

Role of autophagy in diabetes and endoplasmic reticulum stress of pancreatic β -cells

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<http://dx.doi.org/10.3858/emm.2012.44.2.030>

Accepted 19 December 2011
Available Online 19 January 2012

Abbreviations: Atg7, autophagy-related 7; DFCP-1, double FYVE-containing protein 1; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; PDI, protein disulfide isomerase; ROS, reactive oxygen species; UPR, unfolded protein response; XBP-1, X-box binding protein 1

Abstract

Type 2 diabetes mellitus is characterized by insulin resistance and failure of pancreatic β -cells producing insulin. Autophagy plays a crucial role in cellular homeostasis through degradation and recycling of organelles such as mitochondria or endoplasmic reticulum (ER). Here we discussed the role of β -cell autophagy in development of diabetes, based on our own studies using mice with β -cell-specific deletion of *Atg7* (autophagy-related 7), an important autophagy gene, and studies by others. β -cell-specific *Atg7*-null mice showed reduction in β -cell mass and pancreatic insulin content. Insulin secretory function *ex vivo* was also impaired, which might be related to organelle dysfunction associated with autophagy deficiency. As a result, β -cell-specific *Atg7*-null mice showed hypoinsulinemia and hyperglycemia. However, diabetes never developed in those mice. Obesity and/or lipid are physiological ER stresses that can precipitate β -cell dysfunction. Our recent studies showed that β -cell-specific *Atg7*-null mice, when bred with *ob/ob* mice, indeed become diabetic. Thus, autophagy deficiency in β -cells could be a precipitating factor in the pro-

gression from obesity to diabetes due to inappropriate response to obesity-induced ER stress.

Keywords: autophagy; diabetes mellitus; endoplasmic reticulum stress; insulin-secreting cells; unfolded protein response

Introduction

Two major types of cell death are apoptosis and necrosis, however, recently another type of cell death, autophagy, was defined. Autophagy is also called as type 2 programmed cell death, in comparison with apoptosis, programmed cell death type 1 (Boya *et al.*, 2005). Although morphologic description of autophagy had been reported decades ago (Ashford and Porter, 1962), its physiologic role has been unknown. However, elucidation of the genes of autophagy using yeast system revolutionized this field and opened a new era of molecular autophagy (Nakatogawa *et al.*, 2009). Mammalian homologues were also identified, and the roles of mammalian autophagy genes in diverse physiological and pathological systems are being intensely investigated. Recent investigations employing techniques of molecular autophagy showed that autophagy protects cells both constitutively and during cellular stress or starvation (Lum *et al.*, 2005). Furthermore, dysregulated autophagy is being implicated in diverse pathological processes such as neurodegenerative diseases, cancer, and infection (Levine and Kroemer, 2008).

Autophagy (derived from the Greek meaning "self eating") exists in all eukaryotic cells and is evolutionarily conserved from yeast to humans (Levine and Klionsky, 2004). Autophagy is a dynamic process involving the rearrangement of subcellular membranes (referred as an autophagosome) to sequester cytoplasm and organelles for delivery to lysosomes, where the sequestered material is degraded and recycled (Klionsky and Emr, 2000). Autophagy is basically cell-protective; however, it may also promote cell death through excessive degradation of cellular constituents, depending on the cellular and environmental context (Levine and Yuan, 2005). Among the three types of cell death,

the roles of apoptosis and necrosis in type 1 or type 2 diabetes have been extensively studied (Kaneto *et al.*, 1995; Kim *et al.*, 1999, 2007a, 2007b). However, the role of autophagy in diabetes or body metabolism has been far from clear, but recent observations suggest that autophagy may be an important factor in the development and prevention of diabetes.

Role of autophagy in pancreatic β -cell physiology

It has been reported that ubiquitinated-protein aggregates were formed in pancreatic β -cells during oxidative stress associated with hyperglycemia and were regulated by autophagy (Kaniuk *et al.*, 2007). Because accumulation of protein aggregates may damage cells, such data suggested that autophagy may contribute to the regulation of β -cell survival and death, but direct relationship between autophagy and β -cell death was not demonstrated. To address this issue, a mouse model whose β -cells were deficient in autophagic activity would be valuable. By crossing *autophagy-related 7* (*Atg7*)-floxed mice (*Atg7^{F/F}*) (Komatsu *et al.*, 2005) with RIP-*Cre* mice, we and others generated β -cell-specific *Atg7*-knockout mice (*Atg7 $\Delta\beta$ -cell*). *Atg7* is an E1-like gene that is essential for the formation and completion of autophagosomes (Komatsu *et al.*, 2005). *Atg7 $\Delta\beta$ -cell* mice showed significant hyperglycemia, glucose intolerance and hypoinsulinemia (Ebato *et al.*, 2008; Jung *et al.*, 2008). Morphologic analysis showed decreased β -cell mass, along with increased β -cell death and reduced β -cell proliferation. Due to the reduced β -cell mass, pancreatic insulin content was also reduced.

When pancreatic islets were isolated from the *Atg7 $\Delta\beta$ -cell* mice and challenged with glucose to investigate glucose-stimulated insulin release *ex vivo*, both the basal and stimulated insulin secretion were significantly attenuated in β -cells of the *Atg7 $\Delta\beta$ -cell* mice compared to control mice. In addition, when glucose-induced changes of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_c$) were measured in viable islet cells, they were significantly impaired in islets of *Atg7 $\Delta\beta$ -cell* mice compared to control islets (Jung *et al.*, 2008).

While soluble short-lived ubiquitinated proteins are removed through proteasomal pathway, insoluble or large long-lived heavily ubiquitinated proteins are preferentially cleared through autophagy pathway (Kirkin *et al.*, 2009). Consistently, Immunohistochemistry of autophagy-deficient β -cells showed a number of large ubiquitinated inclusion bodies inside the cells. In addition, p62 which is a polyubiquitin-

binding protein and also a specific substrate of autophagy itself (Komatsu *et al.*, 2007), also accumulated in autophagy-deficient β -cells. Confocal immunofluorescent microscopy revealed the ubiquitin and p62 were co-localized in the same inclusion bodies (Jung *et al.*, 2008). Hence, large inclusion bodies were formed in autophagy-deficient β -cells that comprise ubiquitin, p62 and degenerated proteins. Pathophysiological roles of inclusion body formation in wild-type cells or autophagy-deficient cells including β -cells await further investigations (Komatsu *et al.*, 2007).

Analysis of the ultrastructural changes using electron microscopy revealed that some β -cells of *Atg7 $\Delta\beta$ -cell* mice contained remarkably reduced numbers of insulin granules compared to wild-type β -cells. Further magnification showed swelling of mitochondria and distension of rough endoplasmic reticulum (ER) and Golgi complex even in apparently normal-looking β -cells of *Atg7 $\Delta\beta$ -cell* mice at lower magnifications (Jung *et al.*, 2008) (Figure 1).

These morphologic findings implied the existence of mitochondrial dysfunction and ER stress, which could lead to β -cell death and malfunction. We speculate that the decreased function and mass of β -cells of *Atg7 $\Delta\beta$ -cell* mice might be due to mitochondrial dysfunction and/or ER stress, because mitochondria and ER are main cellular organelles rejuvenated by autophagy and critical for β -cell function and survival (Silva *et al.*, 2000; Back *et al.*, 2009). These results suggest that autophagy is necessary to maintain structure, mass and organelle function of β -cells. While these results suggest an important role for autophagy in β -cell homeostasis, the role for autophagy in diabetes has not been fully elucidated. Because ER and autophagy are closely related to each other, and ER stress is

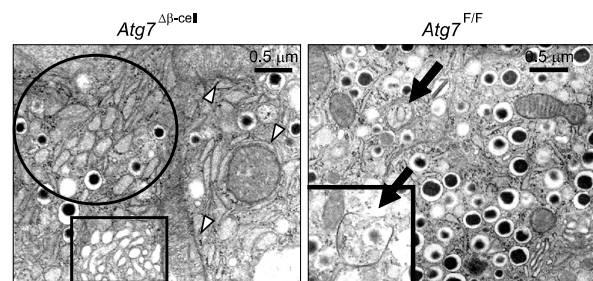


Figure 1. Electron microscopic analysis of the pancreas at 20 weeks of age. Mitochondria (white arrowheads), rough ER (circles) and Golgi complex (rectangles). Those in β -cells of *Atg7 $\Delta\beta$ -cell* mice were swollen and distended compared to those of control *Atg7^{F/F}* mice. A classical autophagosome, an organelle surrounded by double membrane structure was seen in wild-type β -cells (black arrow) (Reproduced from the original paper in *Cell Metabolism* 8:318-325, 2008).

important in the pathogenesis of diabetes, β -cell autophagy may be related with ER stress response, also called unfolded protein response (UPR), which is a cellular response to ER stress induced by accumulation of unfolded proteins in ER lumen. The following sections will deal with relationships between autophagy, UPR, ER stress, and diabetogenesis.

ER and autophagy

Evidences that ER is involved in autophagic process are as follows: First, ER is one of the most important sources of isolation membrane, the starting structure of autophagosomes. Previous delicate 3D electron tomography and immunolabelling on thin slice sections showed that protein disulfide isomerase (PDI), an ER-resident protein, exists in both sides of membranes that sandwiched isolation membrane, suggesting that isolation membrane is forming autophagosome cradle stacked between two ER membranes (Hamasaki and Yoshimor, 2010). This structure is called omegasomes, and double FYVE-containing protein 1 (DFCP-1) has been used as a marker molecule of omegasomes (Matsunaga *et al.*, 2010). In such a way, aggregated or terminally misfolded proteins in ER lumen may be able to be cleared by autophagic process as follows. Usually misfolded or excessive unfolded proteins are cleared through ubiquitination and proteasomal degradation in the cytoplasm in a process called ER-associated protein degradation (ERAD) (Smith *et al.*, 2011). Thus, misfolded protein should be unfolded again and be transported across ER membrane back to cytoplasm in a process called retrograde translocation. However, terminally misfolded protein cannot be unfolded for retrograde translocation and ERAD. Hence, terminally misfolded protein or insoluble protein aggregates cannot be easily processed by ERAD, and autophagy may be the only way to remove such protein aggregates. For example, terminally misfolded proteins in the ER lumen may be able to move into autophagosomes stacked between two ER membranes as described above without traversing ER membrane or retrograde translocation. Second, ER is one of the important target organelles of autophagy in a process called "ER-phagy" or "reticulophagy" (Bernaes *et al.*, 2007). Third, we have also observed distention of ER in autophagy-deficient β -cells (Jung *et al.*, 2008). Finally, the close relationship between ER and autophagy was demonstrated by recent reports showing activation of autophagic process by ER stress, which was mediated by induction of Atg12 expression, JNK

activation or mTOR inhibition (Ogata *et al.*, 2006; Yorimitsu *et al.*, 2006; Kouroku *et al.*, 2007). The significance of such findings is not clear, but may be related to the increased demand for autophagic removal of misfolded proteins in ER lumen in the process of ER stress or accumulation of unfolded proteins.

ER stress and diabetes

Two most important pathgenetic mechanisms of type 2 diabetes are insulin resistance and β -cell failure (DeFronzo *et al.*, 1979). Numerous theories have been proposed to explain insulin resistance and β -cell failure. Several theories proposed different mechanisms for insulin resistance and β -cell failure. However, some theories suggested the same pathogenetic mechanism could explain both insulin resistance and β -cell failure, which is simple and more attractive, from the point of view of Occam's razor. ER stress theory is one of such a unitarian theory. ER stress by lipid injury or cytokines and subsequent JNK activation have been implicated as a cause of insulin resistance (Ozcan *et al.*, 2004, 2006), although effector molecules or lipid metabolites causing direct ER stress has not been identified. ER stress has also been reported to be important in β -cell failure. Pancreatic β -cells are constantly exposed to a great demand for insulin production and, thus, unusually heavy load for UPR. Hence, control of ER stress or UPR is particularly important in β -cells. Furthermore, β -cell response not only to high glucose but also to low glucose may take a form of ER stress response in β -cells (Back *et al.*, 2009). High glucose induces increased insulin synthesis which will lead to an increased amount of unfolded protein and activation of PERK, while low glucose leads to decreased synthesis of insulin through eIF2 α phosphorylation (Gomez *et al.*, 2008). Therefore, pancreatic β -cells are particularly susceptible to ER stress, which is aggravated by obesity, insulin resistance, lipids and other physiologic or pathologic stimuli (Scheuner *et al.*, 2005). ER stress or subsequent JNK activation can lead to decreased insulin production, reduced β -cell mass or even β -cell death (Kaneto *et al.*, 2002; Solinas *et al.*, 2006). Thus, meticulous and continuous control of UPR is needed in β -cells to ensure appropriate insulin release according to ambient glucose level, and failure of such an exquisite control would lead to dysregulated glucose homeostasis and β -cell failure or death.

Compromised UPR machinery in autophagy-deficient β -cells

Because of the aforementioned close relationship between UPR and ER stress and that between diabetes and ER stress, we studied the expression of UPR genes in autophagy-deficient β -cells. In fact, this is the reverse question of aforementioned ER stress-induced autophagy induction. In other word, it is well known that ER stress induces autophagy, then what is the effect of autophagy on ER stress or UPR? In our experiment to address these questions, we unexpectedly observed significantly decreased expression of almost all UPR genes in autophagy-deficient β -cells (Quan *et al.*, 2011), which was contrary to our expectation that UPR gene expression would be upregulated due to ER stress which was suspected on the basis of ER distention in autophagy-deficient β -cells. Then, we realized that UPR is a response to ER stress, but not ER stress itself. While investigators usually suspect the existence of ER stress when they observe ER stress response, the relationship between ER stress and ER stress response is not always linear. For instance, appropriate UPR is an adaptive

response to ER stress, and deficient UPR in the face of ER stress could be a real danger to cells (Merksamer *et al.*, 2008). Thus, we hypothesized that autophagy is important in the maintenance of appropriate UPR machinery and autophagy deficiency could lead to impaired UPR response and vulnerability toward ER stress. In our experiment to prove our hypothesis, we observed that autophagy-deficient β -cells are more prone to cell death when they are treated with ER stressors such as thapsigargin *in vitro* (Figure 2).

The mechanism of the compromised UPR gene expression in autophagy-deficient β -cells is not clear. We observed impaired expression of noncatalytic regulatory subunits of PI3K, p85 and p85 β (Quan *et al.*, 2011) that bind to X-box binding protein 1 (XBP-1) in an insulin-dependent manner and are important in the basal and stimulated expression of diverse UPR genes (Park *et al.*, 2010; Winnay *et al.*, 2010). Thus, deficient p85 and p85 β expression might be a cause of compromised UPR in autophagy-deficient β -cells.

Lipid and ER stress

While thapsigargin is a classical pharmacological ER stressor (Li *et al.*, 1993), lipid is a physiological ER stressor that is relevant to the pathogenesis of

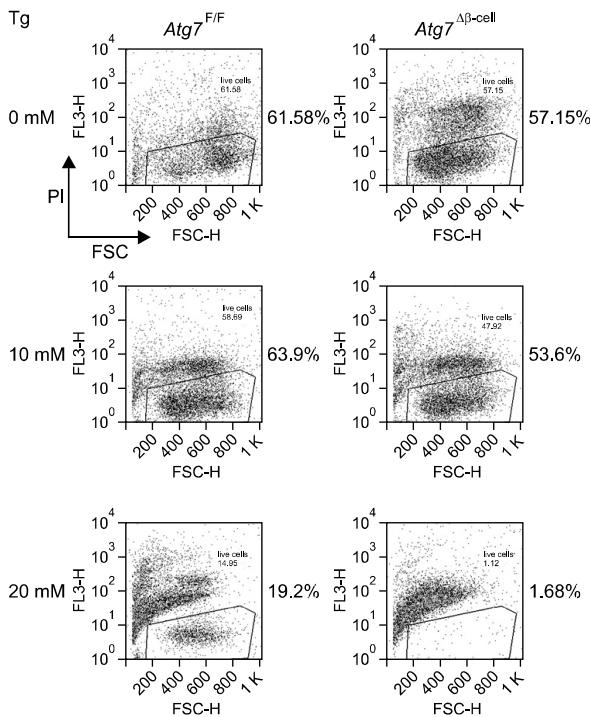


Figure 2. Viability of autophagy-deficient islet β -cells from $Atg7^{\Delta\beta\text{-cell}}$ mice after treatment with thapsigargin (Tg), a classical ER stressor. Viability presented in this figure as the number on each panel was measured by FACS analysis after staining with propidium iodide. Autophagy-deficient β -cells were more susceptible to thapsigargin-induced cell death, probably because of insufficient UPR gene expression.

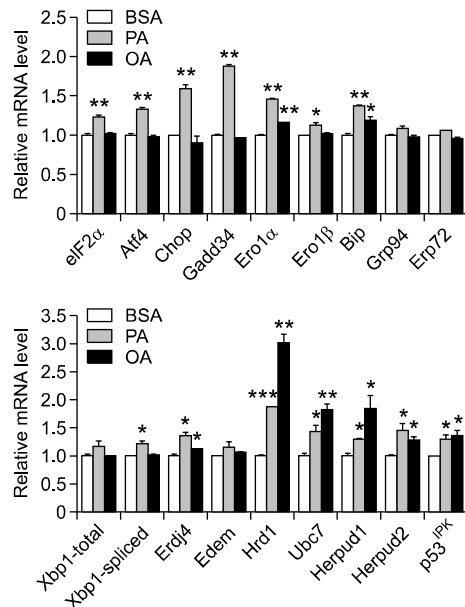


Figure 3. Induction of UPR gene expression in insulinoma cells by palmitic acid (PA) or oleic acid (OA). Increased expression of UPR genes by free fatty acids suggests that lipids are physiological ER stressors and demand for UPR is increased by lipids. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ VS BSA. BSA, bovine serum albumin; PA, palmitic acid; OA, oleic acid.

diabetes and metabolic syndrome (Cunha *et al.*, 2008). As an *in vivo* counterpart, obesity is a well-known inducer of ER stress, while the effector molecules or lipid metabolites directly causing ER stress is not yet clearly demonstrated. The mechanism of ER stress by lipid injury or obesity might entail altered ER luminal homeostasis by lipid overload, changes of lipid composition of ER membrane, or sequestration of ER machinery to accommodate lipid droplets. When we studied the impact of lipids on ER in our system, we confirmed that lipids induce expression of diverse UPR genes both in insulinoma cells and primary islet cells, showing that lipids are ER stressors and increase demand for UPR by lipids in those cells (Figure 3). Induction of UPR gene expression by lipid was significantly lower in autophagy-deficient β -cells compared to wild-type β -cells, suggesting that demand for UPR is increased by lipid injury and the increased demand for UPR is unmet in autophagy-deficient β -cells. After confirming that lipid injury is an ER stressor, we investigated whether autophagy-deficient β -cells with compromised UPR machinery is more susceptible to treatment with lipids *in vitro*. As hypothesized, we found that autophagy-deficient β -cells were more susceptible to injury by high doses of palmitic acid, the most abundant saturated fatty acid *in vivo* (Quan *et al.*, 2011). These results show that autophagy-deficient β -cells with compromised UPR machinery are more vulnerable to physiological ER stressors as well as pharmacological ER stressors.

Role of β -cell autophagy in diabetes and UPR of β -cells *in vivo*

The functional significance of the increased susceptibility of autophagy-deficient β -cells to ER stressors *in vitro* should be tested *in vivo*. Animal

models for this purpose were generated by breeding mice with β -cell-specific *Atg7*-deficiency (*Atg7* ^{$\Delta\beta$ -cell} mice) with *ob/ob* mice. It has been reported that obesity imposes ER stress in β -cells *in vivo* (Scheuner *et al.*, 2005). Increased UPR gene expression of β -cells of *Atg7*-wild type (*Atg7*^{F/F})-*ob/ob* mice was confirmed, which is consistent with previous reports (Scheuner *et al.*, 2005) and suggests increased demand for UPR by obesity *in vivo*. Intriguingly, *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice developed severe diabetes, while littermate *Atg7* ^{$\Delta\beta$ -cell}-*ob/w* mice or *Atg7*^{F/F}-*ob/ob* mice developed only mild hyperglycemia (Quan *et al.*, 2011). Random blood glucose level was very high in *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice, while it was only mildly increased in littermate *Atg7* ^{$\Delta\beta$ -cell}-*ob/w* or *Atg7*^{F/F}-*ob/ob* mice (Figure 4). These results suggest that autophagy-deficient β -cells are susceptible to ER stress imposed by obesity *in vivo* probably due to compromised UPR machinery. This hypothesis was supported by increased β -cell apoptosis and marked accumulation of reactive oxygen species (ROS) in pancreatic islets, and also severely impaired β -cell function of *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice (Quan *et al.*, 2011). Consistently, fasting serum insulin level was significantly reduced in *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice compared to *Atg7*^{F/F}-*ob/ob* mice that showed high serum insulin level in compensation for obesity-induced insulin resistance (Figure 4). Electron microscopy demonstrated severe ER distention in β -cells of *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice, in comparison with minimal and moderate ER distention in *Atg7*^{F/F}-*ob/ob* and *Atg7* ^{$\Delta\beta$ -cell}-*ob/w* mice, respectively (Figure 5). These results are in line with a previous report that autophagy-deficient β -cells showed defects in compensatory increase of β -cell mass in response to high-fat diet (Ebato *et al.*, 2008). However, diabetes was not observed in those mice fed high-fat diet.

In summary, β -cell autophagy is necessary for

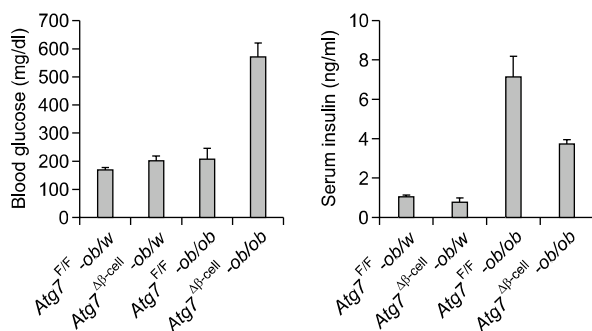


Figure 4. Metabolic profile of *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice and their control mice. Random blood glucose level (left) and fasting serum insulin level (right) in each type of male mice at 12 weeks of age.

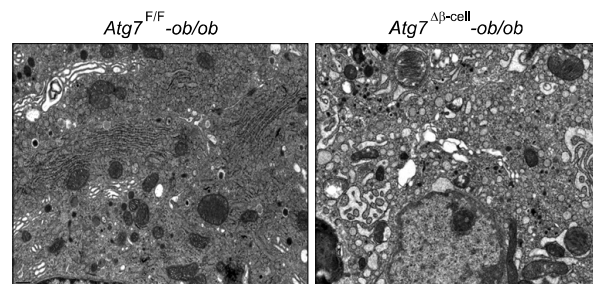


Figure 5. Electron microscopic analysis showed that ER in pancreatic islets of *Atg7* ^{$\Delta\beta$ -cell}-*ob/ob* mice was severely distended (right), while only minimal distention was observed in islets of *Atg7*^{F/F}-*ob/ob* mice (left). ER in islets of *Atg7* ^{$\Delta\beta$ -cell}-*ob/w* mice showed moderate distention (data not shown), similar to that in *Atg7* ^{$\Delta\beta$ -cell} mice (See Figure 1).

appropriate UPR machinery constitutively and in response to ER stress such as lipid injury. Lipid or obesity appears to increase demand for UPR, which was unmet in autophagy-deficient β -cells of $Atg7^{\Delta\beta\text{-cell}}\text{-ob/ob}$ mice. Observation that overt diabetes developed in $Atg7^{\Delta\beta\text{-cell}}\text{-ob/ob}$ mice but not in $Atg7^{\Delta\beta\text{-cell}}$ mice or ob/ob mice, suggest the possibility that autophagy deficiency in pancreatic β -cells due to genetic predisposition or other causes such as aging could be a factor in the progression from obesity to diabetes.

Lipid and autophagy

Now we understand that autophagy is a protective mechanism against ER stress imposed by obesity and its deficiency leads to compromised UPR in response to ER stress. Then, we next asked the reverse question. What is the effect of lipid injury on autophagy level or activity? This is not a simple question, and consensus has not been reached regarding the effect of lipid on autophagy level or autophagic activity. Some papers showed increased autophagy level by lipid, while others reported otherwise. Different levels of autophagy depending on the stages or ages of the experimental animals have also been suggested (Ebato *et al.*, 2008; Singh *et al.*, 2009; Koga *et al.*, 2010; Shibata *et al.*, 2010; Yang *et al.*, 2010). Such inconsistencies are partly due to technical difficulties associated with assay of *in vivo* autophagy level and autophagic activity (Klionsky *et al.*, 2008). Particularly, differentiation of autophagy level and autophagic activity is difficult *in vivo*, as explained below. Thus, we employed $GFP\text{-}LC3^+$ mice that express GFP fused to LC3 globally and could provide reliable results regarding *in vivo* autophagic activity (Hosokawa *et al.*, 2006; Klionsky *et al.*, 2008). In those mice, GFP puncta represent autophagosomes and, thus, direct visualization of autophagy level and quantitation of autophagosome number are possible *in vivo* (Mizushima *et al.*, 2004). To determine the changes in autophagy level by obesity *in vivo*, we crossed ob/w mice to $GFP\text{-}LC3^+$ mice to finally derive $GFP\text{-}LC3^+\text{-ob/ob}$ mice. The number of GFP puncta reflecting autophagy level was apparently increased in pancreatic islets of $GFP\text{-}LC3^+\text{-ob/ob}$ mice compared to $GFP\text{-}LC3^+\text{-ob/w}$ mice (Quan *et al.*, 2011), suggesting that autophagy level is increased by obesity *in vivo*. However, the number of LC3 puncta and autophagosomes could be apparently increased by the blockade of autophagic process at the lysosomal step due to clamp effect, rather than true increase in autophagic activity. Thus, we studied cleavage of GFP in $GFP\text{-}LC3^+$ mice which re-

flects autophagic degradation or cleavage of substrates in lysosomes and, thus, autophagic activity (Hosokawa *et al.*, 2006). Indeed, Western blotting demonstrated that GFP cleavage has occurred in islets of $GFP\text{-}LC3^+\text{-ob/ob}$ mice which was not observed in those of $GFP\text{-}LC3^+\text{-ob/w}$ mice (Quan *et al.*, 2011), suggesting that lysosomal steps of autophagic process had occurred and autophagic activity was indeed increased in pancreatic islets of ob/ob mice. Therefore, our data show that both autophagy level and autophagic activity are increased by lipid or obesity in multiple tissues *in vivo*. However, the level of p62 level, a specific substrate of autophagy (Komatsu *et al.*, 2007), was elevated in islets of $GFP\text{-}LC3^+\text{-ob/ob}$ mice compared to $GFP\text{-}LC3^+\text{-ob/w}$ mice, which is apparently inconsistent with increased autophagic activity (Klionsky *et al.*, 2008). To resolve this inconsistency, we performed a proteolysis assay that represents the eventual degradation of autophagic protein substrates (Klionsky *et al.*, 2008). Lysosomal degradation of long-lived proteins measured by counting release of incorporated C^{14} -leucine was significantly inhibited by palmitic acid or oleic acid ($P < 0.01\text{-}0.001$) (Quan *et al.*, 2011), suggesting that while lipids enhance apparent autophagic activity to eliminate lipids through 'lipophagy' (Singh *et al.*, 2009), lipid overload decreases proteolysis, probably due to shunting or sequestration of the autophagic machinery toward lipophagy. This phenomenon could be a 'trade-off' between removal of lipid and proteolysis, which may be occurring in recent era due to excessive energy supply. Thus, the relationship between lipid and autophagy is complex and may be dependent on cell types, experimental conditions or assay methods. Further exquisite studies employing diverse advanced technology will answer the exact relationship between lipid and UPR and its significance in pathophysiological contexts.

Autophagy and Insulin resistance

In addition to β -cell dysfunction, dysregulated autophagy may possibly be involved in insulin resistance as well, because ER stress has been implicated not only in β -cell dysfunction but also in insulin resistance (Ozcan *et al.*, 2004). Because autophagy deficiency could lead to abnormal ER stress or ER stress response, impaired autophagy may affect insulin resistance. A recent paper reported that autophagy is deficient in the liver of obese mice and overexpression of $Atg7$ restored insulin sensitivity together with alleviated expression of ER stress markers (Yang *et al.*, 2010). However,

it is still controversial whether ER stress is a cause of insulin resistance or simply associated with insulin resistance. Recently, autophagy was reported to participate in the down-regulation of insulin receptors and ER stress-mediated insulin resistance as an adaptive process in ER stress (Zhou *et al.*, 2009). Conversely, insulin resistance has been reported to suppress autophagy (Liu *et al.*, 2009). Although these findings suggest that autophagy may be related to insulin resistance, direct causal relationship between them would require further investigation.

Conclusion and future perspective

While obesity is a well-known risk factor for diabetes, obesity and diabetes are not the same. Not all human subjects with obesity or experimental animals with obesity develop diabetes. Our results suggest the possibility that deficiency of β -cell autophagy could be a factor in the progression from obesity to diabetes.

Because of the elucidation of the machinery of molecular autophagy and its relevance to the maintenance of functions of organelles such as ER, autophagy is emerging as a new target of intense researches and drug discovery aiming at diseases that are caused by or associated with ER dysfunction such as diabetes. If pathogenetic role of dysregulated autophagy in the development of natural human diabetes can be confirmed, a new class of drugs will be possible based on a new principle.

Acknowledgements

This study was supported by the Bio R&D Program (2008-04090) and the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Korea (A080967). Lee M-S is the recipient of the Global Research Laboratory Grant of the National Research Foundation of Korea (K21004000003-10A0500-00310) and a grant from the 21C Frontier Functional Proteomics Project of the Korean Ministry of Science & Technology (FPR08B1-210).

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