Important role of autophagy in regulation of metabolic processes in health, disease and aging

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Summary

Autophagy is the basic catabolic mechanism that involves degradation of dysfunctional cellular components through the action of lysosome as well as supplying energy and compounds for the synthesis of essential biomacromolecules. This process enables cells to survive stress from the external environment like nutrient deprivation. Autophagy is important in the breakdown of proteins, carbohydrates and lipids as well. Furthermore, recent studies have shown that autophagy is critical in wide range of normal human physiological processes, and defective autophagy is associated with diverse diseases, including lysosomal storage disease, myopathies, neurodegeneration and various metabolic disorders. This review summarizes the most up-to-date findings on what role autophagy plays in metabolism.

Key words:

autophagy, lysosome, metabolism, autophagosome, protein, lipid, carbohydrate

Introduction

Autophagy, or the process of degradation of intracellular components in lysosomes, has been traditionally linked to cellular energy balance and nutritional status (Mizushima et al., 2004). Lysosomes are cellular organelles responsible for an important portion of the degradative activity that is necessary for maintaining cellular homeostasis and defense (Dell'Angelica et al., 2000). Plasma membrane proteins and their ligands are targeted into lysosomes by the endosomal pathway when destined for degradation. Extracellular pathogens reach lysosomes for destruction as part of cellular defense via fusion of phagosomes with lysosomes. Intracellular components that need to be degraded, either because they are dysfunctional or to meet cellular energetic or metabolic demands, reach lysosomes by several pathways collectively termed "autophagy" (Eskelinen and Saftig, 2009). The discovery of the autophagic molecular machinery has been rapidly followed by numerous studies supporting the occurrence of autophagic alteration in different common human disorders such as cancer, neurodegenerative and muscular diseases, metabolic syndrome, and infectious disorders, among others. Most of these connections of autophagy with cellular physiology and disease have emphasized the important function of autophagy in quality control and clearance of altered and damaged intracellular proteins and organelles, its contribution to cellular remodeling through degradation of structural components, or its role in cellular defense as part of both innate and acquired immunity (Mizushima et al., 2008).

Autophagic pathways

The process of autophagy is conserved from yeast to mammals. During the past decade researchers have uncovered the existence of yeast autophagy genes and the molecular mechanisms of autophagy have been studied extensively in *Saccharomyces cerevisiae*. These

discoveries were followed by the identification of mammalian orthologs with similar roles and provide a series of reagents for characterizing the molecular machinery of the autophagy system (Klionsky, 2007). The process of autophagy is similar in yeast and mammalian cells, but there are some distinct differences.

Autophagy, the definition of which is "self-eating", can be induced by starvation or other forms of nutrient deprivation to supply a variety of substrates for cellular energy generation (Finn and Dice, 2006). Three forms of autophagy have been defined: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy. In microautophagy, organelles or protein are taken up within an invagination of the lysosomal membrane for breakdown (Yorimitsu and Klionsky, 2005). CMA is a specific process that removes individual proteins that contain a specific peptide motif recognized by the chaperone protein Hsp70 (70 kDa heat shock cognate protein). The chaperone-protein complex translocates to the lysosome where it binds to lysosome-associated membrane protein 2A (LAMP-2A) for protein internalization and degradation (Orenstein and Cuervo, 2010).

Macroautophagy is a non-specific process occurring when a portion of cytosol is engulfed by a double-membrane structure, termed an autophagosome, that fuses with a lysosome whose enzymes degrade the cellular constituents sequestered in the autophagosome (Mehrpour et al., 2010). The regulation of this process is complex and controlled by the coordinated actions of autophagy-regulated genes (Atgs), over 30 of which have been identified both in yeast and humans (Mizushima and Levine, 2010). Studies in yeast indicate that an initial structure called an isolation membrane, or phagophore, becomes a nascent autophagosome whose ends elongate until they form the completely enclosed autophagosome. The source of the double membrane is controversial, but it might be derived from the endoplasmatic reticulum (ER), mitochondria or plasma membrane (Hamasaki and Yoshimori, 2010). The exact mechanism for the formation and elongation of the autophagosomal double membrane is unclear.

However, a number of multi-protein complexes are known to be involved in these processes, as discussed below.

There are three major pathways that regulate the process of macroautophagy (Fig 1). The first one is dependent on the target of rapamycin complex 1 (TORC1) pathway. In direct response to nutrients supply, or nutrient-induced insulin secretion, class I phosphatidylinositol 3-kinase (PI3K) activates protein kinase B (Akt) and TORC1. This signaling pathway blocks macroautophagy through the ability of TORC1 (mammalian homologue of mammalian target of rapamycin mTOR) to inhibit Atg1 (mammalian homologue ULK1/2) from recruiting its partner Atg13 (mammalian homologue mAtg13) and Atg17 (mammalian homologue FIP200) (Neufeld, 2010). The Atg1-Atg13-Atg17 (ULK1/2-mAtg13-FIP200) complex recruits and organizes other proteins for the developing autophagosome.

The second pathway that regulates autophagy is mediated by Atg6 (mammalian homologue beclin1), which forms a complex with the class III PI3K Vps34. Activation of the Atg1-Atg13-Atg17 (ULK1/2-mAtg13-FIP200) complex leads to organization of the Atg6 (beclin1)-Vps34 complex on the lipid membrane. Vps34 produces phosphatidylinositol 3-phosphate, which is involved in recruiting other proteins to the autophagy complex (Cheong et al., 2005). It is important to distinguish this PI3K from the insulin-activated, class I PI3K, which activates mTOR. Vps34 is the target of the widely used pharmacologic inhibitor of autophagy 3-methyladenine (Seglen and Gordon, 1982). Beclin 1 is an important interface between the autophagic and cell death pathway because the anti-apoptotic protein Bcl-2 and Bcl-X_L bind beclin 1 to inhibit autophagy (Levine et al., 2008).

The third pathway that mediates autophagosome formation and elongation involves 2 ubiquitin-like conjugation processes that generate membrane-bound protein complexes. In the first, Atg7 and Atg10 mediate the conjugation of Atg12 to Atg5, which subsequently interact with Atg16. The Atg12-Atg5 complex associates with the membrane and then dissociates

when the autophagosome is fully formed. Another critical conjugation reaction involves Atg8 (mammalian analog microtubule-associated protein 1 light chain 3, LC3). In nutrient-rich condition Atg8 labels the PAS to the vacuole. Moreover, upon starvation, the Atg8 translokate the PAS to autophagosomes (Cheong and Klionsky, 2008). LC3 is constitutively cleaved by Atg4 to produce LC3-I. When autophagic signal is induced, Atg7 and Atg3 mediate the conjugation of LC3-I to the membrane lipid phosphatidylethanolamine, to form LC3-II (Ichimura et al., 2000). LC3-II associates with the autophagosomal membrane, where the lipidated protein can mediate membrane elongation and closure. LC3-II is degraded late in the autophagic pathway after autophagosome fusion with the lysosome (Tanida et al., 2005).

Taken together, the maturation and degradation of autophagosome and autolysosome is a complicated process regulated by many autophagy-associated proteins. Physiologically, autophagy occurs at a low basal level, which is enhanced during starvation as well as in the response to the accumulation of non-required cellular components in order to accomplish its homeostatic mission. Pathologically, however, dysfunctions of autophagy are the cause of several diseases.

Autophagy in carbohydrate homeostasis

The regulatory function of autophagy has a role in glucose metabolism, particularly in glycogenolysis. Glycogen degradation by phosphorylase and debranching enzymes occurs primarily in cytosol, but lysosomal acid glycosidases also contribute to glycogen breakdown. There is an important role for autophagy in regulating cellular glycogen stores, and indeed, glucagon, one of the most important hormones controlling glycogen metabolism, was the first hormone known to activate autophagy (Schworer et al., 1979). Autophagy is acutely required during childbirth in which the neonatal liver induces autophagy to mobilize glycogen stores to increase availability of glucose (Kalamidas and Kotoulas, 2000). At birth the cardiac muscle

of mice has a very high glycogen content, but glycogen levels fall coinciding with the appearance of glycogen-containing autophagic vacuoles. Similarly, glucagon and adrenalin enhance the breakdown of cardiac muscle glycogen by increasing the number, size, and total volume of glycogen-containing autophagic vacuoles, and the activity of acid glucosidase (lysosomal glycogen-hydrolyzing enzyme) (Kotoulas et al., 2004). Lysosomal mannose 6- and glucose 6-phosphatase modulate the phosphorylation of glucose favoring its exit from the lysosome.

Autophagy can indirectly impact glucose metabolism by modulating pancreatic β-cell mass and function. Mice with β-cell-specific inhibition of macroautophagy reveal progressive β cell degradation and decreased insulin secretion. These models demonstrated that the absence of autophagy within β-cells resulted in the impaired insulin secretion, as well as overall impaired glucose homeostasis in the animal (Ebato et al., 2008). β-cells are characterized by active mitochondrial respiration. Several independent lines of evidence indicate that autophagy, particularly mitophagy (autophagy of mitochondria), is of essential importance for β-cell homeostasis as it removes damaged mitochondria. Accordingly, any disruption of this quality control mechanism is expected to result in accumulation of damaged mitochondria and increases in the accompanying negative consequences, such as, elevated ROS production, severe oxidative stress and β-cell apoptosis. Another essential role of autophagy lies in the regulation of intracellular insulin stores and, more generally, in whole protein turnover (Marsh et al. 2007). Rab3A knockout mouse exhibits a dysfunction in insulin secretion due to a defect in β-cell granule transport concomitantly with a normal rate of proinsulin synthesis and processing. Despite this disconnection between insulin production and secretion, insulin content in isolated islets from Rab3A knockout mice is normal due to a marked up-regulation of autophagy that keeps the granule number constant.

Studies in *db/db* mice with diet-induced obesity and insulin resistance have revealed increased autophagosome formation and β-cell expansion. It is possible that autophagy protects against chronic lipid stress in the pancreas in these settings, particularly since β-cell autophagy deficient rodents failed to display similar increases in β-cells mass (Jung et al., 2008). Closer examination of the β-cells revealed these cells had accumulated aggregates of polyubiquinated proteins and structurally abnormal mitochondria. These results are consistent with the housekeeping and known recycling function of autophagy. As such, these models suggest that a functional autophagic system is necessary to maintain β-cell health and that distruption of autophagy can lead to profound metabolic impairment.

Autophagy in protein catabolism

Although lysosomes contain a broad array of hydrolases (lipases, proteases, glycosidases and nucleotidases) that allows them to degrade many different molecules, most of the functional studies on autophagy have focused on protein breakdown. In fact, for a long time, changes in the rate of degradation of long-lived proteins were used to monitor autophagy. In the liver, autophagy was estimated to degrade from 1.5 to 5 % of total proteome per hour under fed or starved conditions (Deter et al., 1967). Autophagy was thus responsible for up to 70 % of intracellular protein breakdown in this organ during prolonged (24 h) fasting, which was later confirmed in mice with hepatic knockouts of essential autophagy genes (Komatsu et al., 2005). There are two purposes for protein breakdown: to utilize amino acids for cellular fueling and to replenish the intracellular pool of amino acids required to maintain protein synthesis. Amino acids can be utilized in fasting to provide substrates for gluconeogenesis and ketogenesis (in liver) and to replenish the intracellular pool of amino acids. The contribution of different proteolytic systems to these processes seems to be timed with the duration of starvation. Studies in cultured cells have shown that the proteasome system

contributes most of the amino acids to this pool during first hours of starvation (Vabulas and Hartl, 2005), whereas macroautophagy starts soon after and reaches a peak at about 6-8 hours later (Deter et al., 1967). In fact, although autophagosomes are still visible up to 24 hours of starvation, the maximal rate of autophagosome formation is reached approximately at the sixth hour of starvation and decline progressively after that. The exact mechanism by which amino acids signal through mTOR to downregulate macroautophagy is still unclear, but the contribution of Vps34, Ras-related small GTPases (that relocate mTOR to the lysosomal compartment) and a bidirectional transporter that exchanges L-glutamine for essential amino acids have all been involved in this signaling process (Nicklin et al., 2009).

Autophagy in lipid metabolism

Lipid droplets (LDs) are intracellular deposits of lipid esters surrounded by a monolayer of phospholipids and separated from hydrophilic cytosolic environment by a coat of structural proteins, known as perilipins (Fujimoto and Parton, 2011). As with many other organelles, LDs have been shown to adapt to changes in the cellular environment and to interact with other intracellular compartments in a regulated manner, with different outcomes. First, the interaction between LDs and other organelles may be a source of membrane lipids. Second, LD interaction with mitochondria or peroxisomes may enhance the provision of lipid for β-oxidation. Finally, lipid droplets originating from the ER and maintaining a close connection with this organelle may facilitate exchange of lipids and proteins between both compartments to meet metabolic requirements of the cell (Ohsaki et al., 2009). Mobilization of the lipids inside the LD occurs through lipolysis that is activated in response to the increased energy demand but also in response to a large affluence of lipids to prevent stores from becoming compromisingly enlarged for cells (Lass et al. 2011). The rate of lipolysis is modulated by the

interaction of lipases present at the surface of the LD with the structural proteins that surround LD and with inhibitory proteins in the cytosol.

Although mobilization of LD by lipolysis has been attributed to the LD-associated cytosolic lipases like adipose triacylglycerol lipase (ATGL) or hormone sensitive lipase (HSL), recent studies have revealed a role for autophagy in LD breakdown. Lipolysis and autophagy share striking similarities. Both are essential catabolic pathways activated in response to nutrient deprivation. They are under identical hormonal control, being inhibited by insulin or activated by glucagon (Finn and Dice, 2006). Intracellular lipids were not previously considered autophagic substrates, but the similarities between lipolysis and autophagy, together with the existence of lysosomal lipases, suggested a possible link between these two pathways. An interrelationship between the two processes has been demonstrated by the finding that autophagy mobilizes lipids from lipid droplets for metabolism, through a process termed, "lipophagy" (Singh et al., 2009) (Fig 2). We have previously shown that manipulation of either autophagy or lysosomal activity independently and comparably affects intracellular lipid degradation and lipid-degradation product formation in primary hepatocytes and liver slices incubated *in vitro*. At least part of TAG in the liver is degraded in lysosomes and normal autophagic flux is necessary for this process (Skop et al. 2012).

The presence of lipases in the lysosomal lumen, along with a large variety of hydrolases such as proteases, glycases and nucleases, has been acknowledged since the discovery of this organelle. Lysosomal lipase was thought to serve mainly for the degradation of lipids contributed by the diet though endocytosis or those present in the membranes of the organelles digested during the autophagic process. The elevation of LD to the category of cytosolic organelles was in part a motivation to address their turnover by autophagy. The first hint that LDs could become substrates of the autophagic process originated from studies in cultured hepatocytes knocked-down for Atg5, one of the genes essential for the formation of

autophagomes. In addition, hepatocytes respond to an acute oleate challenge by increasing lipolysis, which would prevent massive enlargement of the LD compartment. An oleate challenge resulted in a marked increase in the number and size of LD in cells with compromised macroautophagy. The same was true *in vivo* when the knockout of another essential autophagy gene (Atg7) in the liver led to an accelerated development of liver steatosis (fatty liver) when compared to control animals (Singh et al., 2009).

Lipophagy is not limited to hepatocytes but occurs in almost every cell type investigated to date. In addition to the initial observation obtained in cultured hepatocytes and embryonic fibroblasts, it has been confirmed that disruption of macroautophagy leads to intracellular accumulation of lipids in endothelial cells, lymphoblasts, dendritic cells, glial cells, and even in neurons (Koga et al., 2010), suggesting a generalized function of macroautophagy in cellular lipid mobilization. The accumulation of LD upon blocking macroautophagy even in the absence of any nutritional challenge, supports the hypothesis that lipophagy is a constitutive process in many cells (Singh et al., 2009).

The determinants for autophagic initiation on the surface of LD remain unknown. Polyubiquitination has been detected in polarized areas of LD in part resulting from the accumulation of clusters of polyubiquitinated apolipoprotein B (ApoB) on their surface (Ohsaki et al., 2006). It seems that ApoB can undergo both proteasomal and lysosomal degradation as proteasome inhibition caused an increase of autophagic vacuoles abundance and ApoB content in lysosomes. Whether or not the lysosomal degradation of ApoB occurs as a result of the activation of lipophagy and how the accumulation of this protein contributes to the initiation of the process require future investigation. Of particular interest is the fact that LDs have been shown to dynamically interact with two of the organelles - the ER and mitochondria - that have been proposed as sites of formation of the limiting membrane of the autophagosomes. Interaction with the ER may be related to LD biogenesis, as this is the

compartment from where these organelles originate, but may also favor the distribution of lipids from the LD towards other organelles through the endosecretory pathway. In the case of mitochondria, the proximity of LDs to the outer-membrane of this organelle could facilitate delivery of the FFA for mitochondrial β-oxidation. However, considering the described association of the autophagic initiation complex to precise area in the membrane of ER and the mitochondria, and the formation of cup-like precursors of the limiting membrane of the autophagosome from these regions, it is tempting to at least propose that the previously described interactions of LD with these organelles could contribute to the initiation of their autophagic degradation.

Mobilization of LD by autophagy was first observed both in cultured hepatocytes in response to fatty acid exposure and in the liver of mice maintained on a diet enriched in fat for prolonged period of time (Singh et al., 2009). The liver responds to the massive influx of lipids from the blood by up-regulating LD biogenesis as a defense mechanism against the toxicity of FFAs, which upon esterification get converted into TAG that are stored in LDs (Lass et al., 2011). However, in order to prevent uncontrolled expansion of LD, activation of lipolysis also occurs under these conditions and contributes to maintaining of LD size. Failure to regulate lipid accumulation in hepatocytes may be the basis of pathologic conditions such as liver steatosis and steatohepatitis (Christian et al., 2013). Autophagy has now been added to the mechanism that controls the growth of the hepatic LD under these conditions (Greenberg et al., 2011).

After the first observations demonstrating the existence of lipophagy and the upregulation of this process in response to lipid challenge, numerous studies have confirmed the stimulatory effect of dietary lipids on the autophagic process. In contrast, an equal number of studies have reported inhibition of autophagy in response to high concentrations of particular lipids. In animals exposed to a high-fat diet for prolonged periods of time, it is possible to detect an

increase in autophagic activity during the first weeks of treatment that is followed by a gradual decrease in autophagy. This decrease in autophagy further contributes to the expansion of the LD compartment, eventually leading to hepatotoxicity and steatosis (Singh et al., 2009). Other results showed that in the liver, the autophagic response to the increased fat supply in the diet is biphasic. At the beginning of high-fat diet feeding autophagy flux is stimulated but over time autophagy flux is nearly completely impaired (Papackova et al., 2012).

Recently, a significant role for autophagy in lipid metabolism has been revealed in human enterocytes (Khaldoun et al., 2014). Enterocytes have to deal with massive alimentary lipids upon food consummation. They orchestrate complex lipid trafficking events that lead to secretion of TAG-rich lipoproteins and the transient storage of lipids in LDs. The authors showed that delivering alimentary lipid micelles to polarized human enterocytes induced an immediate autophagic response. This was accompanied by rapid capture of newly synthesized LDs by nascent autophagosomal structures at the ER membrane and hence targeting them to the lysosomes. They proposed an interesting hypothesis according to which the autophagosomes, despite their primary lysosomal-delivery function, could also be used as a "hiding" compartment in the cell in order to avoid excessive TAG accumulation from the membranes where lipid biosynthesis occurs. Such a local program could act as a global protection and adaptation response to the arrival of neutral lipids, as is the situation for enterocytes during the postprandial phase (Khaldoun et al., 2014).

Autophagy in other disease

A basal and constitutive level of autophagy is indispensable for intracellular homeostasis and quality control for healthy individuals. The molecular dissection of autophagy and the growing number of physiological function attributed to this process are leading to a better

understanding of the role of autophagy in disease. Mounting evidence has demonstrated that many of its physiological function is strongly related to human diseases such as cancer, myopahies, bacterial and viral infections and neurodegenerative, liver and heart diseases (Fig 3).

Direct evidence for impaired autophagy in a myopathy was first obtained when the gene encoding a lysosomal membrane protein (*lamp2*) was knocked out in mice (Saftig et al., 2001). The predominant phenotype of these mice is a massive accumulation of autophagic vacuoles in cells of the liver, muscle and heart. Despite the increased number of autophagic vesicles, the rate of lysosomal degradation of proteins is reduced because the clearance of autophagosomes through lysosomal fusion is impaired. Autophagy of glycogen is not limited to the liver. In fact, altered autophagic degradation of glycogen stores may underlie the basis of some muscle disorders that are now classified as autophagic vacuolar myopathies such as Danon disease, X-linked vacuolar myopathy with excessive autophagy and Pompe disease (Fukuda et al., 2007). Glycogen granules accumulate in muscles of Danon disease patients resulting in cardiomyopathy, proximal muscle weakness and mental retardation (Nishino et al., 2000). The histological resemblance of the skeletal and heart muscles from the *lamp-2* knockout mice to those from patients with Danon disease led to the identification of mutations in *LAMP2* as the primary defect in this lysosomal storage disease (Yamamoto et al., 2001).

Degradation of proteins by autophagy contributes to quality control and prevents proteotoxicity associated with accumulation of abnormal proteins. In fact, defective autophagy often associates with formation of proteins aggregates and it is likely the basis for protein conformational disorders such as Alzheimer´s, Huntington´s and Parkinson´s disease (Komatsu et al., 2006). Although the mutated protein is different in each of these disorders, the sequence of events leading to protein aggregation is apparently exactly the same and proceeds as follows: 1) the abnormal conformation of affected protein exposes normally

hidden hydrophobic residues; 2) the cell responds to these abnormal proteins by activating chaperone system and cytosolic proteases; and 3) in the initial stages of the disorder, chaperones and proteases can sometimes revert, or at least slow down, protein aggregation. However, as the levels of the pathogenic protein increase, the process becomes irreversible and even the "helpful" proteins become trapped in the aggregates. Although this might have originally been a defensive mechanism against hydrophobic patches, the hydrophobic nature of these proteins makes them resistant to attack by cytosolic proteases, leaving removal by macroautophagy as the only viable possibility (Michalik and Van Broeckhoven, 2003). Recent studies show that, at least in experimental systems, the activation of macroautophagy facilitates the removal of newly formed aggregates (Webb et al., 2003).

Insulin signaling is critical for the regulation of glucose homeostasis in the adipose and muscle. Eating an unhealthy diet and obesity contribute to the development of insulin resistance where hepatic, adipose and muscle tissues no longer respond to insulin signaling, thus resulting in hyperglycemia (Martyn et al., 2008). Recently, a role for autophagy in insulin signaling has been discovered. Intriguingly, it seems that the role of autophagy in the regulation of insulin signaling in different tissues may be opposite. Hyperinsulinemic high-fat diet fed mice and *ob/ob* mice both display impaired hepatic autophagy activity indicated by decreased expression of autophagy markers and increased levels of p62, a protein that is normally degraded by autophagy. Decreased autophagy in *ob/ob* mice led to a decreased insulin signaling and induction of ER stress, both of which were rescued by atg7 overexpression (Yang et al., 2010). This suggests that insulin signaling down-regulates autophagy activity in the liver during a hyperinsulinemic state. In contrast, autophagy was shown to be up-regulated in adipose tissue of obese and T2D patients. Kovsan (2011), in a human study, reveals direct correlation between different types and degrees of obesity and autophagic activity and fat deposits size. Surprisingly, autophagy was extreme high in

omental fat tissue extract from obese individuals and was also increased in insulin-resistant obese subjects (Kovsan et al., 2011). It has been suggested that diabetic state is associated with pseudo starved state, leading to increased adipocyte autophagy, elevated TAG hydrolysis and enhanced plasma fatty acid concentration (Ost et al., 2010).

One of the prominent features of T2D clinical manifestation is the defective angiogenesis and consequent microvascular complications. Some conditions that are tightly associated with T2D, like inflammation or chronic hyperglycemia, are connected also with alterations in autophagy regulation, but the relationship between altered autophagy and the development of these complications is still not fully understood. It has been reported that inflammatory molecules as MCP-1, TNF-α, IL-1β and IL-8 are known to promote angiogenesis. Roy et al. (2012) demonstrated that all these pathways converge at the MCP-induced protein (MCPIP), that is able to switch on the cascade of oxidative stress, ER stress, autophagy and angiogenic differentiation in HUVEC cells. Interestingly, inhibition of each particular step caused inhibition of each subsequent step that was postulated. These data suggest that cellular stress evoked by the diabetic milieu may be translated into *de novo* angiogenesis (Roy and Kolattukudy, 2012). Somewhat contradictory results were reported by Liu (2012), who showed that methylglyoxal (MGO) (a small carbohydrate compound that is elevated in T2D) stimulated autophagic degradation of VEGFR2 in endothelial cells. Suppression of autophagy either by inhibitors or siRNA, but not of the proteasome and caspase, normalized both the VEGFR2 protein levels and angiogenesis. Conversely, induction of autophagy either by rapamycin or overexpression of LC3 and Beclin-1 reduced VEGFR2 and angiogenesis (Liu et al., 2012).

Retinopathy belongs to the serious pathologies associated with T2D progression. Most retinal cells are fully or terminally differentiated cells; therefore, a steady supply of nutrients, cell growth and cell cycle control are important for long-term survival (Dyer and Cepko, 2000;

Lee et al. 2006) and make these cells extremely sensitive to metabolic disturbance. Recently, it has been published that the crucial role in the process of retinopathy plays a pro-oxidant and pro-apoptotic thioredoxin interacting protein (TXNIP) (Singh, 2013). TXNIP contributes to the oxidative and nitrosative stress by significant attenuation of antioxidant cell capacity, induction of mitochondrial damage and consequent stimulation of mitophagy. In the end, this cascade of events may program autophagic cell death involving caspase-3 or cellular energy collapse. Nonetheless, in the broader context the autophagy could serve as an adaptive and even protective mechanism preventing excessive bursts of reactive oxygen species production and necrotic cell death.

Aging

Morphological alterations in the lysosomal system and changes in its enzymatic content are common in almost all tissues from older mammals (Ward, 2002). Considering the physiological functions of autophagy, the cellular consequences of diminished autophagy flux are easily inferred and include inefficient removal of damaged intracellular structures, alterations in cellular homeostasis, inability to adapt to extracellular changes and poor defensive response against damaging agents. Decreased protein degradation plays an important role in aging with regard to prolonging protein lifespan, which increases the probability of their undesired alteration. The defects in the autophagic/lysosomal proteolytic system may be the main cause of reduced protein degradation, since the proteasomal system cannot digest larger proteins or impaired organelles. Indeed, there is evidence of gradual reduction in autophagy with age (Cuervo et al., 2005). Autophagy activation may protect an organism from aging due to the increased ability to get rid of damaged proteins and organelles. Caloric restriction, the only intervention that delays aging and increases lifespan, reverses the decline in autophagy that occurs with age and may come about through reduced

insulin/IGF-1 signaling (Kenyon, 2005). Fasting can promote longevity, but it may cause potentially adverse effects of caloric restriction on human health and hence alternative approaches are currently being studied that mimic the beneficial effects if caloric restriction. The application of antilipolytic drugs that increase autophagy and extend longevity is a good example (Bergamini, 2005).

Conclusion

Autophagy has been shown to complement "classical pathways" in the catabolism of carbohydrates, proteins and mitochondria in fasting and nutrient deficiency. It is now proposed that autophagy also participates in the regulation of lipid metabolism. This finding necessitates a re-evaluation of much of the knowledge and assumptions about LD metabolism in light of this new alternative pathway of lipolysis. Lipophagy is likely to be an important metabolic pathway in the supply of energy for specific cellular function. The findings to date indicate that further investigations of lipophagy might increase our understanding of the role of LD breakdown in cell physiology and provide new avenues to treat diseases resulting from defects in lipid metabolism or storage. Autophagy is necessary for regulation of metabolic processes. In addition, malfunctions of autophagy have been implicated in the development of several diseases and so manipulation of this critical cellular process might be a promising therapeutic target.

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Figure legends

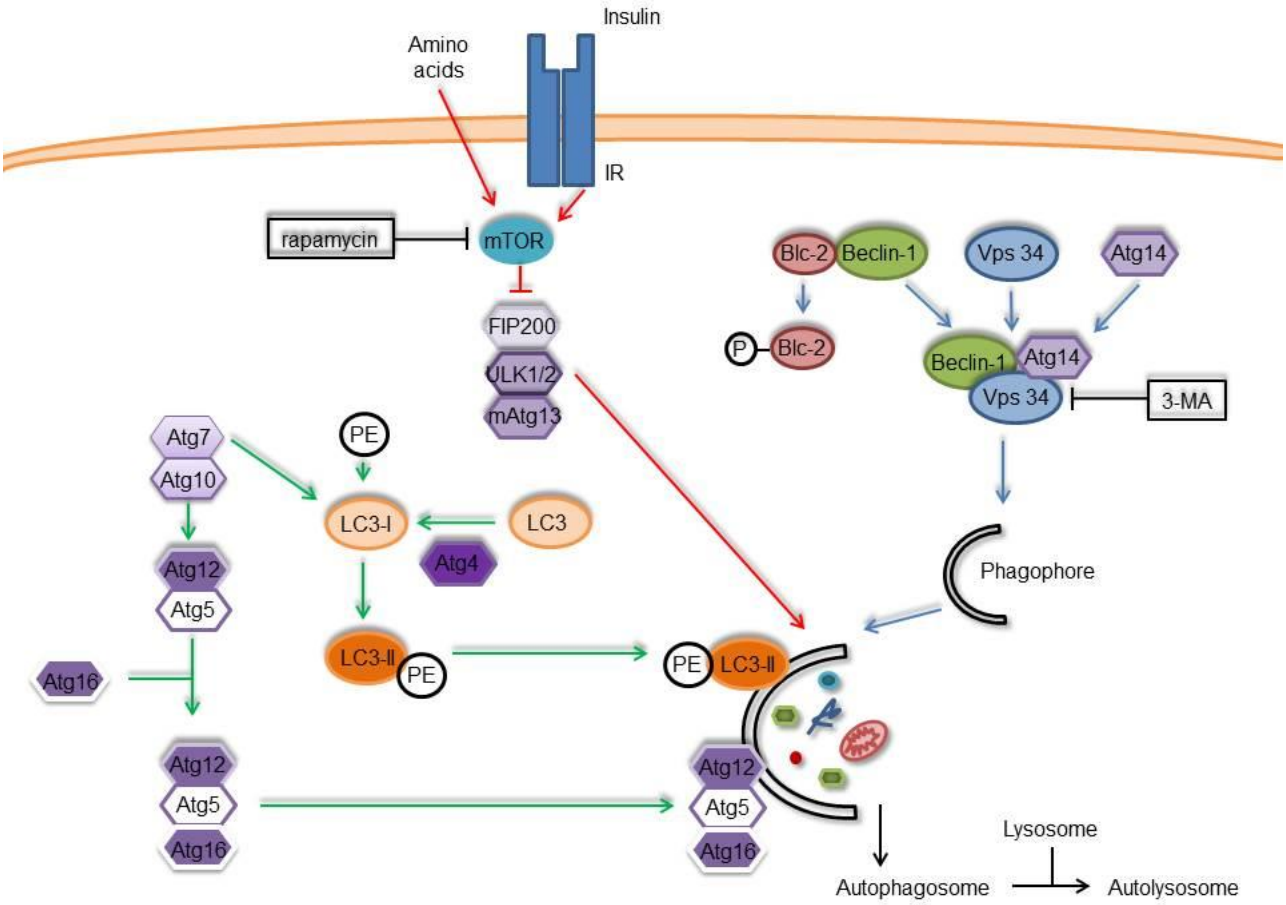

Figure 1: The three major pathways that regulate process of macroautophagy. The first is an inhibitory pathway in which nutrient or insulin stimulation of the mTOR signaling pathway blocks autophagosome formation (red line). Two other pathways are stimulatory. The first, phosphorylation of Bcl-2 dissociates it from beclin-1, which allows beclin-1 to form complex with Vps34 and Atg14, which is required for induction of autophagy (blue line). The other pathway involves a series of conjugation steps that generate LC3-II and the Atg5-Atg12-Atg16 protein complex, which are both necessary for autophagosome formation (green line). IR: insulin receptor, PE: phosphatidylethanolamine, 3-MA: 3-methyladenine.

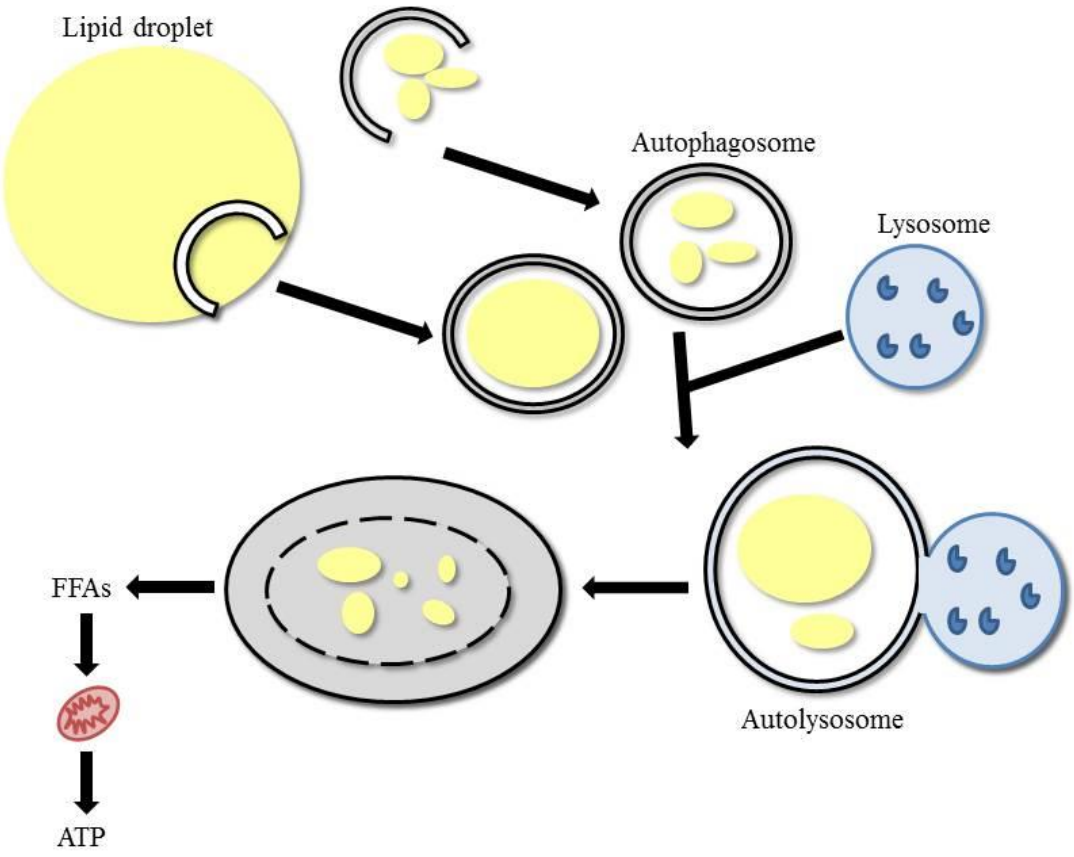

Figure 2: Process of LD breakdowm by lipophagy. Portion of large LD or small LD are enclosed with an autophagosomal double- membrane. Autophagosomes fuse with lysosomes to form autolysosomes and lysosomal enzymes are mixed with the autophagosomal cargo. It leads to degradation of lipids and releases FFAs into the cytoplasm. FFAs are important for stable mitochondrial β oxidation for generation of ATP to maintain cellular energy homeostasis.

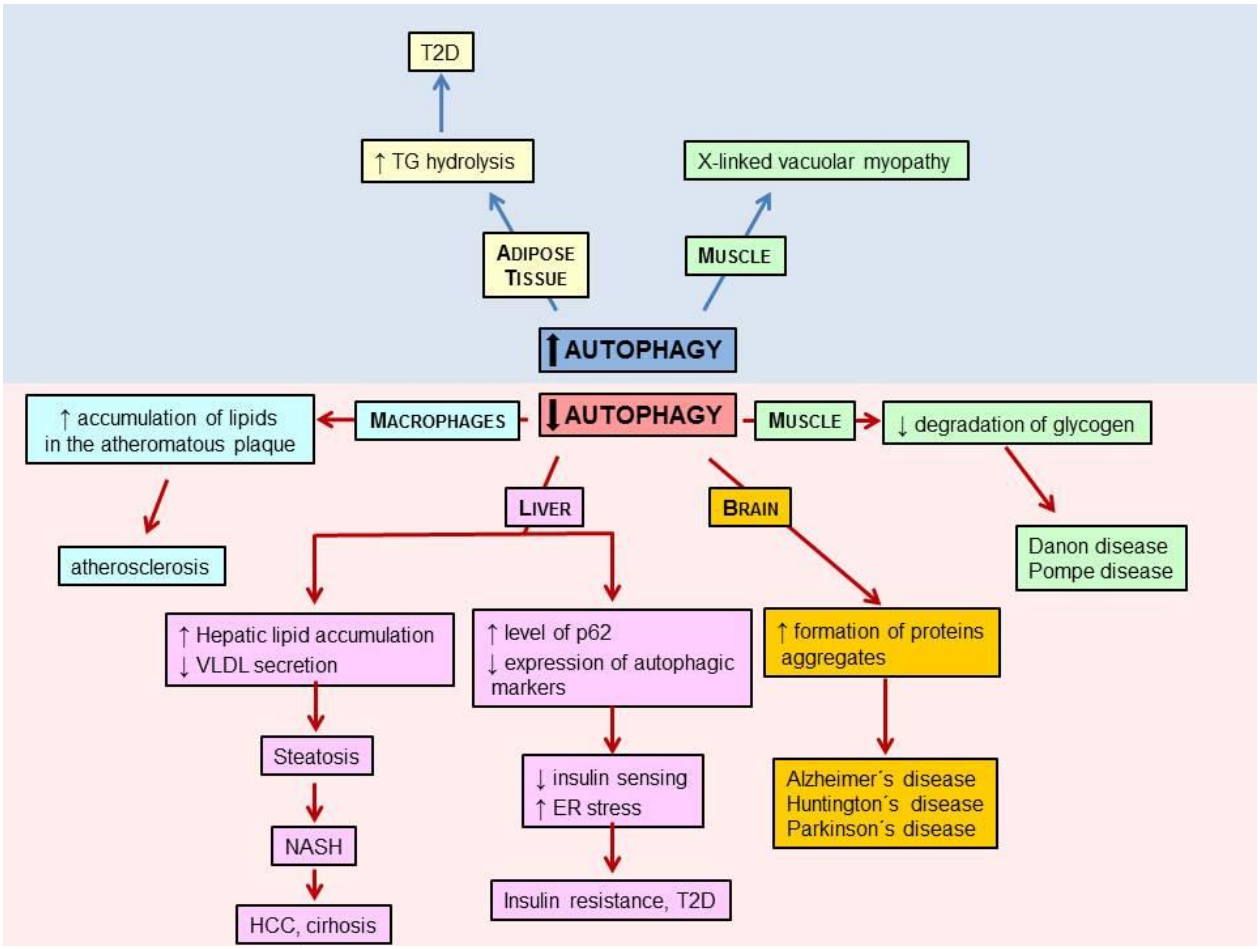

Figure 3: Autophagy in disease. Intensity of autophagy is important for the homeostasis in all organisms. Decreased or excessive increased autophagic activity could progress various disorders.