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A role for autophagy in β-cell life and death

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Autophagy is a vacuolar, self-digesting mechanism responsible for the removal of organelles and defined regions of the cytoplams. This process has, in general, a beneficial role for the cell, since it regulates the turnover of aged proteins and eliminates damaged structures. However, cells that undergo altered autophagy may be triggered to die in a non-apoptotic manner. As a matter of fact, in recent years it has become clear that dysregulated autophagy may be implicated in several disorders, such as cancer, neurodegenerative diseases and hepatic encephalopathy. We have recently shown that β-cells of type 2 diabetic subjects show signs of autophagy associated death, which may contribute to the overall loss of β-cell mass in type 2 diabetes. In addition, studies with cell lines and rodent models have demonstrated the importance of autophagy in β-cell function and survival. Altogether, the available evidence supports the view that autophagy is implicated in β-cell pathophysiology, and suggests that addressing the molecular mechanisms involved in autophagic regulation might provide clues for preventing or treating β-cell damage in diabetes.

Key words: autophagy, β-cells, type 2 diabetes, gene expression, free fatty acids

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Introduction

Type 2 diabetes arises from a combination of decreased insulin sensitivity and the inability of pancreatic β-cells to respond normally to glucose and other nutrients or hormones by appropriately increasing insulin secretion.[1,2] While the relative contribution of these two defects continues to be debated, longitudinal studies have shown that β-cell failure is fundamental for the onset and progression of the disease. β-cell defects in type 2 diabetes occur at both the survival and functional levels, leading to reduced β-cell mass and insufficient insulin secretion.[1-4] Reduction of β-cell mass in type 2 diabetes is generally attributed to increased apoptosis (not compensated for by adequate β-cell regeneration).[1-4] However, other β-cell death mechanisms might be involved, including the form of cell loss characterized by massive cytoplasmic vacuolization and absent or scarse nuclear chromatin condensation, named autophagy associated cell death.

Autophagy

In response to changes in their environment and intracellular milieu, cells can modulate their anabolic and catabolic capacities. Two major pathways are involved in the catabolism of cellular material: the multi-enzyme ubiquitin-proteasome system and autophagy, this latter culminating in lysosomal degradation.[5,6] Whereas both proteasome and autophagy are able to degrade proteins, only autophagy can eliminate also macromolecules and even entire organelles. Actually, the term autophagy includes several mechanisms, such as microautophagy, macroautophagy and chaperone-mediated autophagy. Commonly, however, macroautophagy is referred to as autophagy.

The process of autophagy begins when a flat membrane cistern wraps around cytoplasmic structures with or without a portion of the cytosol, forming closed double membrane vacuoles (autophagic vacuoles). These structures then mature in a stepwise process involving direct fusion events with primary lysosomes (forming autophagosomes, also named autolysosomes), which

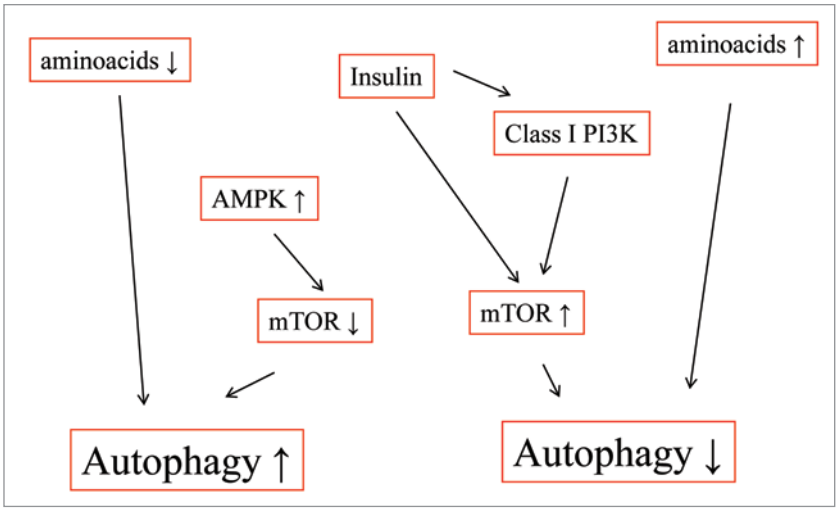

Figure 1. Simplified overview of autophagy regulation.

deliver the hydrolytic enzymes responsible of the degradation of the engulfed material. The final degradation step occurs when lysosomal enzymes digest the content of the autolysomes, and the resulting molecules are released into the cytoplasm.

Several molecules are involved in the regulation and execution of autophagy (**Fig. 1**).[5-8] Essentially, the control of this phenomenon is characterized by being inhibited by mTOR and $PI_{3,4,5}P3$ (produced by class I PI3K), and by requiring PI_3P (produced by class III PI3K). In turn, mTOR is inhibited by amino acid depletion and AMPK activity, that thus promote autophagy. In this framework, insulin has important roles, since it mainly inhibits autophagy by inducing mTOR and activating class I PI3K.[9] However, insulin also seems to be able to activate class III PI3K as well, probably via IRS2, which would favour autophagy by production of PI_3P.[10]

In turn, the main target of mTOR is the protein kinase Atg1 (ULK1), which is inactivated by phosphorylation. Formation and completion of membranes for sequestration of cytoplasmic components require the integrated activity of several additional Atgs including Atg6—also known as beclin-1, and Atg8 (also known as LC3). Then, fusion of vacuoles with lysosomes occurs, followed by degradation by hydrolases and recycle of "building blocks" (e.g., amino acids) to the cytosol (mediated by specific effluxers, such as Atg22).

In general, the process of autophagy is beneficial for the cell, since it maintains the energy level under nutrient-depleted conditions, regulates the turnover of aged or abnormal proteins, and eliminates damaged organelles. However, if altered, autophagy may be implicated in various diseases, including cancer, muscle and liver disorders, pathogen invasion and neurodegenerative diseases.[5-8] In addition, as mentioned above, autophagy can trigger a form of cell death known as autophagic cell death, morphologically characterized by the presence of massive vacuoles engulfment.[11,12]

Autophagy and the Pancreatic β-Cell

In a recent study, we evaluated some features of autophagy in β-cells from type 2 diabetic patients.[13] Using electron microscopy, we observed an increased amount of dead β-cells in diabetic than control islets. As expected, several (approximately 50%) of these β-cells showed clear signs of apoptotic cell death (marked chromatin condensation and/or blebs). However, the remaining β-cells were characterized by massive vacuoles overload without nuclear alterations, suggesting autophagy-associated cell death. When we assessed transcription of molecules involved in the autophagic machinery, we observed unchanged expression of beclin-1 and Atg1 (involved in early steps of autophagy), and reduced transcription of LAMP2 and cathepsin B and D (involved in later steps). Exposure of non-diabetic islets to increased non-esterified fatty acids caused a marked increase of vacuoles accumulation, together with enhanced β-cell death and decreased LAMP2 expression. In addition, we found that metformin was able to improve the alterations observed in diabetic β-cells and those in non-diabetic islets pre-exposed to fatty acids.[13] In an effort to investigate on the possible mechanisms involved in metformin effects we have successively measured mRNA expression of AMP-Activated Protein Kinase (AMPK) by real-time quantitative reverse transcription reaction (RT-PCR). The method as used in our laboratory has been described in detail.[13,14] Total RNA was extracted from human type 2 diabetic islets either not exposed or pre-exposed for 24 h to a therapeutical concentration of metformin (2.4 μg/ml). Extraction was performed using the RNeasy Protect Mini Kit (QIAGEN Inc., Valencia, CA), and RNA was quantified by absorbance at A_{260}/A_{280} (ratio >1.65) nm in a Perkin-Elmer spectrophotometer. Integrity was assessed after electrophoresis in 1.0% agarose gels by ethidium bromide staining. cDNA synthesis was then performed from 2 μg of total RNA and the oligonucleotides of interest were obtained from Assay-on-Demand Gene Expression Products (Applied Biosystem). The UniGene ID for AMP-K was Hs.591439 (NM 006252.2). Following labeling with FAM at the 5' end and TAMRA at the 3' end, amplification was performed in a total volume of 25 μl containing 1 μl cDNA sample, 200 nmol/l of primer, 100 nmol/l of the corresponding probe, and 12.5 μl of TaqMAn Universal PCR Master Mix. Polymerase was activated by preincubation at 95°C for 10 min. Messenger mRNA level of target genes was quantified (cycle thresholds below 30) and normalized for β-actin. It was in this way found that the expression of AMPK was 0.39 ± 0.04 (ratio over β-actin) in untreated diabetic islets, and increased to 1.1 ± 0.37 ($p < 0.05$ by the two tailed Student's t-test) following treatment with metformin. This confirms that the drug can induce and potentiate the action of AMPK, as widely accepted.[15] Since as mentioned above AMPK can inhibit mTOR, which in turn is the major brake on autophagy, we hypothesize that met-

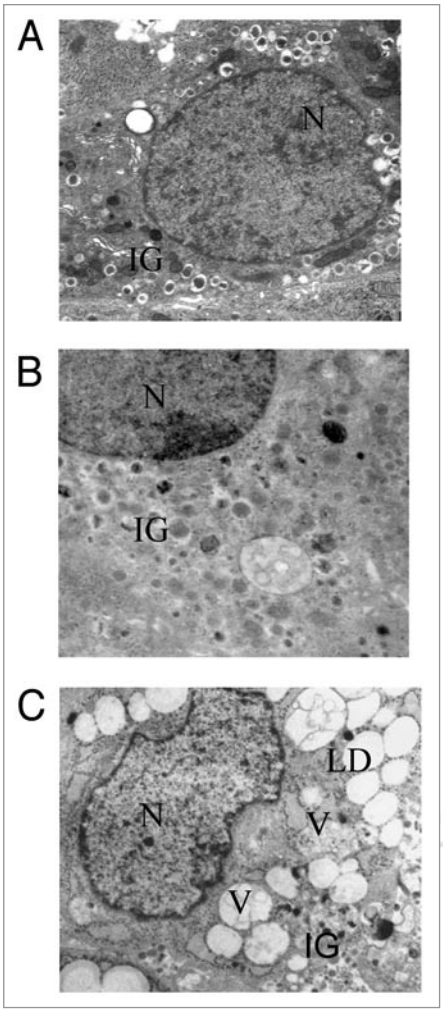

Figure 2. Micrographies of β-cells from control β-cell (A), β-cell pre-exposed to high glucose levels (B) and β-cell cultured in the presence of high free fatty acids (C). Vacuolization is evident in (C) but not in (A) and (B). Lipid droplets (LD) are also shown in (C) (note the lack of membrane). N, nucleus; IG, insulin-granules. Magnification x7,000.

formin can protect β-cells by promoting removal of accumulated vacuoles.

We were also interested in assessing whether metabolic perturbations other than lipotoxicity influence autophagy in human islets. Therefore, isolated islets were cultured for five days with either M199 medium containing 5.5 mmol/l glucose or M199 medium containing 5.5 or 16.7 mmol/l glucose, alternating every 24 h. By electron microscopy examination, it was confirmed that the glucotoxic conditions caused increased amount of β-cell apoptosis (not shown). However, the quantity of β-cell with signs of autophagy associated death did not change and the density volume of autophagic vacuoles (control islets: 0.3 ± 0.03 ml%; high glucose exposed islets: 0.2 ± 0.06 ml%) and autolysosome (control islets: 3.2 ± 0.8 ml%; high glucose exposed islets: 3.2 ± 0.5 ml%) remained unaltered. The ultrastructure of control β-cells and β-cells exposed to FFA or high glucose is reported in **Figure 2**. Similar results have been reported with isolated rodent islets,[16] and altogether the results suggest that β-cell autophagy can be affected more heavily by prolonged exposure to free fatty acids and perhaps other lipids[17] than by culture in the presence of high glucose concentration.

Other recent articles have underlined the importance of autophagy in the pathophysiology of the β-cell (reviewed in ref. 18). In particular, Jung et al. have reported impaired glucose tolerance and lower insulin levels in β-cell specific Atg7 knockout mice.[19] This was accompanied by vacuolar changes, mitochondrial swelling and endoplasmic reticulum distension. Similar findings were reported by Ebato and coll, who confirmed glucose intolerance and blunted insulin secretion in Atg7 knockout rodents.[16] In addition, they observed that high-fat diet stimulated β-cell autophagy in control mice, but induced profound deterioration in Atg7 deficient animals, again suggesting that maintained autophagy is important for β-cell adaptation to metabolic insults.

There is therefore growing evidence that autophagy plays an important role in the pathophysiology of the pancreatic β-cells. Whereas more work is needed to better understand the underlying mechanisms, it is suggested that preservation of the β-cell can require remedies directed at multiple cell-death pathways.

References

1. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005; 365:1313-33.
2. Marchetti P, Dotta F, Lauro D, Purrello F. An overview of pancreatic beta-cell defects in human type 2 diabetes: Implications for treatment. Regul Pept 2008; 146:4-11.
3. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52:102-10.
4. Meier JJ. Beta cell mass in diabetes: a realistic therapeutic target? Diabetologia 2008; 51:703-13.
5. Todde V, Veenhuis M, van der Klei IJ. Autophagy: principles and significance in health and disease. Biochim Biophys Acta 2009; 1792:3-13.
6. Cuervo AM. Autophagy: many paths to the same end. Mol Cell Biochem 2004; 263:55-72.
7. Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 2007; 8:931-7.
8. Eisenberg-Lerner A, Bialik S, Simon H-U, Kimchi A. Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death Differ 2009; 16:966-75.
9. Codogno P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ 2005; 12:1509-18.
10. Maffucci T, Nrancaccio A, Piccolo E, Stein RC, Falasca M. Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation. EMBO J 2003; 22:4178-489.
11. Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L, et al. Cell death modalities: classifcation and pathophysiological implications. Cell Death Differ 2007; 14:1237-43.
12. Bredesen DE, Rao RV, Mehlen P. Cell death in nervous system. Nature 2006; 443:796-802.
13. Masini M, Bugliani M, Lupi R, del Guerra S, Boggi U, Filipponi F, et al. Autophagy in human type 2 diabetes pancreatic beta-cells. Diabetologia 2009; 52:1083-6.
14. Marco Bugliani, Matilde Masini, Robin Liechti, Lorella Marselli, Ioannis Xenarios,Ugo Boggi, et al. The direct effects of tacrolimus and cyclosporin A on isolated human islets: a functional, survival and gene expression study. Islets 2009; 2:103-7.
15. Rutter GA, da Silva Xavier G, Leclerc I. Roles of 5-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. Biochem J 2003; 375:1-16.
16. Ebato C, Uchida T, Arakawa M, Komatsu M, Ueno T, Komiya K, et al. Autophagy is important in islet homeostasis and compensatory increase of beta-cell mass in response to high fat diet. Cell Metab 2008; 8:325-32.
17. Cnop M, Grupping A, Hoorens A, Bouwens L, Pipeleers-Marichal M, Pipeleers D. Endocytosis of low-density lipoprotein by human pancreatic beta cells and uptake in lipid-storing vesicles, which increase with age. Am J Pathol 2000; 156:237-44.
18. Hartley T, Brumell J, Volchuck A. Emerging roles for the ubiquitin-proteasome system and autophagy in pancreatic beta-cells. Am J Physiol Endocrinol Metab 2008; 296:1-10.
19. Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, Tanaka K, et al. Loss of autophagy diminishes pancreatic beta-cell mass and function with resultant hyperglycemia. Cell Metab 2008; 8:318-24.