Molecules and Cells

Minireview

The Role of Autophagy in Systemic Metabolism and Human-Type Diabetes

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Autophagy is critical for the maintenance of organelle function and intracellular nutrient environment. Autophagy is also involved in systemic metabolic homeostasis, and its dysregulation can lead to or accelerate the development of metabolic disorders. While the role of autophagy in the global metabolism of model organisms has been investigated mostly using site-specific genetic knockout technology, the impact of dysregulated autophagy on systemic metabolism has been unclear. Here, we review recent papers showing the role of autophagy in systemic metabolism and in the development of metabolic disorders. Also included are data suggesting the role of autophagy in human-type diabetes, which are different in several key aspects from murine models of diabetes. The results shown here support the view that autophagy modulation could be a new modality for the treatment of metabolic syndrome associated with lipid overload and human-type diabetes.

Keywords: amyloid, autophagy, diabetes, inflammasome, metabolism

INTRODUCTION

Autophagy, literally meaning 'self-eating,' is a cellular process of degradation of the cell's own internal material, such as proteins or organelles, in lysosomes. There are three major types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Among them, macroautophagy (hereafter referred to as autophagy) is a process involving the rearrangement of subcellular membranes, sequestering the cytoplasm and organelles, which leads to the formation of an autophagosome surrounded by a double membrane. When autophagosomes fuse with lysosomes, autophagolysosomes are formed in which proteolysis of the sequestered material by lysosomal enzymes occurs (Klionsky and Emr, 2000). The main purpose of autophagy is quality control of organelles or proteins, and protection of the intracellular nutrient environment. Thus, in the case of nutrient deficiency, intracellular macromolecules are degraded by autophagy to meet cellular requirements for energy and vital elements, suggesting that adaptive autophagy may have evolved from ancient cellular machinery supplying nutrients during energy crises in primordial unicellular organisms such as yeast. The autophagy machinery has been extensively characterized by many scientists, and the 2016 Nobel Prize for Physiology or Medicine was awarded to Yoshinori Ohsumi as a tribute to his seminal discovery of the autophagy-related (Atg) conjugation system. Outlining the detailed molecular and cellular mechanisms of autophagy is beyond the scope of this paper, and the readers are encouraged to consult excellent, previously published reviews (Klionsky, 2016; Mizushima and Komatsu, 2011).

Since autophagy is critical for the maintenance of cellular homeostasis and organelle function, it affects almost all physiological processes and, furthermore, is expected to influence the pathogenesis of a diverse range of diseases. Thus, dysregulated autophagy may lead to or be associated

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with a variety of diseases, including metabolic disorders such as type 2 diabetes (T2D) or metabolic syndrome, neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease or Huntington's disease, immune/inflammatory disorders, infectious diseases, cancer and aging. Accordingly, autophagy modulation may have therapeutic potential on such disorders and conditions (Eisenberg et al., 2009; Rubinsztein et al., 2012; Shoji-Kawata et al., 2013; Zhang et al., 2007).

In this review, we summarize the recent progress made in understanding the role of systemic and local autophagy in the pathogenesis of metabolic disorders, and the prospect of using autophagy modulators as a new therapeutic option against diabetes and metabolic disorders.

DIVERSE METABOLIC PHENOTYPES OF MICE WITH SITE-SPECIFIC AUTOPHAGY DISRUPTION

Two main pathogenic axes in the development of T2D are insulin resistance and β-cell failure. Numerous theories have been proposed as mechanisms causing insulin resistance and β-cell failure such as lipid overload, reactive oxygen species, ER stress, mitochondrial dysfunction and low-grade metabolic inflammation (Johnson and Olefsky, 2013). Besides such well-recognized factors, dysregulated autophagy and altered gut microbiota have recently been implicated as contributing factors (Kim and Lee, 2014; Yang et al., 2017). While extensively studied, the role of autophagy in the maintenance of metabolic homeostasis and in the pathogenesis of metabolic disorders has been controversial, which is partly due to the neonatal lethality of mice with systemic autophagy-knockout. Thus, the *in vivo* role of autophagy in metabolic disorders has been studied mostly using site-specific gene knockout mouse models which have diverse metabolic features depending on the site and severity of autophagy knockout (Kim and Lee, 2014). For example, mice with knockout of *Atg7*, an essential autophagy gene in pancreatic β-cells producing insulin ($Atg7^{\Delta b\text{-}cell}$ mice), show structural and functional defects of pancreatic β-cells, resulting in glucose intolerance and susceptibility to diabetes in the presence of metabolic stress (Ebato et al., 2008; Jung et al., 2008; Quan et al., 2012a). While $Atg7^{\Delta b\text{-}cell}$ mice develop hyperglycemia but not diabetes, they do develop severe diabetes when crossed to *ob/ob* mice, suggesting that autophagy is crucial in the metabolic adaptation of β-cells to metabolic stress (Quan et al., 2012a). On the other hand, autophagy knockout in skeletal muscle cells leads to the induction of fibroblast growth factor 21 (FGF21) as a 'mitokine' due to mitochondrial stress, and resistance to diet-induced obesity or insulin resistance (Kim et al., 2013a), contrary to the expectation that autophagy deficiency associated with mitochondrial dysfunction in insulin target tissues would lead to insulin resistance. Autophagy knockout in the liver leads to the accumulation of lipid droplets due to impaired lipophagy (Singh et al., 2009a) or reduced hepatic lipid content after starvation or high-fat diet (HFD) feeding, due to enhanced lipid catabolism by fibroblast growth factor 21 (FGF21) from autophagy-deficient hepatocytes (Kim et al., 2013b; Shibata et al., 2009), similar to the findings in skeletal muscle-specific autophagy knockout. In mice with white adipose tissue-specific autophagy knockout, reduced fat mass due to defective adipocyte differentiation was observed, which was accompanied by the browning of white adipose tissue and resistance to diet-induced obesity or insulin resistance (Singh et al., 2009b; Zhang et al., 2009). In contrast, mice with autophagy deletion in brown adipose tissue show defective brown adipose tissue differentiation but paradoxically increased body temperature due to increased fatty acid oxidation in beige adipocytes (Martinez-Lopez et al., 2013). Autophagy knockout in hypothalamic neurons has also been conducted, which led to obesity or leanness depending on the location of autophagy deletion (proopiomelanocortin vs. agouti-related protein neurons) (Coupe et al., 2012; Kaushik et al., 2011; 2012; Quan et al., 2012b).

METABOLIC CHANGES IN MICE WITH GLOBAL AUTOPHAGY INSUFFICIENCY

Such diverse metabolic features of mice with autophagy knockout in various metabolic tissues raise a question - what is the effect of global autophagy insufficiency of a physiological level on systemic metabolism and the development of metabolic disorders, since there is neither 'tissue-specific' autophagy nor 'gene knockout' in the real world. To answer this question, mice with systemic autophagy insufficiency of a physiologically relevant degree have been generated (Lim et al., 2014). Such $Atg7^{+/-}$ heterozygote or haploinsufficient mice showed no apparent abnormalities in their systemic metabolic profile in an unstressed or basal metabolic state. However, when $Atg7^{+/-}$ mice were crossed with leptin-deficient *ob/ob* mice or fed a HFD, imposing metabolic stress, metabolic changes were observed. $Atg7^{+/-}$-*ob/ob* mice developed more severe and persistent diabetes compared to autophagy-competent *ob/ob* mice, which was accompanied by aggravated glucose intolerance and decreased insulin sensitivity. Histologically, more severe fat buildup in the liver and an increased number of crown-like structures (macrophages aggregating around dead adipocytes, indicating metabolic inflammation) in adipose tissue were noted, which was accompanied by an increased serum level of alanine aminotransferase (ALT)/aspartate aminotransferase (AST), suggesting increased liver damage and a stronger induction of inflammatory cytokines in adipose tissue. The increased lipid accumulation in the livers of $Atg7^{+/-}$-*ob/ob* mice compared to autophagy-competent *ob/ob* mice might be related to compromised lipophagy. When the mechanism of increased metabolic inflammation was studied, enhanced inflammasome activation, characterized by increased caspase-1 cleavage and pro-IL-1β maturation to IL-1β, was observed in the stromal vascular fraction of adipose tissue from $Atg7^{+/-}$-*ob/ob* mice compared to that of autophagy-competent *ob/ob* mice. Increased infiltration of macrophages expressing IL-1β into the adipose tissue of $Atg7^{+/-}$-*ob/ob* mice was also observed by confocal microscopy (Fig. 1). The enhanced inflammasome activation in the adipose tissue of $Atg7^{+/-}$-*ob/ob* mice was probably due to aggravated mitochondrial dysfunction in metabolically-stressed

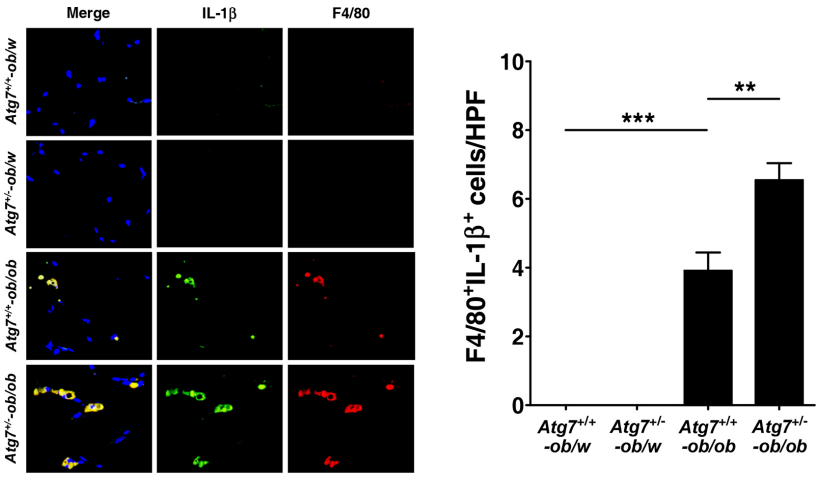

Fig. 1. Infiltration of IL-1-producing macrophages into the adipose tissue of *Atg7*$^{+/-}$*-ob/ob* mice. Paraffin-embedded adipose tissue sections were stained with anti-IL-1β and anti-F4/80 antibodies as the primary antibodies, and confocal microscopy was done (left). The number of IL-1β-producing macrophages in the adipose tissue of *Atg7*$^{+/-}$*-ob/ob* mice was higher than in the adipose tissue of *Atg7*$^{+/+}$*-ob/ob* mice (right). (HPF, high-power field)

macrophages, which play a crucial role in inflammasome activation by various NACHT, LRR and PYD domains-containing protein 3 (NLRP3) activators (Misawa et al., 2013; Vandanmagsar et al., 2011; Wen et al., 2011). Mitochondrial reactive oxygen species (ROS) production and the cellular fraction with lower mitochondrial potential observed after treatment with palmitic acid (PA) in combination with lipopolysaccharide (LPS) were increased in macrophages from autophagy-insufficient mice compared to those from autophagy-competent mice, probably due to less efficient removal or rejuvenation of stressed mitochondria in autophagy-insufficient macrophages. The relationship between autophagic activity, mitochondrial stress, and inflammasome activation associated with lipid overload was more directly demonstrated in myeloid cell-specific *Atg7*-knockout mice (Lee et al., 2016). In these mice, metabolic deterioration, characterized by aggravated insulin resistance and elevated glucose levels, was observed after crossing them with *ob/ob* mice, similar to systemic *Atg7*-haploinsufficient mice. *Atg7*-knockout macrophages showed increased mitochondrial stress and inflammasome activation after treatment with a combination of PA and LPS *in vitro*, as expected. Myeloid cell-specific deletion of *Atg5*, another critical autophagy gene, also led to the development of steatohepatitis associated with M1 polarization when the mice were fed a HFD and challenged with LPS (Liu et al., 2015), and aggravated atherosclerosis when they were crossed with LDL receptor-knockout mice and fed a Western diet (Liao et al., 2012).

Thus, increased inflammasome activation due to inefficient clearance of dysfunctional mitochondria acting as a hub for inflammasome activation (Misawa et al., 2013) and increased lipid content probably due to compromised lipophagy are the main culprits leading to the aggravated metabolic deterioration seen in *Atg7*$^{+/-}$*-ob/ob* mice compared to autophagy-competent *ob/ob* mice. Furthermore, lipid accumulation can aggravate metabolic inflammation and inflammasome activation, since fatty acids can be activators of NLRP3 (Wen et al., 2011), linking the two events observed in *Atg7*$^{+/-}$*-ob/ob* mice (lipid accumulation and inflammasome activation). These results suggest that autophagy insufficiency of a physiologically relevant degree might not cause significant problems in managing basal metabolic stress, but can cause difficulty in dealing with increased metabolic stress, and autophagy insufficiency due to aging, genetics, or exogenous agents can be a factor in the development or aggravation of metabolic syndrome or diabetes associated with lipid overload or obesity. This suggestion is consistent with previous papers demonstrating that overexpression of *Atg5* improves the metabolic profile of aged mice (Pyo et al., 2013), and that mice that are defective in stimulus-induced autophagy show defective exercise-mediated protection against glucose intolerance after HFD feeding (He et al., 2012). These data also suggest the possibility that systemically-enhanced autophagic activity by pharmacological agents may have beneficial effects on body-wide metabolism during metabolic stress. Indeed, imatinib and trehalose have been shown to increase autophagic activity (Castillo et al., 2013; Dehay et al., 2010; Lin et al., 2015; Xie et al., 2017), and were able to ameliorate the metabolic profile of *ob/ob* mice with systemic autophagy insufficiency, as demonstrated by increased autophagic activity and reduced inflammasome activation or cytokine expression in metabolic tissues (Lim et al., 2014). Natural products identified from a plant metabolite library have also been shown to improve metabolic profile by enhancing systemic autophagy (Fan et al., 2017). In line with these reports, spermidine that has been reported to enhance autophagy by modulating acetylproteome (Morselli et al., 2011), could improve cardiac function and extend the lifespan of aged mice (Eisenberg et al., 2016). These results imply that if bona fide autophagy enhancers without adverse effects could be developed, they could be candidates for novel therapeutic agents against diabetes, and metabolic syndrome associated with lipid overload or cardiovascular diseases.

ROLF OR AUTOPHAGY IN HUMAN-TYPE DIABETES

So far, we have discussed the role of autophagy in diabetes based on data generated using animal models. However, human diabetes and murine diabetes differ in several as-

pects. One of the key differences is the accumulation of amyloids in islets that occurs in human diabetes but not in murine diabetes. Islet amyloid stained with Congo red is found in 90% of human subjects with diabetes (Kahn et al., 1999). This striking dissimilarity is due to the differences in the amino acid sequence of islet amyloid polypeptide (IAPP) between mice and humans. Human IAPP (hIAPP) is amyloidogenic, and murine IAPP (mIAPP) is nonamyloidogenic (Westermark et al., 2011), although it remains unclear why humans acquired IAPP amyloidogenicity during evolution. Amyloidogenic or aggregate-prone proteins are preferentially cleared by autophagy, while nonamyloidogenic or soluble proteins can be cleared by both autophagy and proteasome pathways (Rubinsztein, 2006). Thus, the role of autophagy in human diabetes would be greater than in murine diabetes. To study the role of autophagy in human-type diabetes, transgenic mice expressing hIAPP in pancreatic β-cells driven by the rat insulin promoter (*hIAPP*$^{+}$ mice) (Janson et al., 1996; Verchere et al., 1996) were employed. *hIAPP*$^{+}$ mice develop only mild hyperglycemia; however, when crossed with RIP-*Cre;* *Atg7*$^{F/F}$ mice to render them autophagy-deficient in β-cells, *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice developed diabetes (Kim et al., 2014; Rivera et al., 2014). These data suggest the importance of β-cell autophagy in protecting against hIAPP-induced diabetes. The role of hIAPP in the development of diabetes was also investigated using *hIAPP* knock-in mice, which could be more physiologically relevant than the transgenic *hIAPP* overexpression model. In line with the results from *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice, *hIAPP* knock-in:*Atg7*$^{\Delta\beta\text{-cell}}$ mice showed more severe metabolic deterioration and further reduction in β-cell mass compared to *Atg7*$^{\Delta\beta\text{-cell}}$ mice when fed a HFD, while overt diabetes was not observed in those mice (Shigihara et al., 2014).

When the mechanism of overt diabetes in *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice was investigated, significant accumulation of hIAPP oligomers stained with an oligomer-specific antibody (A11 or I11 antibody) and islet amyloids stained with Thioflavin S or (E,E)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB) were observed by confocal microscopy in the pancreatic islets of *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice (Kim et al., 2014) (Fig. 2). Accumulation of hIAPP oligomers can be an important event leading to overt diabetes in *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice, since it is hypothesized that hIAPP oligomers, rather than hIAPP amyloids, may be a toxic effector molecule inducing β-cell apoptosis according to the 'toxic oligomer hypothesis' (Haataja et al., 2008). Indeed, increased apoptosis of pancreatic β-cells was observed in *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice compared to *hIAPP*$^{+}$*Atg7*$^{F/F}$ or *hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice. Moreover, when monkey islet cells expressing amyloidogenic IAPP in a manner similar to human islet cells were incubated with bafilomycin, blocking the lysosomal step of autophagy, accumulation of intracellular IAPP oligomers occurred with resultant apoptosis *in vitro* (Kim et al., 2014). The types of hIAPP oligomers accumulating when autophagy was blocked were also investigated. For this purpose, an HA tag was attached to pro-hIAPP and pro-mIAPP. When INS-1 insulinoma cells were transfected with pro-mIAPP, only pro-mIAPP monomers were observed via immunoblot analysis using anti-HA antibody. In contrast, when pro-hIAPP was expressed,

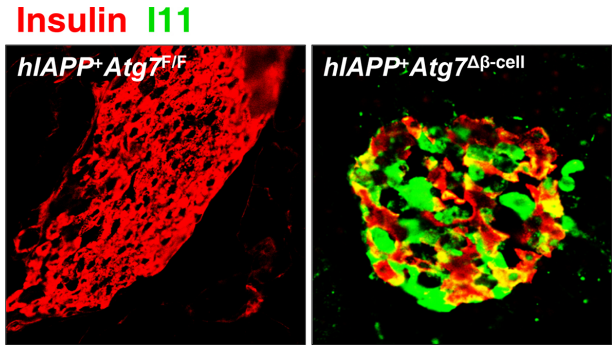

Fig. 2. Accumulation of hIAPP oligomers in *hIAPP*$^{+}$ mice with β-cell autophagy knockout (*hIAPP*$^{+}$*Atg7*$^{\Delta b\text{-cell}}$ mice). Confocal microscopy after immunofluorescent staining of pancreas sections employing anti-insulin and anti-hIAPP oligomer (I11) antibodies. hIAPP oligomer accumulation was not seen when autophagy is competent, despite transgenic expression of *hIAPP* (left).

pro-hIAPP dimers were observed in addition to pro-hIAPP monomers. When autophagy was blocked with 3-methyladenine (3-MA), accumulation of pro-hIAPP dimers increased and a protein band consistent with pro-hIAPP trimers was also seen on immunoblot. Accumulation of pro-hIAPP dimers or trimers was more pronounced in the detergent-insoluble fraction compared to the detergent-soluble fraction, suggesting the possibility that pro-hIAPP dimers may be initially formed in membrane-rich compartments (e.g., secretory granule membranes) and translocate to the soluble fraction or proceed to form pro-hIAPP trimers in the membrane fraction. Accumulation of pro-hIAPP dimers or trimers disappeared when three amino acids critical for amyloid formation were changed to prolines, as seen in nonamyloidogenic pro-mIAPP. These results indicate that the amino acid sequence in this critical region determines the amyloidogenicity of IAPP and suggest that pro-hIAPP dimers or trimers could be the initial seed proceeding to high-*n* hIAPP oligomers and hIAPP amyloids. These results are also consistent with a report that pro-hIAPP aggregates are the dominant form in the initial phase of islet amyloid formation, and may act as niduses for advanced intracellular amyloid and extracellular amyloid deposition in the later stages, together with mature IAPP (Paulsson et al., 2006). The biochemical reason for preferential pro-hIAPP dimer or trimer formation in the initial phase of islet amyloid formation could be the presence of a heparin binding domain in the N-terminal cleavage site and the higher isoelectric point of pro-hIAPP compared to mature hIAPP, which could enhance association of hIAPP with negatively charged membranes (Jayasinghe and Langen, 2005; Park and Verchere, 2001; Paulsson et al., 2006). Further studies will be required to elucidate the detailed molecular and structural mechanisms governing the formation and progression of hIAPP amyloids.

Since autophagy deficiency can lead to hIAPP oligomer formation, β-cell apoptosis and, finally, diabetes, the possibility that autophagy enhancement could improve β-cell function by accelerating clearance of hIAPP oligomers has been

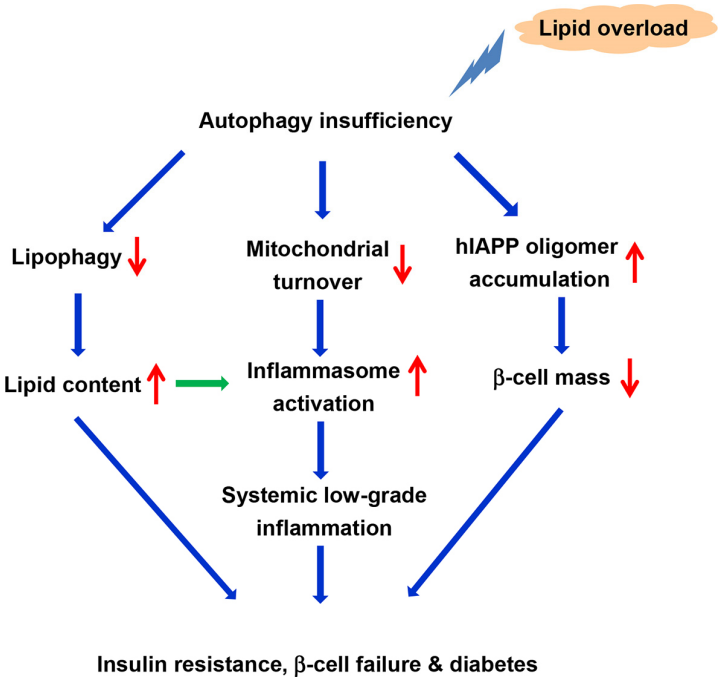

Fig. 3. Proposed model for the development of diabetes with lipid overload or human-type diabetes with autophagy insufficiency. Metabolic stress in the presence of autophagy insufficiency causes increased lipid accumulation due to compromised lipophagy. Autophagy insufficiency leads to delayed clearance of dysfunctional mitochondria, which causes increased inflammasome activation when the cell is challenged with an inflammasome activator, such as lipids (green arrow). Autophagy insufficiency also leads to accumulation of human IAPP (hIAPP) oligomers in pancreatic β-cells, since hIAPP clearance is dependent on autophagy. The combined effects of these three axes and their interactions finally culminates in the development of insulin resistance, β-cell failure and diabetes.

tested. To this end, *hIAPP*$^{+}$ mice were fed a HFD to make them diabetic. When trehalose was administered for four weeks to *hIAPP*$^{+}$ mice given a HFD, their glucose profile significantly improved which was accompanied by reduced accumulation of hIAPP oligomers and islet amyloids, decreased β-cell apoptosis, and an enhanced insulinogenic index representing β-cell function (Kim et al., 2014). These results suggest that autophagy enhancers could be used as a novel therapeutic agent not only against murine diabetes with lipid overload, but also against human-type diabetes characterized by islet amyloid accumulation.

While these results show that enhancement of β-cell autophagy leads to improvement of β-cell function by clearing hIAPP oligomer, a recent paper suggested a different role of starvation-induced autophagy of pancreatic β-cells such as degradation of insulin granules inhibiting excessive release of insulin during fasting (Goginashvili et al., 2015). Thus, further studies will be required to unravel the functional complexity regarding the role of β-cell autophagy in the physiological condition and in the pathological context.

CONCLUSION

A number of mouse studies employing tissue-specific knockout of essential autophagy genes have revealed the important role and function of autophagy in controlling metabolic function. However, it has not been clear from those genetic studies how dysregulated autophagy could affect global or systemic metabolic features. Considering recent evidence that autophagy and mitophagy declines in aging (Sun et al., 2015), such a question could be a practical one related to the effect of aging and organelle dysfunction on body metabolism. Now, it is considered very likely that autophagy deficiency due to aging, genetic causes, or other factors could compromise an organism's ability to adapt to metabolic stress, and predispose it to the development of metabolic syndrome and diabetes due to increased lipid accumulation and inflammasome activation when confronted by metabolic or other types of stress (Fig. 3). Autophagy deficiency could be a predisposing factor for human-type diabetes in particular, since hIAPP oligomers or amyloids are preferentially cleared by autophagy (Fig. 3). Thus, it is highly likely that enhancement of autophagy could be a novel strategy against a global increase in the incidence of metabolic syndrome and diabetes.

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REFERENCES

Castillo, K., Nassif, M., Valenzuela, V., Rohas, F., Matus, S., Mercado, G., Court, F.A., van Zundert, B., and Hetz, C. (2013). Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. Autophagy *9*, 1308-1320.

Coupe, B., Ishii, Y., Dietrich, M.O., Komatsu, M., Horvath, T.L., and Bouret, S.G. (2012). Loss of autophagy in pro-opiomelanocortin neurons perturbs axon growth and causes metabolic dysregulation. Cell Metab. *15*, 1-9.

Dehay, B., Bove, J., Rodriguez-Muela, N., Perier, C., Recasens, A., Boya, P., and Vila, M. (2010). Pathogenic lysosomal depletion in Parkin's disease. Neurobiol. Dis. *30*, 12534-12544.

Ebato, C., Uchida, T., Arakawa, M., Komatsu, M., Ueno, T., Komiya, K., Azuma, K., Hirose, T., Tanaka, K., Kominami, E., et al. (2008). Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. Cell Metab. *8*, 325-332.

Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., Ring, J., Schroeder, S., Magnes, C., Antonacci, L., et al. (2009). Induction of autophagy by spermidine promotes longevity. Nature Cell Biol. *11*, 1305-1314.

Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A., et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat. Med. *22*, 1428-1438.

Fan, Y., Wang, N., Rocchi, A., Zhang, W., Vassar, R., Zhou, Y., and He, C. (2017). Identification of natural products with neuronal and metabolic benefits through autophagy induction. Autophagy *13*, 41-56.

Goginashvili, A., Zhang, Z., Erbs, E., Spiegelhalter, C., Kessler, P., Mihlan, M., Pasquier, A., Krupina, K., Schieber, N., Cinque, L., et al. (2015). Insulin granules. Insulin secretory granules control autophagy in pancreatic β cells. Science *347*, 878-882.

Haataja, L., Gurlo, T., Huang, C.J., and Butler, P.C. (2008). Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocr. Rev. *29*, 303-316.

He, C., Bassik, M.C., Moresi, V., Sun, K., Wei, Y., Zou, Z., An, Z., Loh, J., Fisher, J., Sun, Q., et al. (2012). Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature *481*, 511-515.

Janson, J., Soeller, W.C., Roche, P.C., Nelson, R.T., Torchia, A.J., Kreutter, D.K., and Butler, P.C. (1996). Spontaneous diabetes mellitus in transgenic mice expressing human islet amyloid polypeptide. Proc. Natl. Acad. Sci. USA *93*, 7283-7288.

Jayasinghe, S.A., and Langen, R. (2005). Lipid membranes modulate the structure of islet amyloid polypeptide. Biochemistry *44*, 12113-12119.

Johnson, A.M.F., and Olefsky, J.M. (2013). The origins and drivers of insulin resistance. Cell *152*, 673-684.

Jung, H.S., Chung, K.W., Kim, J.W., Kim, J., Komatsu, M., Tanaka, K., Nguyen, Y.H., Kang, T.M., Yoon, K.H., Kim, J.W., et al. (2008). Loss of autophagy diminishes pancreatic b-cell mass and function with resultant hyperglycemia. Cell Metab. *8*, 318-324.

Kahn, S.E., Andrikopoulos, S., and Verchere, C.B. (1999). Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. Diabetes *48*, 241-253.

Kaushik, S., Rodriguez-Navarro, J.A., Arias, E., Kiffin, R., Sahu, S., Schwartz, G.J., Cuervo, A.M., and Singh, R. (2011). Autophagy in hypothalamic AgRP neurons regulates food intake and energy balance. Cell Metab. *14*, 173-183.

Kaushik, S., Arias, E., Kwon, H., Lopez, N.M., Athonvarangkull, D., Sahu, S., Schwartz, G.J., Pessin, J.E., and Singh, R. (2012). Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. EMBO Rep. *13*, 258-265.

Kim, K.H., and Lee, M.-S. (2014). Autophagy-a key player in cellular and body metabolism. Nat. Rev. Endocrinol. *10*, 322-337.

Kim, K.H., Jeong, Y.T., Oh, H., Kim, S.-H., Cho, J.M., Kim, Y.-N., Kim , S.S., Kim, D.H., Hur, K.Y., Kim , H.K., et al. (2013a). Autophagy deficiency leads to protection from obesity and insulin resistance by inducing FGF21 as a 'mitokine' Nat. Med. *19*, 83-92.

Kim, K.H., Jeong, Y.T., Oh, H., Kim, S.H., Cho, J.M., Kim, Y.-N., Kim, S.S., Kim, D.H., Hur, K.Y., Kim, H.K., et al. (2013b). Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. Nat. Med. *19*, 83-92.

Kim, J., Cheon, H., Jeong, Y.T., Quan, Y., Kim, K.H., Cho, J.M., Lim, Y.-M., Oh, S.H., Jin, S.-M., Kim, J.H., et al. (2014). Amyloidogenic peptide oligomer accumulation in autophagy-deficient b-cells leads to diabetes. J. Clin. Invest. *125*, 3311-3324.

Klionsky, D.J., and Emr, S.D. (2000). Autophagy as a regulated pathway of cellular degradation. Science *290*, 1717-1721.

Klionsky, D.J., Abdelmohsen, K., Abe, A., Abedin, M.J., Abeliovich, H., Acevedo Arozena, A., Adachi, H., Adams, C.M., Adams, P.D., Adeli, K., et al. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy *12*, 1-222.

Lee, H.-Y., Kim, J., Quan, Y., Lee, J.-C., Kim, M.-S., Km, S., Bae, J.-W., Hur, K.Y., and Lee, M.S. (2016). Autophagy deficiency in myeloid cells increases susceptibility to obesity-induced diabetes and experimental colitis. Autophagy *12*, 1390-1403.

Liao, X., Sluimer, J.C., Wang, Y., Subramanian, M., Brown, K., Pattison, J.S., Robbins, J., Martinez, J., and Tabas, I. (2012). Macrophage autophagy plays a protective role in advanced atherosclerosis. Cell Metab. *15*, 545-553.

Lim, Y.-M., Lim, H.-J., Hur, K.Y., Quan, W., Lee, H.-Y., Cheon, H., Ryu, D., Koo, S.H., Kim, H.L., Kim, J., et al. (2014). Systemic autophagy insufficiency compromises adaptation to metabolic stress and facilitates progression from obesity to diabetes Nat. Commun. *5*, 4934.

Lin, F., Ghislat, G., Luo, S., Renna, M., Siddiqi, F., and Rubinsztein, D.C. (2015). XIAP and cIAP1 amplifications induce Beclin 1-dependent autophagy through NFκB activation. Hum. Mol. Genet. *24*, 2899-2913.

Liu, K., Zhao, E., Ilyas, G., Lalazar, G., Lin, Y., Haseeb, M., Tanaka, K.E., and Czaja, M.J. (2015). Impaired macrophage autophagy increases the immune response in obese mice by promoting proinflammatory macrophage polarization. Autophagy *11*, 271-284.

Martinez-Lopez, N., Athonvarangkul, D., Sahu, S., Coletto, L., Zong, H., Bastie, C.C., Pessin, J.E., Schwartz, G.J., and Singh, R. (2013). Autophagy in Myf5+ progenitors regulates energy and glucose homeostasis through control of brown fat and skeletal muscle development. EMBO Rep. *14*, 795-803.

Misawa, T., Takahama, M., Kozaki, T., Lee, H., Zou, J., Saitoh, T., and Akira, S. (2013). Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat. Immunol. *14*, 454-460.

Mizushima, N., and Komatsu, M. (2011). Autophagy: renovation of cells and tissues. Cell *147*, 728-741.

Morselli, E., Mariño, G., Bennetzen, M.V., Eisenberg, T., Megalou, E., Schroeder, S., Cabrera, S., Benit, P., Rustin, P., Criollo, A., et al. (2011). Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J. Cell Biol. *192*, 615-629.

Park, K., and Verchere, C.B. (2001). Indentification of a heparin binding domain in the N-terminal cleavage site of pro-islet amyloid polypeptide. J. Biol. Chem. *276*, 16611-16616.

Paulsson, J.F., Anderson, A., Westermark, P., and Westermark, G.T. (2006). Intracellular amyloid-like deposits contains unprocessed pro-islet amyloid polypeptide (proIAPP) in beta cells of transgenic mice overexpressing the gene for human IAPP and transplanted human islet. Diabetologia *49*, 1237-1246.

Pyo, J.O., Yoo, S.M., Ahn, H.H., Nah, J., Hong, S.H., Kam, T.I., Jung, S., and Jung, Y.K. (2013). Overexpression of Atg5 in mice activates autophagy and extends lifespan. Nat. Commun. *4*, 2300.

Quan, W., Hur, K.Y., Lim, Y., Oh, S.H., Lee, J.-C., Kim, H.C., Kim, G.-H., Kim, S.-H., Kim, H.L., Lee, M.-K., et al. (2012a). Autophagy

deficiency in beta cells leads to compromised unfolded protein response and progression from obesity to diabetes in mice. Diabetologia *55*, 392-403.

Quan, W., Kim, H.-K., Moon, E.-Y., Kim, S.S., Choi, C.S., Komatsu, M., Jeong, Y.T., Lee, M.-K., Kim, K.-W., Kim, M.-S., et al. (2012b). Role of hypothalamic proopiomelanocortin neuron autophagy in the control of appetite and leptin response. Endocrinology *153*, 1817-1826.

Rivera, J.F., Costes, S., Gurlo, T., Glabe, C., and Butler, P.C. (2014). Autophagy defends pancreatic b-cells from human islet amyloid polypeptide-induced toxicity. J. Clin. Invest. *124*, 3489-3500.

Rubinsztein, D.C. (2006). The roles of intracellular protein-degradation pathways in neurodegeneration. Nature *443*, 780-786.

Rubinsztein, D.C., Codogno, P., and Levine, B. (2012). Autophagy modulation as a potential therapeutic target for diverse diseases. Nat. Rev. Drug Discov. *11*, 709-730.

Shibata, M., Yoshimura, K., Furuya, N., Koike, M., Ueno, T., Komatsu, M., Arai, H., Tanaka, K., Kominami, E., and Uchiyama, Y. (2009). The MAP1-LC3 conjugation system is involved in lipid droplet formation. Biochem. Biophys. Res. Com. *382*, 419-423.

Shigihara, N., Fukunaka, A., Hara, A., Komiya, K., Honda, A., Uchida, T., Abe, H., Toyofuku, Y., Tamaki, M., Ogihara, T., et al. (2014). Human IAPP-induced pancreatic beta-cell toxicity and its regulation by autophagy. J. Clin. Invest. *124*, 3634-3644.

Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G.R., Zou, Z., Kinch, L., Wilkins, A.D., Sun, Q., Pallauf, K., MacDuff, D., et al. (2013). Identification of a candidate therapeutic autophagy-inducing peptide. Nature *494*, 201-206.

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

Singh, R., Xiang, Y., Wang, Y., Baikati, K., Cuervo, A.M., Luu, Y.K., Tang, Y., Pessin, J.E., Schwartz, G.J., and Czaja, M.J. (2009b). Autophagy regulates adipose mass and differentiation in mice. J. Clin. Invest. *119*, 3329-3339.

Sun, N., Yun, J., Liu, J., Malide, D., Liu, C., Rovira, I.I., Holmström, K.M., Fergusson, M.M., Yoo, Y.H., Combs, C.A., et al. (2015). Measuring In vivo mitophagy. Mol. Cell *60*, 685-696.

Vandanmagsar, B., Youm, Y.-H., Ravussin, A., Galgani, J.E., Stadler, K., Mynatt, R.L., Ravussin, E., Stephens, J.M., and Dixit, W.D. (2011). The NLRP3 inflammasome instigate obesity-induced inflammation and insulin resistance. Nat. Med. *17*, 179-188.

Verchere, C.B., D'Alessio, D.A., Palmiter, R.D., Weir, G.C., Bonner-Weir, S., Baskin, D.G., and Kahn, S.E. (1996). Islet amyloid formation associated with hyperglycemia in transgenic mice with pancreatic beta cell expression of human islet amyloid polypeptide. Proc. Natl. Acad. Sci. USA *93*, 3492-3496.

Wen, H., Gris, D., Lei, Y., Jha, S., Zhang, L., Huang, M.T., Brickey, W.J., and Ting, J.P. (2011). Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat. Immunol. *12*, 408-415.

Westermark, P., Andersson, A., and Westernark, G.T. (2011). Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol. Rev. *91*, 795-826.

Xie, Q., Lin, Q., Li, D., and Chen, J. (2017). Imatinib induced autophagy via upregulating XIAP in GIST882 cells. Biochem. Biophys. Res. Com. *488*, 584-589.

Yang, B.-G., Hur, K.Y., and Lee, M.S. (2017). Alterations of gut microbiota and immunity by dietary fat. Yonsei Med. J. *58*, 1083-1091.

Zhang, L., Yu, J., Pan, H., Hu, P., Hao, Y., Cai, W., Zhu, H., Yu, A.D., Xie, X., Ma, D., et al. (2007). Small molecule regulators of autophagy identified by an image-based high-throughput screen. Proc. Natl. Acad. Sci. USA *104*, 190223-119028.

Zhang, Y., Goldman, S., Baerga, R., Zhao, Y., Komatsu, M., and Jin, S. (2009). Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. Proc. Natl. Acad. Sci. USA *106*, 19860-19865.