The importance and practical application of autophagy in human health

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Introduction

When we consider the important roles various parts of our cells have, it is often the classic organelles that come to mind—nuclei and DNA storage, mitochondria and energy production, endoplasmic reticulum and molecular transport, and the various others. Our recognition of their importance is not misplaced in the least, for without these structures our cells would cease to function. However, research over the past few decades, facilitated by advancements in technology and techniques, have uncovered other equally important cellular structures on which the cell relies upon for its maintained health and function. One such structure is the autophagosome, a small vesicle-like structure that engulfs damaged organelles and other cytoplasmic material for the transport to the lysosome for digestion.

A large amount of today's research on autophagy is pioneered by Noboru Mizushima, a nominee for the 2015 Nobel Prize in Medicine or Physiology. Mizushima was first intrigued by autophagy when he read an article about newly identified autophagy genes with unique amino-acid sequences ("Autophagy: Noboru Mizushima Interview - Special Topic of Autophagy," 2009). Inspired, he joined Dr. Yoshinori Ohsumi, the author of the article, in researching the molecular mechanism and physiological role of autophagy in yeast and mammals. Once there, Mizushima began to explore the physiological roles of autophagy. At its most basal level, autophagy is a mechanism by which other organic material is recycled. However, this design is modified in different cases to suit various functions. Mizushima categorizes autophagy into groups (microautophagy, macroautophagy, and chaperone-mediated autophagy) that each have specialized methods for delivering certain molecules to the lysosome (Mizushima, Levine, Cuervo, & Klionsky, 2008). These specialized autosomes can be distributed to various parts of the body depending on their function. For example, autophagy in the liver is stimulated by amino acids and autophagy in the muscles is instead stimulated by insulin (Naito, Kuma, & Mizushima, 2013).

The importance of autophagy lies in its various roles. Autosome activity is governed by a multitude of genes that are specific to certain parts of the body. The basal regulators in this process are the autophagy-related (ATG) genes, which determine how selective autosomes are and the amount of activity expressed at any point in time (Mizushima et al., 2008). Mizushima explains that given the destructive potential of autophagy, regulation of this process is necessary in order to prevent damage. In one of his experiments, Mizushima found that autophagy is stimulated during starvation, leading to the breakdown of organic molecules into their constituent amino acids (Sahani, Itakura, & Mizushima, 2014). The SQSTM1 gene is upregulated by the availability of these amino acids, and SQSTM1 protein, which has an inhibitory effect on autophagy, is synthesized. While starvation induces autophagy, the autophagy pathway itself produces a negative-feedback loop in what is suggested to be a self-regulatory process to avoid excess self-digestion.

Autophagy is necessary for the proper cellular function, and knowledge of its physiological roles can be applicable to medical research. One of the potential applications for autophagy that Mizushima suggests is its use against tumor cells in cancer patients. He states that an ATG gene knockout can upregulate autophagy and stimulate apoptosis (Mizushima et al., 2008). Autophagosomes that target tumor cells could theoretically be stimulated through such a gene knockout, and a natural autoimmune response would destroy the tumorous cells. In another study by Mizushima, it was discovered that static encephalopathy was linked with autophagy suppression (Saitsu et al., 2013). Patients with encephalopathy expressed a mutation in the autophagy gene WDR45, which resulted in defective production of autophagosomes. Some neurodegenerative diseases are caused by the build-up of ubiquitin plaques in the brain (i.e. Alzheimer's disease), which might normally be prevented through autophagy recycling. A treatment restoring autophagy might be effective in reversing neurodegeneration, though the means for accomplishing this would have to be investigated.

History of autophagy

A core component of the autophagic pathway is the fusion of the autophagosome to the lysosome, where the ingested molecules are then degraded by hydrolytic enzymes. Discovering the lysosome was the first step in understanding the role autophagy occupies, and was done by Christian de Duve, a Nobel Prize winner, once the electron microscope was commercially available (Appelmans, Wattiaux, & de Duve, 1955). The lysozyme's name was given based upon the hydrolytic enzymes presence within the newly discovered granules (de Duve, Pressman, Gianetto, Wattiaux, & Appelmans, 1955). Around the same time, Hruban et al. discovered that cellular material can be sequestered into lysosomes in a processes they called focal cytoplasmic degradation (FCD) (1963). de Duve renamed this process "autophagy" when he realized that it was an integral part of lysosome function (Deter, Baudhuin, & de Duve, 1967). From this evidence, de Duve correctly implied the autosome-lysosome role in digesting injured cell matter, facilitating reuse of cellular components, and facilitating cell death (de Duve, 1963).

Introduction to autophagy

There are currently three recognized forms of autophagy: micro-, macro-, and chaperone-mediated autophagy (Mizushima et al., 2008). Microautophagy refers to the sequestration of cytosolic components directly by lysosomes through invaginations in their limiting membrane, but this process has not been observed in multicellular eukaryotes. Macroautophagy involves the sequestration of material into a double-membrane autophagosome and its transfer to a lysosome for degradation. Finally, chaperone-mediated autophagy involves direct translocation of unfolded substrate proteins across the lysosome membrane with the use of the protein chaperone hsc70 and integral membrane receptor LAMP-2A (Mizushima et al., 2008). However, for the purposes of this essay, only the importance of macroautophagy will be discussed since the majority of Noboru Mizushima's research is concerned with this form.

Autophagosomes are first generated as single

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membrane structures called autophagosome precoursers (Mizushima, Ohsumi, & Yoshimori, 2002). It is not yet fully understood from where these young autophagosomes are derived in eukaryotes, but research in yeast cells show that a structure near the vacuole called the pre-autophagosome structure (PAS) generates them. A combination of Apg12-Apg5•Apg16 protein complex and LC3 proteins initiate the elongation of the membrane as it isolates and engulfs the target molecule, forming the autophagosome. It is then recruited to the lysosome, where the two membranes fuse and the ingested molecules are released into the lysosome for degradation. The material ingested by the autophagosome may or not be damaged, since macroautophagy has lately been found to play a role in a variety of maintenance activities, including starvation response, cell death, and disease prevention (Mizushima et al., 2008, 2002).

Functions of autophagy

(I) Response to starvation

Recent research indicates that autophagy is upregulated as a response to starvation (Mizushima et al., 2008, 2002; Sahani et al., 2014; Tekirdag, Korkmaz, Ozturk, Agami, & Gozuacik, 2013). Because consistent protein synthesis and metabolic rate is necessary for overall cellular health, macroautophagy is used in the recycling of unnecessary material to increase the availability of amino acids. This is beneficial in the case of short-term starvation when further nutrient intake is expected in the near-future. Sahani, Itakura, and Mizushima has produced evidence that de novo amino acid production is indeed used in protein synthesis following short-term starvation (2014). Because this process is inherently destructive to the cell, a regulatory system would be necessary to prevent excessive damage that would result in cell death. Tekirdag et al. has identified one of possible ways autophagic activity can be downregulated (2013). miRNAs have been implicated in the suppressive targeting of ATG proteins necessary for autophagy, and one of these, MIR181A, directly restricts ATG5 expression and attenuates autophagy. Thus, it is clear that autophagy functions to temporarily supply the cell with an emergency supply of amino acids during starvation.

(II) Cell death

Just as it is important to maintain cellular health, the occasional death of specific cells is needed for the proper function of the organism. During embryonic development, for example, apoptosis is used in the formation of different organs and structures. Apoptosis is typically regulated through various types of signaling pathways, but Denton et al. has shown that mid-gut disintegration during Drosophila metamorphosis is autophagy-mediated (2009). Though in vitro research suggests the involvement autophagy may have in programmed cell death, this study was the first to demonstrate it naturally in vivo.

Research also suggests that autophagy is involved in the control and suppression of tumor cells. Some ATG genes such as atg4c exert tumor suppression effects, implying that tumor regulation may be signaled at certain steps of the autophagy pathway (Mizushima et al., 2008). Mutations in genes that regulate autophagy thus appear to promote tumor generation. For example, the deletion of beclin 1 is observed in 40–70% of human breast, ovarian, and prostate cancer (Mizushima et al., 2008). Therefore, it is apparent that autophagy plays an important role in promoting cell death in scenarios that benefit the organism's health.

(III) Disease mitigation

One of the most important functions autophagy has is the prevention of many types of illnesses and disease. Through either engulfing bacteria and virons for lysosome delivery or removing particulates that would be toxic in high amounts, autophagy can directly mitigate disease. Xenophagy refers to a specialized form of autophagy in which autophagosomes specifically target foreign bodies such as bacteria or virons (Mizushima et al., 2008). In some cases, autophagy is used to bolster the immune response. For example, autophages are needed to traffic viral RNA to the endosomal toll-like receptor TLR7 and activation of type I interferon signaling (Mizushima et al., 2008).

Because diseases like encephalopathy and Alzheimer's are associated with the buildup of ubiquinated protein and other material, autophagy may play an important role in preventing the development of these illnesses. Saitsu et al. discovered that a mutation in WDR45, a gene responsible in part for autophagy activity, is found in patients with encephalopathy (2013). This study provides a link between the subsequent loss of autophagy to this disease. Likewise, research suggests that the deletion of Atg5 and Pik3c3 genes, also necessary for autophagy function, is implicated in the development of age-related cataracts (Morishita et al., 2013). Since autophagy removes damaged particulate from the cell, the accelerated onset of cataracts is likely due to the unrestricted buildup of these damaging molecules.

Current and future work

Macroautophagy research has potential application towards the development of various health treatments. Saitsu et al.'s demonstration that mutations in WIPI4 inhibit autophagy (2013) suggests the importance further research can have on encephalopathy and other neurodegenerative treatments. However, restoration of autophagy through treatment may not always lead to medical success, for an inhibition of macroautophagy itself does not always cause illness, rather that it might be merely correlated with it (Morishita et al., 2013). Thus it is important that the mechanisms influencing autophagy be further investigated in order to understand the connections autophagy has with various illnesses.

Recently, more experiments on autophagy have been directed towards understanding the factors influencing autophagy. Dutta et al. showed that autophagy improves mitochondrial function and protects the cell from oxidative damage (2013). The accumulation of ubiquinated proteins (which create toxic oxygen species) following antimycin A (AMA) treatment is reversed when autophagy is induced by rapamycin. In this case, the damage caused by oxidative stress can be reversed by inducing autophagy. While this information is indicative that autophagy-inducing treatments can be beneficial, Mizushima explains that tumorous cells can be dependent upon autophagy to prevent their own apoptosis (Mizushima et al., 2008). To prevent cellular damage, these cells must employ feedback mechanisms to maintain homeostasis. As previously discussed, Tekirdag et al. discovered that the miRNA MIR181A is involved in one of these mechanisms (2013). This miRNA works in overexpression to inhibit starvation and rapamycin-induced autophagy. Larson had found that the miR-181 family of miRNAs are downregulated in myeloid leukemia (2010), which in light of Tekirdag et al.'s new information may suggest that enhanced autophagy and leukemia viability may correlate. Vakana et al. has also demonstrated interesting results correlating leukemic cell health with autophagy. They determined that several signaling pathways support the health of leukemic cells. Fusion tyrosine kinase BCR-ABL is a hallmark fusion gene

present in abnormal leukemic chromosomes and is an important inducer of several signaling cascades, one of which includes the mTOR pathway that promotes BCR-ABL expression via mRNA translation (Vakana, Sassano, & Platanias, 2014). Experimentally inhibiting mTORC2 and mTORC1 with OSI-027 expressed inhibitory effects on leukemic cells, but because mTOR is a negative regulator of autophagy, OSI-027 treatment upregulated autophagy and only produced limited apoptosis. However, directly inhibiting autophagy with chloroquine and mTOR with OSI-027 together fully induced apoptosis (Vakana et al., 2014). The authors suggest that autophagy inhibition must be used alongside with mTOR inhibition to promote leukemic cell apoptosis (Vakana et al., 2014).

Because the cellular processes involving macroautophagy are still not fully understood, research should be encouraged in order to potentially discover new treatment options for debilitating diseases like leukemia. Mizushima specializes in autophagy because of it can be applied to a variety of scientific fields, and thus further research can be done any of them. In particular, autophagy research can be applied to diseases like Alzheimer's and various forms of cancer, which are rising in prevalence. Thus, I consider these diseases to be worth targeting in future research. In the following section, I suggest a few experiments that can be applied to combating these types of diseases and improving health.

(I) Elimination of β-amyloid plaques via macroautophagy

The buildup of β-amyloids (Aβ) contribute to plaque formation in the brain, leading to Alzheimer's disease. Protein fragments such as Aβs would theoretically be consumed by autophagosomes and then digested, but a study by Yu et al. showed that autophagy is induced in Alzheimer's-afflicted mice, but autophagosomes unnaturally build up (2005). They hypothesize that the formation of lysosomes from these autophagic vacuoles is inhibited. To investigate the cause of this inhibition, APP/PS1 mutant mice, which exhibit Aβ-buildup, should be used in the same manner of Yu et al. as a model organism. Fortunado et al. show that autolysosome fusion is impaired by a depletion in the membrane protein Lamp-2 (2009). Differences in membrane proteins in AD and control mice would be explored in addition to possible defects in their corresponding regulatory genes. Any observed reductions in proteins or differential expression of related genes could indicate a cause of impaired autophagosome-lysosome fusion.

(II) Non-invasive induction of apoptosis in leukemic cells

Tekirdag et al. showed that miRNAs are downregulated in leukemic cells (2013), which would correlate with an increase in autophagic activity. Because autophagy prolongs cellular health, inhibition of autophagy would eventually lead to apoptosis. Engineered ATG-knockout mice can be induced with leukemia to investigate whether or not a loss of autophagy in leukemic cells induces apoptosis. According to Tang et al., several herpes simplex viruses inhibit the Beclin-1 protein necessary for autophagy regulation (2012). If apoptosis is observed in the knock-out mice, engineering a herpes simplex virus or some other vector to target leukemic cells can be used to non-invasively inhibit autophagy. This type of treatment could be used in conjunction with typical radiation or chemotherapy to potentially enhance results.

(III) Retrovirus inhibition through autophagy upregulation

Retroviruses reproduce by parasitizing a host cell and forcing it to produce viral proteins using a reverse-transcripted code of DNA. Some retroviruses like hepatitis and HIV are of great concern to medical industries and improved methods of treatment are desired. Since autophagosomes are used in the isolation of viral genetic material, it may be possible to use autophagy to remove retrovirus RNA or reverse transcriptase from the cell. Inhibition of mTOR signaling may be one option to determine if an upregulation of autophagy retards the viral infection. Autophagy enhancer drugs, like carbamazepine and others described by Chu et al. (2014), may also achieve similar effects.

(IV) Autophagy as a mechanism for cardiovascular health

There is growing support that autophagy is an important mechanism outside of debris removal. Since autophagy leads to the breakdown of unneeded molecules into useful components (i.e. proteins → free amino acids), autophagy may play an important role in nutrient metabolism. Ouimet et al. (2011) shows that autophagy is stimulated in the presence of low-density lipoproteins, facilitating the breakdown of lipid droplets from atheroscleric plaques. Furthermore, mTOR inhibitors have been found to prevent development of atherosclerosis, attenuate plaque progression, and reduce cholesterol content mouse models (Ouimet et al., 2011). Therefore, it seems likely that autophagy can function to promote cardiovascular health. If autophagy helps remove excess lipids from circulation, then the heart would be subjected to less stress and the likelihood for the development of heart disease would be reduced. Furthermore, since autophagy is important for increasing the availability of amino acids in muscle tissue (Naito et al., 2013), heart health should be improved in this regard as well. An experiment similar to Ouimet et al.'s but with a focus on the progression of heart disease would discern whether or not autophagy can improve heart health.

Conclusion

More and more research is indicating that autophagy, once considered a simple maintenance pathway, serves important roles in preventing metabolic dysfunction and illness. Its function can vary slightly in different systems throughout the body, and thus autophagy has great potential to be foundational in future treatments in the medical field. Although Noboru Mizushima did not win the Nobel Prize for 2015, his continued contributions to medicinal research still are widely recognized and demonstrates the usefulness of autophagy in various fields of research. To improve our understanding of autophagy for therapeutic purposes, it is necessary to support further research on the various regulatory pathways and other factors influencing its activity. This is all the more important because autophagy can sometimes be a double-edged sword, and may be counter-effective, such as in the case of tumor cells, when designing plans for therapy. There are still many questions left unanswered, and it will necessary to be persistent in our research to overcome the issues preventing our full understanding of the functional pathways of autophagy.

References

Appelmans, F., Wattiaux, R., & de Duve, C. (1955). Tissue fractionation studies. 5. The association of acid phosphatase with a special class of cytoplasmic granules in rat liver. *Biochemical Journal*, *59*(3), 438–445.

Autophagy: Noboru Mizushima Interview - Special Topic of Autophagy. (2009). Retrieved August 11, 2014, from http://archive.sciencewatch.com/ana/st/autophagy/09augSTAutoMizu/

Chu, A., Perlmutter, D., & Wang, Y. (2014). Capitalizing on the Autophagic Response for Treatment of Liver Disease Caused by Alpha-1-Antitrypsin Deficiency and Other Genetic Diseases. *BioMed Research International*, 2014, 459823. doi:10.1155/2014/459823

De Duve, C. (1963). General properties of lysosomes. In *Ciba Foundation Symposium-Lysosomes*, 1–35.

De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., & Appelmans, F. (1955). Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochemical Journal*, *60*(4), 604–617.

Denton, D., Shravage, B., Simin, R., Mills, K., Berry, D. L., Baehrecke, E. H., & Kumar, S. (2009). Autophagy, not apoptosis, is essential for midgut cell death in Drosophila. *Current Biology* : *CB, 19*(20), 1741–6. doi:10.1016/j.cub.2009.08.042

Deter, R., Baudhuin, P., & de Duve, C. (1967). Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *The Journal of Cell Biology, 35*(2).

Dutta, D., Xu, J., Kim, J., Dunn, W. A., & Leeuwenburgh, C. (2013). Upregulated autophagy protects cardiomyocytes from oxidative stress-induced toxicity. *Autophagy, 9*(3), 328–344.

Fortunato, F., Bürgers, H., Bergmann, F., Rieger, P., Büchler, M. W., Kroemer, G., & Werner, J. (2009). Impaired autolysosome formation correlates with Lamp-2 depletion: role of apoptosis, autophagy, and necrosis in pancreatitis. *Gastroenterology, 137*(1), 350–60, 360.e1–5. doi:10.1053/j.gastro.2009.04.003

Hruban, Z., Spargo, B., & Swift, H. (1963). Focal cytoplasmic degradation. *The American Journal of Pathology and Zoology, 42*(6), 657–683.

Larson, R. (2010). Micro-RNAs and copy number changes: new levels of gene regulation in acute myeloid leukemia. *Chemico-Biological Interactions, 184*(1-2), 21–25. doi:10.1016/j.cbi.2009.10.002.MicroRNAs

Mizushima, N., Levine, B., Cuervo, A. M., & Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature, 451*(7182), 1069–1075. doi:10.1038/nature06639

Mizushima, N., Ohsumi, Y., & Yoshimori, T. (2002). Autophagosome formation in mammalian cells. *Cell Structure and Function, 27*(6), 421–429.

Morishita, H., Eguchi, S., Kimura, H., Sasaki, J., Sakamaki, Y., Robinson, M. L., … Mizushima, N. (2013). Deletion of autophagy-related 5 (Atg5) and Pik3c3 genes in the lens causes cataract independent of programmed organelle degradation. *Journal of Biological Chemistry, 288*(16), 11436–11447.

Naito, T., Kuma, A., & Mizushima, N. (2013). Differential contribution of insulin and amino acids to the mTORC1-autophagy pathway in the liver and muscle. *Journal of Biological Chemistry, 288*(29), 21074–21081.

Ouimet, M., Franklin, V., Mak, E., Liao, X., Tabas, I., & Marcel, Y. L. (2011). Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase. *Cell Metabolism, 13*(6), 655–67. doi:10.1016/j.cmet.2011.03.023

Sahani, M. H., Itakura, E., & Mizushima, N. (2014). Expression of the autophagy substrate SQSTM1/p62 is restored during prolonged starvation depending on transcriptional upregulation and autophagy-derived amino acids. *Autophagy, 10*(3), 8–18.

Saitsu, H., Nishimura, T., Muramatsu, K., Kodera, H., Kumada, S., Sugai, K., … Matsumoto, N. (2013). De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. *Nature Genetics, 45*(4), 445–449.

Tang, S., Ducroux, A., Jeang, K., & Neuveut, C. (2012). Impact of cellular autophagy on viruses : Insights from hepatitis B virus and human retroviruses. *Journal of Biomedical Science, 19*(1), 1. doi:10.1186/1423-0127-19-92

Tekirdag, K. A., Korkmaz, G., Ozturk, D. G., Agami, R., & Gozuacik, D. (2013). MIR181A regulates starvation- and rapamycin-induced autophagy through targeting of ATG5. *Autophagy, 9*(3), 374–85.

Vakana, E., Sassano, A., & Platanias, L. C. (2014). Induction of autophagy by dual mTORC1-mTORC2 inhibition in BCR-ABL-expressing leukemic cells. *Autophagy, 6*(7), 966–967. doi:10.4161/auto.6.7.13067

Yu, W. H., Cuervo, A. M., Kumar, A., Peterhoff, C. M., Schmidt, S. D., Lee, J.-H., … Nixon, R. a. (2005). Macroautophagy—a novel β-amyloid peptide-generating pathway activated in Alzheimer's disease. *The Journal of Cell Biology, 171*(1), 87–98. doi:10.1083/jcb.200505082