The Atg1/unc-51-like kinase (ULK) complex; (b) two ubiquitin-like protein (Atg12 and Atg8/LC3) conjugation systems which involve conjugating proteins like Atg7 and Atg10; (c) the class III phosphatidylinositol 3-kinase (PtdIns3K)/Vps34 complex I; and (d) two transmembrane proteins, Atg9/mAtg9 (and associated proteins involved in its movement such as Atg18/WIPI-1) and VMP1. The proposed site for autophagosome formation,
Metabolic Roles of Autophagy

1. Mitophagy (autophagy of mitochondria): While mitochondria perform essential functions for the cell, notably ATP production via oxidative phosphorylation, any damage to the mitochondrial outer membrane leads to release of cytochrome c, triggering caspase activation and apoptosis. More catastrophic stresses can lead to pathologic increase in mitochondrial permeability, accompanied by transient but massive release of reactive oxygen species (ROS) and calcium. These events can trigger neighboring mitochondria to do the same, culminating in activation of calcium-dependent proteases (calpains) and lipases (cPLA2) that together ensure the necrotic destruction of the cell. Impaired mitochondrial function has been suggested to be a significant pathophysiological process. Indeed, the accumulation of intracellular fatty acids and diacylglycerol, which may cause mitochondrial dysfunction can ultimately lead to suppression of insulin sensitivity. Therefore, a defect in mitochondrial function may be responsible for cellular insulin resistance. Moreover, since mitochondrial dysfunction has been found to occur in organs such as skeletal muscle, liver, pancreas and smooth vascular cells, mitochondrial dysfunction could play a critical role in the occurrence of metabolic disorders.

2. AMPK Pathway: The AMP-activated protein kinase (AMPK) is another sensor of cellular bioenergetics, specifically in response to energy stress. During nutrient and energy depletion, AMPK is activated by a decreased ATP/AMP ratio through the upstream LKB1 kinase (encoded by the Peutz-Jeghers syndrome gene). Active AMPK leads to inhibition of mTORC1 activity either via phosphorylation of TSC1/2 or phosphorylation of Raptor, a subunit of mTORC1. Thus, AMPK serves as a positive regulator of autophagy. Research by the Shaw and Guan groups show yet another, more direct, mechanism whereby AMPK regulates autophagy through phosphorylation of ULK1. Taken together, these findings increase our understanding of the mechanisms that connect cellular energy homeostasis and autophagy.

Metabolic Regulators of Autophagy

Autophagy is a stress-induced catabolic process that is tightly regulated by two major energy and nutrient sensing signaling pathways:

1. mTOR (mTORC1) pathway: Nutrient starvation, stress, or reduced availability of growth factors alarm eukaryotic cells to adjust their metabolism in order to survive. Among the numerous components involved in the regulation of autophagy and growth, mTORC1 (mammalian target of rapamycin) is a key component that coordinates the balance between growth and autophagy in response to intracellular and environmental conditions. The activity of mTORC1 is inhibited under nutrient starvation, which is a crucial step for autophagy induction in eukaryotes. Recent studies indicate that, the first signaling component downstream of mTORC1 in the autophagy pathway is Atg1, an evolutionarily conserved serine/threonine kinase. Atg1 likely plays a key role at the earliest step in the initial stages of autophagy induction such as the nucleation (the early event when membrane structures are initiated) and formation of the preautophagosomal structures (PAS). mTORC1 inhibits Atg1 (or ULK1) by either phosphorylating its activators like Atg13 at multiple residues causing a reduced affinity between Atg1 and its binding proteins, or by phosphorylating Atg1 itself leading to repression of autophagy. Hormonal inhibition and stimulation of autophagy by insulin and glucagon, respectively leads to opposing regulation of mTORC1 in fed and starved states.

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where most of the core Atg proteins are recruited, is termed the phagophore assembly site (PAS).
diseases. Autophagy of mitochondria helps to reduce mitochondrial stress by recycling damaged mitochondria before they can trigger an apoptotic cascade thus preventing tissue damage. In fact, livers of Atg7 deficient mice develop massive hepatomegaly (enlarged liver) marked by accumulation of deformed mitochondria. The regulation of ULK-1 by AMPK has been proposed to link mitochondrial damage to mitophagy.

2. Removing Protein Aggregates:
Continuous removal of unfolded and redundant proteins is necessary for the survival of non-dividing cells such as neurons and hepatocytes since they accumulate these protein aggregates during their long life span. Notably, Atg7 null mice that have defective autophagy accumulate polyubiquitinated proteins that can lead to hepatomegaly. One such aggregated protein, p62/SQSTM1, may induce increased oxidative stress and even tumorigenesis. Similarly, stimulation of autophagy has shown to be beneficial in the treatment of genetic disorders like α1-antitrypsin deficiency in which protein degradation fails to occur. Active mechanisms of lipolysis, exist within these tissues such as adipose tissue and pancreatic β-cells. At the physiological level, autophagy promotes metabolic homeostasis and prevents degenerative disease and cancer and thus opens a new field of therapeutic intervention for the treatment of metabolic disorders, as summarized in Table 1.

3. Cellular injury and tumorigenesis:
Basal levels of autophagy are also required for maintenance of pancreatic β-cells and hepatocytes in the mammals. Loss of autophagy is associated with mitochondrial abnormalities and large aggregates of polyubiquitinated proteins, including p62. Interestingly, increased accumulation of p62 also has been shown to be a key initiator of tumorigenesis in autophagy-deficient livers. Similarly, basal autophagy can protect plaque cells against oxidative stress by degrading damaged intracellular material, in particular polarized mitochondria. In this way, successful autophagy of the damaged components promotes cell survival and may reduce cardiovascular complication associated with atherosclerosis.

Pathophysiology of Defective Autophagy:

1. Pancreatitis: Acute pancreatitis is an inflammatory disorder that is believed to be initiated by self-digestion of the pancreatic acinar cells following inappropriate activation of enzymes, particularly trypsin. In a normal acinar cell, autophagy mediates the degradation of trypsinogen-containing zymogen granules that are not secreted from the apical membrane. In pancreatitis, a block in enzyme secretion leads to an accumulation of intracellular zymogen granules, which result in increased uptake of these structures by autophagosomes. Following autophagosome-lysosome fusion, degradation of cargo fails to occur. Active enzymes from these enlarged autolysosomes are released into the cytoplasm to initiate pancreatic cellular injury.

2. Alcoholic and Non-Alcoholic fatty liver disease: Recent studies have shown that alcoholic injury of the liver is associated with an up-regulation of autophagy. Increased autophagy serves as a protective stress response to injury and is selective towards removing damaged mitochondria and lipid droplets. Similarly, defective autophagy is thought to be a major cause of non-alcoholic fatty liver disease (NAFLD). Lipid degradation by autophagy or "lipophagy" may be inhibited by chronic hepatic lipid overload leading to steatosis and insulin resistance.

Conclusion
Autophagy is a major contributor to cellular metabolism and survival. It serves to provide fuel for energy production under starvation while monitoring and clearing damaged and non-functional organelles; thus providing an essential means for refreshing and remodeling cells. It is required for normal development, especially for metabolic tissues such as adipose tissue and pancreatic β-cells. At the physiological level, autophagy promotes metabolic homeostasis and prevents degenerative disease and cancer and thus opens a new field of therapeutic intervention for the treatment of metabolic disorders, as summarized in Table 1.
Metabolic diseases potentially involving Autophagy

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<thead>
<tr>
<th>DISEASE</th>
<th>PATHOPHYSIOLOGIC CONSEQUENCES</th>
<th>THERAPEUTIC INTERVENTION</th>
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<tbody>
<tr>
<td>Diabetes</td>
<td>Decreased insulin production and hepatic insulin resistance</td>
<td>Increasing autophagy may help expand β-cell mass, provide resistance against stress and enhance hepatic insulin sensitivity.</td>
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<tr>
<td>Nonalcoholic fatty liver disease</td>
<td>Increased steatosis, hepatocyte injury and predisposition to cirrhosis and hepatic cancer</td>
<td>Increasing autophagy would help getting rid of liver fat and inflammation together with protection against tumorogenesis.</td>
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<tr>
<td>Pancreatitis</td>
<td>Increased cellular injury due to defective autophagy</td>
<td>Decreasing autophagy may help to prevent or treat pancreatitis.</td>
</tr>
<tr>
<td>Cardiovascular complications</td>
<td>Atherosclerosis</td>
<td>Increasing autophagy may safeguard plaque cells against cellular distress, in particular oxidative injury.</td>
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<tr>
<td>Hypercholesterolemia, Hypertriglyceridemia</td>
<td>Increased circulating and tissue cholesterol and triglycerides</td>
<td>Increasing autophagy may help to degrade hepatic triglyceride and regulate reverse cholesterol transport (RCT) from the macrophage.</td>
</tr>
<tr>
<td>Obesity</td>
<td>Hypertrophy in white adipose tissue</td>
<td>Regulated decrease in autophagy could be beneficial in limiting white adipose tissue mass and lipid content.</td>
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References


Dr. Rohit Anthony Sinha did his PhD in Molecular Endocrinology at Sanjay Gandhi Post Graduate Institute of Medical Sciences, India supported by national research fellowships from the Council of Scientific and Industrial Research (CSIR, India) and Indian Council of Medical Research (ICMR). Presently he is working as a Research Fellow in the Laboratory of Hormonal Regulation headed by Dr. Paul M. Yen at Duke-NUS Graduate Medical School, Singapore. His current area of interest is in understanding hormonal regulation of metabolic signaling in mammals with special focus on regulation and effect of autophagy in metabolic disorders.