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A role for Glucagon-Like Peptide-1 in the regulation of β-cell autophagy

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Abstract

Autophagy is a highly conserved intracellular recycling pathway that serves to recycle damaged organelles / proteins or superfluous nutrients during times of nutritional stress to provide energy to maintain intracellular homeostasis and sustain core metabolic functions. Under these conditions, autophagy functions as a cell survival mechanism but impairment of this pathway can lead to pro-death stimuli. Due to their role in synthesising and secreting insulin, pancreatic β-cells have a high requirement for robust degradation pathways. Recent research suggests that functional autophagy is required to maintain β-cell survival and function in response to high fat diet suggesting a pro-survival role. However, a role for autophagy has also been implicated in the pathogenesis of type 2 diabetes. Thus, the pro-survival vs pro-death role of autophagy in regulating β-cell mass requires discussion. Emerging evidence suggests that Glucagon-Like Peptide-1 (GLP-1) may exert beneficial effects on glucose homeostasis via autophagy-dependent pathways both in pancreatic β-cells and in other cell types. The aim of the current review is to: i) summarise the literature surrounding β-cell autophagy and its pro-death vs pro-survival role in regulating β-cell mass; ii) review the literature describing the impact of GLP-1 on β-cell autophagy and in other cell types; iii) discuss the underlying mechanisms.

Highlights

Pancreatic β-cell mass is regulated by autophagy
Stimulation of autophagy by GLP-1 promotes β-cell survival
GLP-1 impacts on autophagy in other cell types
Uncovers a potential therapeutic pathway to modulate β-cell mass

Keywords

Glucagon-like peptide-1
Autophagy
Pancreatic β-cells

Abbreviations

Akt: protein kinase B; Ambra1: Autophagy And Beclin 1 Regulator 1; AMPK: 5' AMP-activated protein kinase; Atg: autophagy related proteins; CAMKK: Ca^{2+}/calmodulin-dependent protein kinase kinase; DFCP1: double FYVE-containing protein 1; DPP4: dipeptidyl peptidase-4; ER stress: endoplasmic reticulum stress; FIP200: Focal adhesion kinase family interacting protein of 200 kDa; FFA: free fatty acids, GFP: green fluorescent protein; GLP-1: glucagon-like peptide 1; HFD: high fat diet; HOPS: homotypic fusion and vacuole protein sorting; IAPP: islet amyloid polypeptide; JNK: c-jun N-terminal kinase; LAMP-2: lysosome-associated membrane glycoprotein-2; LC3 : microtubule-associated protein 1 light chain 3 beta; LMP: lysosomal membrane permeabilization; 3-MA: 3-methyladenine; MCOLN1: mucolipin 1; mRFP: monomeric red fluorescent protein; mLST8: mTOR associated protein subunit LST8; mTORC1: mammalian target of rapamycin (mTORC1); PI3P: phosphatidylinositol 3-phosphate; PtdIns3K: class III phosphatidylinositol 3-kinase; Rheb: ras homolog enriched in brain; SNARE: soluble N-ethylmaleimide sensitive factor attachment protein receptor; Tfeb: transcription factor EB; TSC: tuberous sclerosis complex; ULK-1/2: uncoordinated-51-like kinase 1 or 2; UVRAG: UV radiation resistance-associated gene protein; Vsp: vacuolar protein sorting; WIPI: WD repeat domain phosphoinositide-interacting protein-1.

Contents

1. Introduction

Macroautophagy (hereafter referred to as autophagy) is a highly conserved intracellular recycling pathway which targets cytosolic components for lysosomal degradation. Classically considered an essential mechanism to promote cell survival during nutrient deprivation, recycling of damaged organelles / proteins via autophagy releases nutrients and metabolites for energy production and biosynthesis to maintain cellular function and promote cell survival [1,2]. Under basal conditions when adequate nutrients are available, autophagy is inhibited although some constitutive activity remains to maintain basic cell function, removing unwanted proteins, organelles and foreign matter [3]. Autophagy can also be stimulated in response to nutrient excess to remove toxic aggregates or in response to increased protein synthesis to remove unfolded proteins, again to promote cell survival [1-4]. Thus, autophagy is primarily a cell survival mechanism acting to promote cellular homeostasis [4]. However, when exacerbated for long periods or when deregulated, autophagy can promote cell death. Hence, autophagy has been described as the 'double-edged sword' with the ability to switch from a pro-survival to pro-death role depending on the cellular environment [1-4].

2. Molecular mechanism of autophagy

The degradation of cytosolic components via autophagy involves complex signalling pathways with the interplay of more than 30 autophagy-related (Atg) proteins which are summarised below (Figure 1). For a comprehensive review of these pathways readers are directed to [1,5,6].

Autophagy is characterised by the formation of autophagosomes which surround and capture cytosolic components destined for recycling and their subsequent fusion with lysosomes to promote degradation and recycling of the contents [1-4]. It can be divided into four main stages: Initiation, Nucleation, Elongation / Completion, and Fusion / Degradation.

i) *Initiation*: This stage is dependent on the activation state of Uncoordinated (Unc)-51-like kinase 1 or 2 (ULK-1/2) which is regulated by mammalian target of rapamycin (mTORC1) and 5' AMP-activated protein kinase (AMPK). Starvation causes activation of ULK-1/2 via the activation of AMPK either through inactivation of mTORC1 or directly [7,8]. Under these conditions, autophagy is initiated.

ii) *Nucleation*: Activation of ULK-1/2 in turn activates the class III phosphatidylinositol 3-kinase (PtdIns3K) complex consisting of Beclin 1, Vsp15, Vsp34, Atg14L and Ambra1, which is critical for formation of the phagophore via the localisation of phosphatidylinositol 3-phosphate (PI3P) and subsequent recruitment of key proteins [9,10].

iii) *Elongation / Completion*: Once formed, the phagophore is elongated to generate the autophagosome which encaptures the cargo targeted for degradation. This stage is dependent on two ubiquitin-like degradation systems Atg16L1-Atg5-Atg12 and phosphatidylethanolamine (PE)-LC3, in addition to the PtdIns3K complex to mediate localisation of key proteins such as LC3-PE to the expanding phagophore membrane [9,11-13].

iv) *Fusion / Degradation*: Fusion of the closed autophagosome with lysosomes to form the autolysosome is dependent on several proteins including homotypic fusion and vacuole protein sorting (HOPS) complex, syntaxin 17 and lysosome-associated membrane glycoprotein-2 (LAMP-2). Following fusion, lysosomal enzymes are activated resulting in degradation of the contents to generate nutrients and metabolites which can be used as building blocks to promote cell survival [13-15].

The pathway outlined above assumes continuous flux through the pathway whereby the contents marked for degradation meet their end. However, if flux through the pathway is blocked due to loss

of fusion between autophagosomes and lysosomes, this impairment in autophagic signalling can contribute to cell death either via autophagic cell death or by stimulation of other cell death pathways [4,16]. Whether the impairment in flux occurs due to alterations in autophagosomal / lysosomal structures or to changes in signalling varies depending on the stimuli and cell type, and in many instances the underlying cause remains unknown.

3. Technical approaches for monitoring of autophagy

To understand how autophagy is regulated, one must first understand how it's highly dynamic nature is evaluated. Comprehensive guidelines have been published [17] and the commonly used accepted measures are outlined below.

i) *LC3 lipidation* – Lipidation of LC3-I to LC3-II is easily identified as distinct bands via western blotting and is regarded as a marker of increased autophagosomal content. However, an increase in LC3 II can result from both increased autophagy but also due to decreased autophagosomal clearance. Assessing LC3 I-II conversion in the absence or presence of lysosomal inhibitors (such as chloroquine or bafilomycin A1) provides some detail on flux [18,19].

ii) *GFP-LC3 localisation to autophagosomes* – Localisation of exogenously expressed GFP-LC3 to autophagosomes can be detected by a punctate pattern using fluorescence microscopy. Detail on flux can be provided using lysosomal inhibitors [19,20].

iii) *Tandem mRFP/mCherry-GFP LC3* – this technique is specifically designed to measure autophagic flux and is based on the pH differences between the neutral autophagosome and acidic autolysosome and pH sensitivity of GFP (sensitive) and mRFP / mCherry (stable). A red signal alone indicates increased autophagic flux whilst colocalisation of green and red signals indicate a blockage. This technique is deemed the most appropriate to monitor flux *in vitro* [21].

iv) *Accumulation of p62* – The prototypic autophagic receptor p62 can be used as a measure of autophagic degradation since it is incorporated into autophagosomes and degraded in autolysosomes. In most tissues, activation of autophagic flux decreases p62 due to its continued degradation whilst accumulation of p62 is indicative of an impairment in flux [22,23].

v) *Transmission electron microscopy* – this approach allows for visualisation of autophagic structures in relation to other cellular structures in isolated tissues. It is often used to determine the effect of stresses / disease on the autophagic pathway but lacks detail on autophagic flux [24,25].

4. Autophagy in pancreatic β-cells

4.1. β*-cell autophagy under basal conditions*

Owing to their high requirements for insulin synthesis, granule formation and insulin release, pancreatic β-cells require robust degradation mechanisms to remove excess insulin and other granule components to maintain normal function and survival [26]. Early studies identified crinophagy (direct fusion of insulin granules with lysosomes) as a mechanism for regulation of insulin stores [27] but latterly, evidence supports a role for autophagy. Autophagy was reported to be pivitol to the regulation of proinsulin content in β-cells [28] and to the degradation of excess insulin in a mouse model of impaired insulin secretory capacity [29]. Further to this, β-cell specific genetic ablation of the key autophagic protein Atg7 caused accumulation of polyubiquinated proteins and damaged organelles when mice were fed a normal diet (16-20wks) [30,31]. These changes were associated with impaired glucose tolerance, decreased glucose-induced insulin secretion [30,31] and also loss of β-cell mass as a consequence of both increased apoptosis and decreased proliferation [30]. These studies support a role for autophagy in regulating β-cell physiology and survival.

4.2. β*-cell autophagy in response to nutrient excess*

A role for autophagy in the pathogenesis of type 2 diabetes has also been studied using high fat diet (HFD) or nutrient excess as models of type 2 diabetes. β-cell specific Atg7 -/- mice showed severe glucose intolerance when exposed to a HFD for 12 weeks [31]. When compared to wild-type animals,

the Atg7-/- mice had a decreased β-cell mass suggesting that autophagy is essential for the compensatory increase in β-cell mass in response to HFD [31]. This hypothesis is supported by numerous studies on *in vitro* β-cell models which showed that excess free fatty acids (FFA) or glucose increased autophagy [32-40], with reports of increased autophagic flux via the use of lysosomal inhibitors [32-35]. Furthermore, an increase in autophagic flux in response to HFD was shown *in vivo* using a mouse model overexpressing GFP-LC3 [41]. Similar to the results in the Atg7-/- mice, chemical or genetic blockage of autophagy increased β-cell death [32,33,40,41]. These studies support a protective role for autophagy in regulating β-cell mass in response to excess nutrients.

However, several studies have shown that tissue from patients with type 2 diabetes show evidence of deregulated autophagy as evidenced by vacuole overload accompanied by increased autophagosomes [42] and also increased accumulation of p62, a marker of deregulated autophagy [43,44], indicating that autophagy may be impaired in type 2 diabetes. Similar findings were evident in animal models [43,45,45]. Studies on β-cell lines and isolated islets showed that although excess FFA initially stimulated autophagic flux, upon sustained exposure, flux was impaired as evidenced by accumulation of p62 and ubiquitinated proteins, decreased protein degradation and loss of autophagosomal-lysosomal co-localisation representing loss of fusion [44, 47-50]. Pharmacological stimulation of autophagic flux with rapamycin or carbamazepine caused clearance of accumulated autophagosomes and an improvement in cell survival [47-49,51] suggesting that the blockage in autophagic flux promoted cell death.

To further investigate this conflicting evidence, we studied autophagic flux in β-cells exposed to excess FFA and high glucose using tandem mCherry-GFP LC3, a system considered the most robust for determination of autophagic flux [17,21]. Using this technique alongside other measures, we showed that nutrient excess does activate autophagy but if sustained causes deregulation of flux [44]. This promotes the accumulation of irregular, large autophagosomes and also enlarged lysosomes with impaired function. This is consistent with previous studies showing that FFA causes lysosomal dysfunction as evident by increased lysosomal pH and decreased cathepsin activity [47,48,52]. We were also able to show how this deregulation directly led to cell death via the loss of lysosomal membrane integrity and release of lysosomal contents into cytoplasmic compartments [44], a process known as lysosomal membrane permeabilisation (LMP) [53]. A role for this mechanism in fuelling nutrient excess-induced cell death was supported by the inhibition of cell death by a cathepsin D inhibitor. These studies thus suggest that it is dysregulation of the lysosomal compartment that fuels cell death, similar to that described in the renal tubule and neuronal cortex [54,55]. This is consistent with recent studies showing that treatment of lipid overloaded β-cells with photoactivatable acidifying nanoparticles were able to restore lysosomal pH and function, and improve glucose-induced insulin secretion [52] whilst exogenous overexpression of the lysosomal transcription factor EB (Tfeb) [56] to promote lysosomal biogenesis prevented FFA-induced β-cell death [46].

We can therefore conclude that β-cell autophagy is essential for the maintenance of β-cell function / mass where it plays a pro-survival role. However, upon prolonged exposure to nutrient excess, this system becomes overloaded leading to deregulation of autophagic flux and accumulation of dysregulated lysosomes which play a pro-death role (Figure 2). Whilst the activation of autophagy has been proposed to be via the AKT/mTORC1 axis, downstream of endoplasmic reticulum (ER) stress and involve JNK activation [33,36,38,44], the mechanisms underlying the loss of autophagosomal-lysosomal fusion is not clear. Pharmacological inhibition of ER stress reversed the FFA-induced defect in lysosomal function [44] supporting a role for ER stress in preventing fusion. Lipid incorporation into autophagic / lysosomal membranes has also been shown to dictate autophagosomal-lysosomal fusion [57]. Treatment of INS-1E with FFA altered the lipid composition of intracellular membranes which was associated with decreased autophagosomal-lysosomal fusion [50]. This was further exacerbated upon inhibition of stearoyl-CoA desaturase, an enzyme involved

in desaturation of fatty acids, supporting a role for lipid incorporation in regulating fusion. Interestingly, studies on transgenic mice expressing human Islet Amyloid Polypeptide (IAPP) showed that degradation of hIAPP by autophagy was essential for β-cell survival and that upon inhibition of autophagy, hIAPP accumulated leading to decreased β-cell mass in response to a HFD [58,59], thus suggesting that hIAPP may contribute to autophagic-dependent cell death. Further work is required to clearly identify the mechanisms involved.

5. Impact of GLP-1 signalling on autophagy

Glucagon-Like Peptide-1 (GLP-1) is released from intestinal L-cells in response to food intake and acts on multiple tissues to lower blood glucose [60]. This has resulted in the development of GLP-1 receptor agonists (such as exenatide, liraglutide) and dipeptidyl peptidase-4 (DDP4) inhibitors (such as sitagliptin), which prevent the rapid degradation of endogenous GLP-1, for the treatment of type 2 diabetes [61]. Despite their application in the clinic, the mechanisms by which these drugs exert their glucose-lowering effects are not well defined. In pancreatic β-cells GLP-1 acts to enhance glucose-stimulated insulin secretion and has also been shown to increase β-cell mass via increased proliferation and decreased apoptosis [62]. Recent studies have implicated a role for GLP-1 in the regulation of cell survival via alterations in autophagic signalling in both pancreatic β-cells (Table 1) and in other cell types (Table 2).

5.1. β-cell autophagy

Under basal, unstressed conditions GLP-1 receptor agonists did not alter β-cell autophagy [44,63,64]. In contrast, when β-cell autophagy was deregulated by chronic exposure to nutrient excess (FFA, high glucose), co-treatment with GLP-1 receptor agonists further stimulated autophagy as determined by increased LC3 II [44,63,64]. Further analysis in our laboratory using the mCherry-GFP-LC3 system, showed that this increase was due to increased autophagic flux, consistent with the decrease in p62 accumulation [44]. This is supported by an *in vivo* model of HFD that showed that treatment with the DPP4 inhibitor MK-626 increased LC3 II and decreased p62 accumulation [65]. Chemical or genetic blockage of autophagy in β-cell models or *in vivo* partially prevented the GLP-1-induced protection over β-cell survival or function [44,64,65] suggesting that stimulation of autophagic flux by GLP-1 is critical for its protective effects over FFA-induced β-cell dysfunction / death. Autophagic-independent mechanisms may also be at play since exendin-4 improved β-cell function and survival in β-cell specific Atg7 -/- mice [43]. However, it should be noted that this study was performed in the absence of nutrient excess in a setting where GLP-1 does not promote autophagy [44,64,64]. Feeding these β-cell specific autophagy deficient mice a HFD to recapitulate the stresses described above would firmly establish the importance of autophagy in the protective role of GLP-1 over β-cell function and survival.

In addition to the stimulation of autophagic pathways, exendin-4 also improved autophagic flux by preventing the impairment in lysosomal function caused by FFA treatment which was associated with an increase in co-localisation between autophagosomes and lysosomes indicating increased autophagosomal-lysosomal fusion [44]. This is consistent with an independent study on tacrolimus-induced diabetes which showed that exendin-4 improved lysosomal function and restored the impairment in fusion [66]. Restoration of autophagosomal-lysosomal fusion was essential for the protective effects of exendin-4 in this model since chemical inhibition of fusion using bafilomycin A1 prevented the protective effect of exendin-4 over apoptosis [66]. These studies highlight the importance of lysosomal function in preserving β-cell survival and show that GLP-1 acts via restoration of this pathway to protect β-cells from insult. Interestingly, in tacrolimus-induced diabetes, exendin-4 did not stimulate autophagy [66] unlike in FFA treated β-cells which showed an increase in LC3 II and puncta formation [44,63-65]. Similarly, in a model of high fructose feeding, treatment with exendin-4 or sitagliptin decreased LC3 II levels suggestive of inhibition of β-cell autophagy which in turn improved β-cell function and mass [67]. Thus, the effectiveness of GLP-1 in

activating autophagy and / or promoting clearance of autophagosomes by improvement in lysosomal function may be dependent on the underlying mechanism of stress.

5.2. non-β-cell autophagy

Consistent with the studies in β-cells, the impact of GLP-1 on autophagy in other cell types appears to be specific to the underlying stress. In conditions of nutrient excess or in models of type 2 diabetes, GLP-1 stimulates autophagy in a wide range of cell types which serves to improve cell function and survival. Studies in liver showed that GLP-1 receptor agonists increase autophagic flux in both rodents exposed to HFD and hepatocytes treated with FFA, which was associated with decreased steatosis and improved survival [68-72]. Furthermore, Roux-en-Y gastric bypass in diabetic mice increased liver autophagy which was deemed to be secondary to the increase in GLP-1 release [73]. Studies in neurones isolated from type 2 diabetic mice showed that exendin-4 also stimulates autophagy in this cell type and that this effect is pro-survival [74], similar to the cardioprotective effects of exenatide and improved endothelial function with sitagliptin in animal models of diabetes via an autophagy-dependent mechanism [75-77]. Further to this, GLP-1 receptor agonists also increased autophagy in neurons from animal models of Parkinson's disease and spinal injury [78,79] as well as in cardiomyocytes following treatment with the chemotherapy drug doxorubicin [80], which conferred protection from cell death.

In contrast, GLP-1 agonists can also inhibit autophagy. In hepatocytes injured by ischaemia in the presence of HFD, exendin-4 decreased autophagy which was associated with an improvement in hepatocyte survival [71]. This suggests that in this hepatic ischaemic environment, autophagy plays a pro-death role and its inhibition by exendin-4 protects from cell death. Similarly, studies in retinal ganglion cells treated with H_2O_2 to increase oxidative stress showed that treatment with liraglutide decreased autophagy which lead to an improvement in cell survival [81]. Liraglutide also inhibited autophagy in the kidney tubule epithelium when exposed to high glucose which prevented cell death [82].

In all the studies detailed above, whether GLP-1 receptor agonists promoted autophagy or inhibited, the overall impact of GLP-1 signalling was a pro-survival response. However, studies in pancreatic exocrine tissue highlight a potentially detrimental effect of GLP-1 which have been associated with an increase in autophagy. Chronic treatment of mice with both exenatide and sitagliptin caused spontaneous changes to the exocrine pancreas including inflammation, atrophy, apoptosis and autophagy, changes that were consistent with acute pancreatitis [83,84]. Further analysis revealed activation of pancreatic acinar autophagy associated with increased p62 accumulation, indicating impairment of flux [85]. However, a causative link between GLP-1 induced autophagy and cell death / dysfunction was not shown [82-84]. Furthermore, meta-analysis of observational studies from humans treated with GLP-1 receptor agonists or DPP4 inhibitors did not highlight an association with acute pancreatitis [86,87]. Clearly, further work is required to determine the impact of GLP-1 regulated autophagy on survival in the exocrine pancreas.

5.3. Mechanisms underlying GLP-1 regulated autophagy

The mechanisms underlying the impact of GLP-1 receptor agonists on both autophagy and lysosomal function are not yet elucidated. Studies in non-β cells support a role for the AMPK / mTORC1 axis in the activation of autophagic flux, whereby mTORC1 activity is inhibited by GLP-1, leading to autophagic activation [73,74,77]. However, several studies in β-cells have shown that treatment with GLP-1 receptor agonists activate mTORC1 which in turn induces proliferation and prevents apoptosis [88-90]. Consistent with this, treatment of the INS-1E β-cell line with exendin-4 in the presence of FFA caused increased phosphorylation of mTORC1 and its downstream target S6 (Zummo, Arden, unpublished data), suggesting that exendin-4 does not stimulate autophagy via inhibition of mTORC1 in β-cells. Similarly, autophagic activation via the JNK / Beclin1 axis is unlikely since numerous reports show that GLP-1 receptor agonists inhibit JNK signalling [91-93] and since exendin-4 increased LC3 I-II conversion in the presence of a JNK inhibitor [44]. Autophagy can also be activated

directly via AMPK / ULK1 in an mTORC1-independent manner (Figure 1). Studies in non-β cells have shown that starvation stimulates autophagy via an increase in intracellular Ca^{2+} which activates Ca^{2+}/calmodulin-dependent protein kinase kinase (CAMKK), leading to activation of the AMPK / ULK1 axis [94-95]. This is important since GLP-1 receptor agonists increase intracellular Ca^{2+} in β-cells [96] and studies have shown that activation of CAMKK is central to GLP-1 regulated β-cell gene expression [97]. However, the impact of GLP-1 receptor agonists on AMPK signalling is contentious with reports of both increased AMPK activity and also inactivation of the pathway [88,98,99].

It may be that GLP-1 alters autophagic signalling via non-canonical pathways. Recent studies have uncovered a novel role for calcineurin in the regulation of autophagy and lysosomal biogenesis [100,101]. During starvation or in response to exercise, Ca^{2+} is released from the lysosome via mucolipin 1 (MCOLN1), a calcium channel located in the lysosomal membrane. This localised calcium spike activates calcineurin which promotes a direct interaction between calcineurin and Tfeb, leading to dephosphorylation of the transcription factor EB (Tfeb) and its subsequent translocation to the nucleus where it promotes transcription of genes involved in autophagosomal and lysosomal biogenesis [91]. In our study we showed that treatment of INS-1E with exendin-4 in the presence of FFA increased Tfeb translocation and downstream transcription of autophagosomal and lysosomal proteins although the underlying mechanism was not explored [44]. A role for calcineurin in regulating GLP-1-induced autophagy is supported by recent findings that GLP-1 mediates beta-cell proliferation via calcineurin signalling in human β-cells from juvenile donors [102] and also that exendin-4 failed to increase LC3 II in β-cell treated with tacrolimus, an inhibitor of calcineurin [66]. However, it should be noted that exendin-4 was able to improve lysosomal function and restore lysosomal clearance in the model of tacrolimus-induced diabetes [66] and was also able to improve β-cell survival in human islets treated with tacrolimus [103]. Clearly further work is required to determine a role for this pathway in GLP-1 regulated autophagy.

The molecular mechanisms of autophagy and endoplasmic reticulum (ER) stress are heavily interlinked [104]. Several studies have reported that GLP-1 receptor agonists decrease ER stress [44,68,105,106] and resolution of ER stress appeared to be essential for both improved lysosomal function [44] and autophagic activation [33,38,44], supporting a role for this pathway in driving GLP-1 induced changes in autophagy. However, the exact mechanisms at play remain undetermined.

5.4. Future directions

Several outstanding questions remain: i) Firstly, what is the mechanism underlying the defect in lysosomal function in models of T2D? Could this be due to alterations in lipid composition of the lysosomal membrane and could this be altered by manipulating lipid handling? Can targeting of lysosomes improve β-cell function in animal models of T2D? How does GLP-1 act on these mechanisms? ii) Secondly, what are the signalling pathways by which GLP-1 activates autophagy in β-cells? It is via canonical or non-canonical signalling? Is this common to other tissues or is it β-cell specific? iii) Thirdly, how essential is autophagy for the protective effects of GLP-1 over β-cell survival and function in models of diabetes? Are GLP-1 receptor agonists able to improve β-cell survival and function in a β-cell model of high fat diet with simultaneous autophagic knockdown? iv) Finally, What is the impact of GLP-1 on autophagy in exocrine tissue? Is there any evidence that GLP-1 receptor agonists contribute to the development of pancreatitis in these studies?

6. Conclusions

There is increasing evidence for a role of deregulated autophagy in the pathogenesis of type 2 diabetes through its impact on β-cell function and survival. These findings have led to the suggestion that pharmacological targeting of autophagic / lysosomal pathways should be explored for the treatment of type 2 diabetes. However, the complex interplay between autophagy promoting cell survival vs death in varying tissues in response to different stimuli, complicates the use of a common pathway targeted approach. Emerging evidence that GLP-1 can regulate autophagy and that through this mechanism can promote cell survival and improve cell function highlights a novel therapeutic

approach. Elucidation of the underlying signalling pathways / mechanisms involved in the regulation of autophagy by GLP-1 is essential to determine the impact of GLP-1 receptor agonists and DPP4 inhibitors on β-cell autophagy and the consequences on other cell types. Understanding these pathways could identify alternative therapeutic targets to enhance β-cell autophagy in the absence of deleterious effects. This avenue of research thus offers an exciting approach for the treatment of type 2 diabetes.

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8. Conflict of interest

The author has no conflict of interests to declare.

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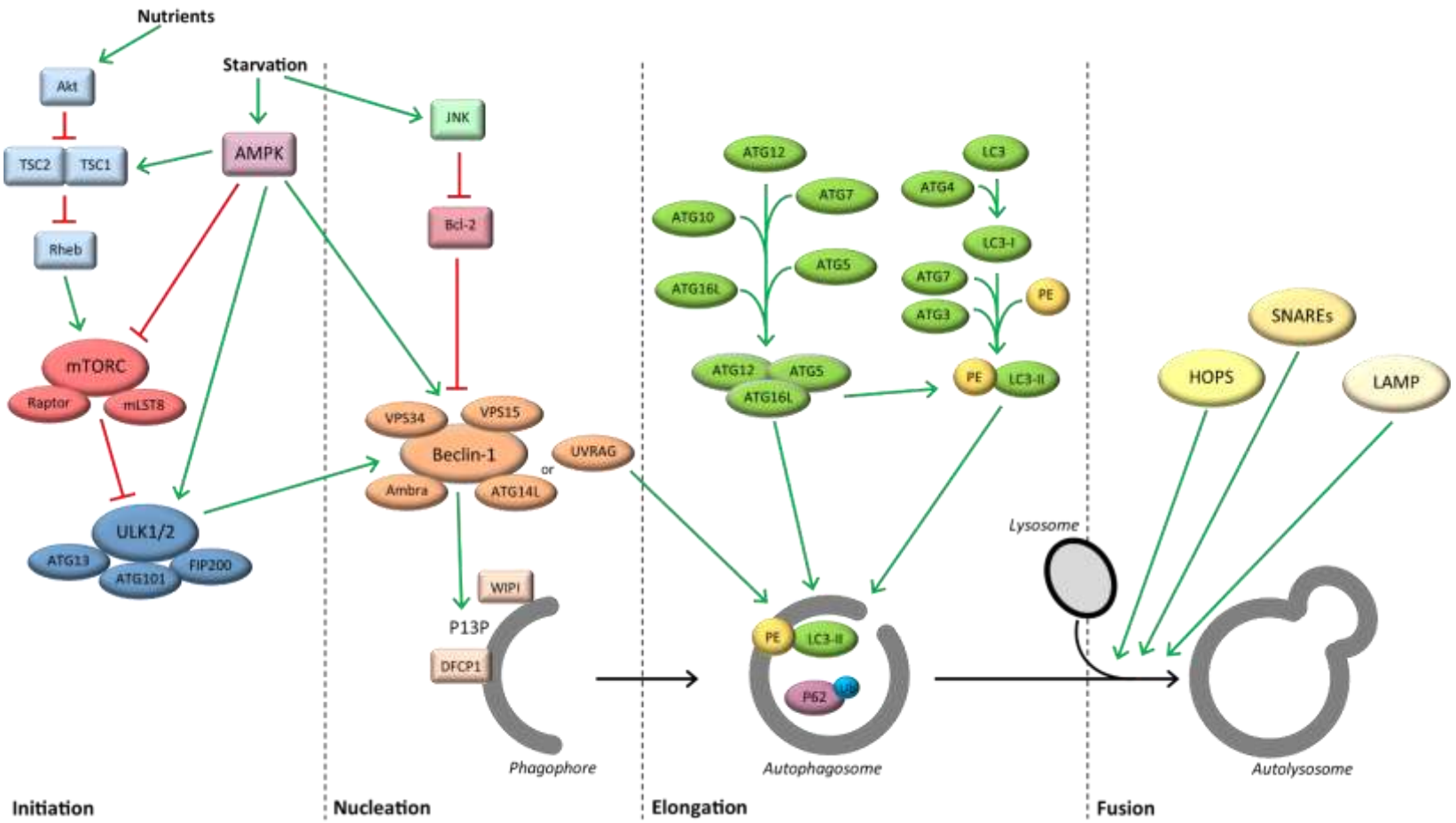
Nutrients
Starvation
Akt
TSC2
TSC1
Rheb
AMPK
JNK
Bcl-2
mTORC
Raptor
mLST8
ULK1/2
ATG13
FIP200
ATG101
VPS34
VPS15
Beclin-1
Ambra
ATG14L
or
UVRAG
WIPI
P13P
DFCP1
Phagophore
ATG12
ATG7
ATG10
ATG5
ATG16L
ATG12
ATG5
ATG16L
LC3
ATG4
LC3-I
ATG7
PE
ATG3
PE
LC3-II
PE
LC3-II
P62
Autophagosome
Lysosome
SNAREs
HOPS
LAMP
Autolysosome
Initiation
Nucleation
Elongation
Fusion

Figure 1: Schematic outlining the main molecular mechanisms and signalling pathways regulating autophagy. Under fed conditions, nutrients, growth factors and insulin inhibit autophagy though the activation of mTORC1 via the class I phosphatidylinositol 3-kinase (PI3K) / AKT / TSC / Rheb pathway. Activation of mTORC1 causes its binding to ULK-1 or 2, preventing autophagy. During starvation, AMPK is activated which initiates autophagy by i) activation of TSC1/2 leading to activation of Rheb and inactivation of mTORC1 which dissociates from ULK-1/2; ii) direct inactivation of mTORC1; iii) direct activation of ULK-1/2. ULK-1/2 is localised to a complex comprised of ULK-1/2, Atg13, Atg101 and FIP200 and its activation marks the initiation of autophagy. The activated ULK-1/2 complex in turn activates the PtdIns3K complex consisting of Beclin1, Vsp15, Vsp34, Atg14L and Ambra1. The activated PtdIns3K complex translocates to the phagophore assembly site (PAS) where it generates PI3P to recruit PI3P binding proteins such as DFCP1 and WIPI2b to PIP3 enriched regions, proteins essential for phagophore formation. Beclin1 can also be activated directly by AMPK and also via the JNK pathway via disruption of the anti-autophagic Bcl-2-Beclin1 complex. The elongation of autophagosomes is dependent on two ubiquitin-like degradation systems, Atg16L1-Atg5-Atg12 and phosphatidylethanolamine (PE)-LC3. Conjugation of Atg proteins to form the Atg-16L1-Atg5-Atg12 complex in the presence of additional Atg proteins mediates LC3-PE formation and its localisation to the expanding phagophore membrane. These processes mediate phagophore elongation to form the autophagosome. In addition to this, replacement of Atg14L with UVRAG in the PtdIns3K complex further promotes autophagosome formation. Fusion of the closed autophagosome with lysosomes to form the autolysosome is dependent on the lipid composition of their membranes and also several proteins including the HOPS complex, the autophagosomal-specific SNARE named syntaxin 17 and LAMP-2 although the precise mechanisms are yet to be identified.

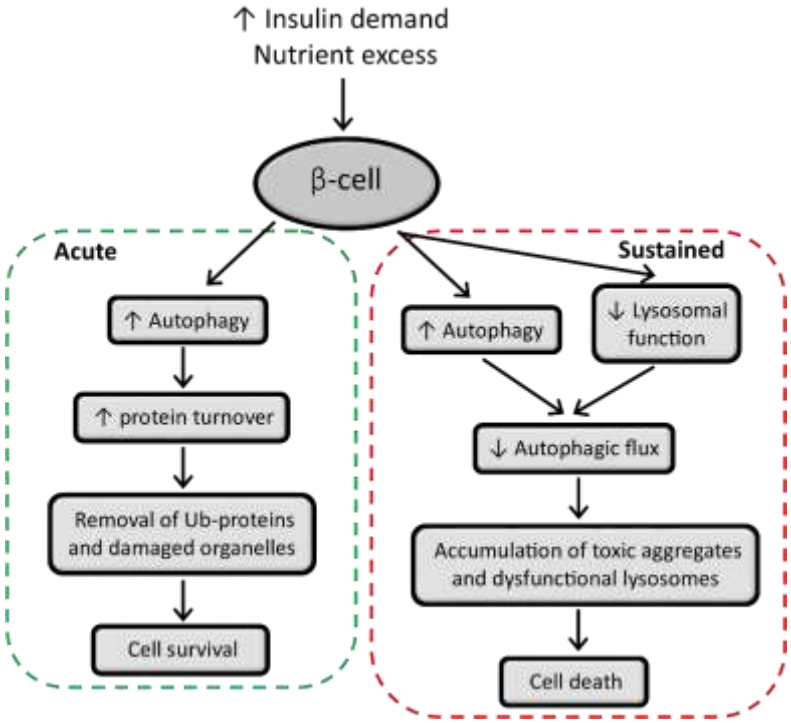

Figure 2: Schematic summarising the autophagic response to nutrient excess in pancreatic β-cells. In the setting of insulin resistance and nutrient excess, the increased demand for insulin and increased circulating nutrients act on the β-cells to activate autophagic flux. This results in an increase in protein turnover and removal of ubiqutinated proteins and damaged organelles from the cytoplasmic compartment. This removal of toxic aggregates promotes cell survival. However, if these stimuli are sustained the autophagic pathway become deregulated. Whilst the excess nutrients continue to activate autophagy and promote formation of autophagosomes, these nutrients also cause damage to the lysosomes causing a decrease in lysosomal function. This leads to loss of fusion between autophagosomes and lysosomes, leading to an impairment in autophagic flux. In the absence of functional autophagy toxic aggregates and dysfunctional lysosomes accumulate, leading to cell death.

Stress	Model	Drug	Effect of GLP-1 receptor agonist / DPP-4 inhibitor		Ref
			on autophagy	on disorder	
None	Atg7 -/- mice	Exendin-4	No effect (Atg7 -/-)	↑ glucose homeostasis ↓ apoptosis	[43]
FFA -/+ HG	INS-1E, islets	Exendin-4	↑ LC3 II, ↓ p62 ↑ flux (tandem LC3) ↑ lysosomal function ↑ AP-lysosome fusion	↑ viability ↓ apoptosis inhibited by Atg7 -/-	[44]
	Ins-1	Liraglutide	↑ LC3 II, AVO's	↑ viability Inhibited by CHQ/3-MA	[63,64]
HFD	C57BL/J6 mice	MK-626	↑ LC3 II, Atg7, ↓ p62	↑ glucose homeostasis ↑ insulin Inhibited by CHQ	[65]
Diabetes	ApolipoproteinE -/- mice HFD + STZ	Liraglutide	↑ AVO's	↑ glucose homeostasis ↓ plasma lipids Inhibited by CHQ	[64]
Tacrolimus	Sprague Dawley, INS-1	Exendin-4	↓ LC3 II, p62, Ub-proteins, AVO's ↑ lysosomal function ↑AP-lysosomal fusion	↑ glucose homeostasis ↑ β-cell mass ↓apoptosis	[66]
Excess fructose	Wistar rats isolated islets	Exendin-4 Sitagliptin	↓ LC3 II, AVO's	↑ glucose homeostasis ↑ β-cell mass ↓ apoptosis	[67]

Table 1: Effect of GLP-1 receptor agonists/DPP-4 inhibitors on pancreatic β-cell autophagy

AVO: autophagic vacuoles; AP: autophagosome; CHQ: chloroquine; FFA: free fatty acids, HFD: high fat diet; HG: high glucose; 3-MA: 3-methyladenine; STZ: streptozotocin; Ub: ubiquitinated proteins.

GLP-1 receptor agonists: Exendin-4, Liraglutide. DPP-4 inhibitors: MK-626, sitagliptin.

Tissue	Stress	Model	Drug	Effect of GLP-1 receptor agonist / DPP-4 inhibitor		Ref
				on autophagy	on disorder	
Liver	none	HepG2	Liraglutide	↑ AVO's	↓ viability, mimicked by rapa	[107]
	FFA	Human hepatocytes	Exendin-4	↑LC3 II, Beclin1, AVO's ↑ flux (LC3 II + Baf)	↓ steatosis ↑ viability	[68]
		L0-2	Liraglutide	↑ LC3 II, ↓p62 ↑ flux (LC3 II + Baf)	↓ steatosis, inhibited by 3-MA	[69,70]
		HuH7	Exendin-4	↑ LC3 II, p62, LC3 puncta ↑ flux (LC3 II + Baf)	↓ cytotoxicity	[71]
	HFD	C57BL/J6 mice	Liraglutide	↑ LC3 II, Beclin1, ↓p62 ↑ flux (LC3 II + CHQ)	↓ steatosis, ↑ glc homeostasis	[68,69, 72]
	Diabetes	Sprague Dawley rat HFD + STZ	Roux-en-Y (↑GLP-1)	↑ LC3 II, Atg7, Beclin1,	↓ steatosis, ↑ glc homeostasis	[73]
	Ischaemia	HuH7 + FFA	Exendin-4	↓ LC3 II, p62, Beclin1, Atg7, LC3 puncta	↓ cytotoxicity	[71]
		C57BL/J6 mice + HFD	Exendin-4	↓ LC3 II	↓ cytotoxicity	[71]
Heart	Diabetes	Zucker Diabetic rat	Liraglutide	↑ LC3 II, LC3 puncta, AVO's, ↓ p62	↑ heart function, inhibited by CHQ	[75]
		Rat cardiomyocytes	Liraglutide	↑ LC3 II, LC3 puncta, ↓ p62	↓ apoptosis, inhibited by CHQ	[75]
	Doxorubicin	Cardiomyocytes	Exenatide	↑ LC3 II, Atg7, Atg5, Beclin1, LC3 puncta	↓ cell death, inhibited by Baf	[80]
		Sprague Dawley rats	Exenatide	↑ LC3 II, Beclin1, ↓ p62	↓ apoptosis	[80]
Kidney	High glucose	HK-2 cell line	Liraglutide	↓ LC3 II, Beclin-1	↑ viability	[82]
Neuronal	Diabetes	Goto-Kakizaki mice	Exendin-4	↑ LC3 II, ↓p62	↓cell death	[74]
	Parkinson's	SD rat + 6-OHDA lesion	DA-JC1	↑ Beclin-1	↑ neuronal function	[78]
	Spinal injury	Sprague Dawley rat	Exendin-4	↑ LC3 II, Beclin-1, LC3 puncta	↑ cell survival	[79]
	Oxidative stress	SH-SY5Y + rotenone	Liraglutide Exenatide	↑ Atg7, Atg3	↑ viability	[108]
		RGC-5 + H_2O_2	Liraglutide	↓ LC3 II, Beclin1, p62, LC3 puncta	↓ cell death, inhibited by rapa	[81]
Monocytes	Obesity	Human monocytes from lean vs obese	Exendin-4 (in vitro)	↑ LC3 II in lean but not obese	↓ foam cell in lean ↑ foam cell in obese	[109]
Endothelial	High glucose	Rat EPC's	Sitagliptin	↑ LC3 II, LC3 puncta, ↓p62	↑ function, survival, inhibited by 3-MA.	[77]
	Diabetes	Zucker Diabetic rat	Sitagliptin	↑ LC3 II, ↓p62	↓ endothelial damage,	[76]
Exocrine pancreas	HFD	C57BL/J6 mice	Exenatide, Sitagliptin	↑ AVO morphology	↑ apoptosis, ↑ necrosis	[83,84]
	Diabetes	SD rats on HFHSD + STZ	Exenatide	↑ LC3 II, p62	B-cell atrophy, ↑ apoptosis	[85]

Table 2: Effect of GLP-1 receptor agonists/DPP-4 inhibitors on autophagy in other cell types.

AVO: autophagic vacuoles; Baf: Bafilomycin; CHQ: chloroquine; EPC's: endothelial progenitor cells; FFA: free fatty acids, glc: glucose; HFD: high fat diet; HFHSD: high fat high sugar diet; 6-OHDA: 6-hydroxydopamine, 3-MA: 3-methyladenine; rapa: rapamycin; SD rat: Sprague Dawley rat; STZ: streptozotocin.

GLP-1 receptor agonists: Exendin-4, Exenatide, Liraglutide. DPP-4 inhibitors: sitagliptin. Dual GLP-1/GIP agonist: DA-JC1.

Unshaded rows: GLP-1-induced autophagy, pro-survival; light shaded bars: GLP-1-inhibited autophagy, pro-survival; dark shaded bars: GLP-1-induced autophagy, pro-death.