Chapter 1

Autophagy in Cell Fate and Diseases

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Abstract

Autophagy pathway has been one of the hot topics during the last decade. From a general notion about its cellular role, autophagy becomes a more sophisticated phenomenon with significant implications in cellular homeostasis. Consequently, autophagy represents an emerging new factor in human diseases. Despite its general task, the bulk degradation of cellular constituents during starvation settings, autophagy possesses important cross talk and interrelationships with several cellular processes such as apoptosis and senescence, among others. This entire panorama gives us a complex but exciting scenario. Consequently, with the aim of encompassing the whole spectrum, in this chapter, we review three main topics: autophagy as a cellular process; autophagy in cell fate; and autophagy in disease. We discuss the emerging role of selective type of autophagy to avoid apoptosis or necrosis and the novel relationship between autophagy and senescence to understand the real extent that autophagy pathway has over cell fate. Finally, we briefly describe the current trends on autophagy in human pancreatic diseases and its role in cancer cell metabolism.

Keywords: VMP1, zymophagy, senescence, acute pancreatitis, pancreatic cancer

1. Introduction

Autophagy is a highly regulated cellular pathway for degrading long-lived proteins and is the only known pathway for clearing cytoplasmic organelles. Autophagy is a major contributor to maintain cellular homeostasis and metabolism.

Autophagy is an evolutionarily conserved and highly regulated lysosomal pathway that degrades macromolecules (e.g., proteins, glycogen, lipids, and nucleotides) and cytoplasmic

organelles [1-3]. This catabolic process is involved in the turnover of long-lived proteins and other cellular macromolecules and it might play a protective role in development, aging, cell death, and defense against intracellular pathogens [4,5]. Moreover, autophagy has been linked to a variety of pathological processes such as neurodegenerative diseases and tumorigenesis, which highlights its biological and medical importance [6,7].

Although autophagy was first identified in mammalian liver upon glucagon treatment approximately 50 years ago, its molecular understanding began only in the past decade, largely based on the discovery of the autophagy-related genes (ATGs) by genetic analyses in yeast. Since the discovery of the yeast ATG proteins, autophagosome formation has been dissected at the molecular level, but a lot of questions about this pathway remain unanswered. In mammalian cells, the sequential association of at least a subset of the ATG proteins leads to the assembly of the pre-autophagosome structure (PAS). PAS formation also requires PtdIns3P generation and it is thought that this lipid is present in specialized subdomains of membranes where the PAS is assembled and autophagosomes are generated.

One of the autophagy-related proteins is VACUOLE MEMBRANE PROTEIN 1 (VMP1), whose expression triggers autophagy in mammalian cells even under nutrient-rich conditions. Conversely, autophagy is completely blocked in the absence of VMP1. VMP1 is required for the biogenesis of autophagosomes in mammalian cells in all conditions underscoring its upstream regulatory function in autophagy. Importantly, VMP1 is also expressed early during the onset of several pathologies including diabetes mellitus, pancreatitis, and pancreatic cancer.

The presence of autophagy has been described in dying acinar cells in tissue from human diseases. Though controversy exists about whether autophagy could be at the same time a survival cell response and a programmed cell death pathway, there is no discussion that autophagy has the power to change the fate of a cell. From an energetic homeostasis point of view, autophagy is a major cellular response against starvation condition and plays key roles in other cellular stress conditions such as oxidative damage. In those cases, autophagy is not only a way to keep energy and nutrition but also a specific mechanism of cell adaptive response.

Increasing evidence suggests that autophagy may influence the pathogenesis of human diseases including cancer, neurodegenerative diseases, inflammatory diseases, and metabolic diseases. Several inherited myopathies are associated with aberrant autophagy. Recent investigations have explored the functions of autophagy in the pathogenesis of metabolic disorders such as diabetes, insulin resistance, and obesity, and the progression of aging.

It is possible to think that different types of autophagy may be involved in the initial cellular events induced by the noxa. Autophagic processes might be triggered in different cells by several diseases and probably they are more complex than we actually understand. These pathways function as adaptive responses that mostly act as protective mechanisms against cell healing. The knowledge of the molecular mechanisms of a sophisticated membrane transport system such as autophagy would provide bases for novel and more rational diagnostic and therapeutic strategies.

2. Autophagy, a complex cell event

Autophagy consists of several sequential steps: induction, autophagosome formation, autophagosome-lysosome fusion, and degradation. Depending on the delivery route of the cytoplasmic material to the lysosome, there are three major types of autophagy in eukaryotes: 1) chaperone-mediated autophagy (CMA), 2) microautophagy, and 3) macroautophagy, hereafter referred to as autophagy [8]. CMA allows the direct lysosomal import of unfolded soluble proteins that contain a particular pentapeptide motif. In microautophagy, cytoplasmic material is directly engulfed into the lysosome at the surface of the lysosome by membrane rearrangement. Finally, autophagy involves the sequestration of cytoplasm into a double-membrane cytosolic vesicle referred to as an autophagosome that subsequently fuses with a lysosome to form an autolysosome for degradation by lysosomal hydrolases [9].

2.1. The autophagic process

Autophagy is characterized by sequestration of bulk cytoplasm and organelles in double-membrane vesicles called autophagosomes that eventually acquire lysosomal-like features [9,10]. The autophagic process is described in Figure 1.

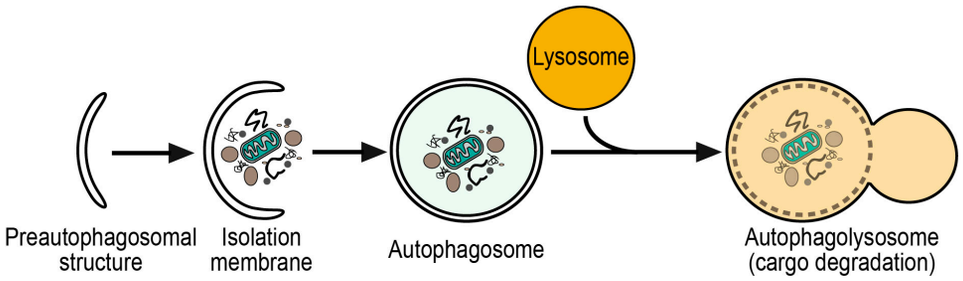

Figure 1. General scheme of autophagic process. During autophagy an isolation membrane forms, invaginates and sequesters cytoplasmic constituents. The edges of the membrane fuse to form the autophagosome. The outer membrane of the autophagosome fuses with the lysosome to form the autolysosome, where the cargo is degraded.

Briefly, sequestration of cytoplasm into a double-membrane cytosolic vesicle is followed by the fusion of the vesicle with a late endosome or lysosome to form an autophagolysosome (or autolysosome). Then, inner membrane of the autophagosome and autophagosome-containing cytoplasm-derived materials are degraded by lysosomal/vacuolar hydrolases inside the autophagosome. The molecular mechanisms underlying the transport and fusion of autophagosomes are just beginning to be understood and through active investigations, several major events involved in the process have recently been clarified including the recycling of lysosomes [11]. In mammalian cells, autophagosome maturation is a prior step for the fusion between autophagosomes and lysosomes. The degradation products, including macromolecules, are then exported to the cytosol for reuse by the cell.

2.2. The autophagosome at a molecular level

Since the discovery of yeast ATG proteins, autophagosome formation has been dissected at the molecular level but a lot of questions about the molecular mechanism underlying this

process remain unanswered. Autophagosomes can be considered unique organelles because they do not contain marker proteins of other subcellular compartments [12]. In mammalian cells, the sequential association of at least a subset of the ATG proteins leads to the assembly of the PAS that is believed to be the site where the precursor structure of the autophagosomes, the phagophores, are generated [13]. The PAS and phagophore formation also requires phosphatidylinositol 3-phosphate (PI3P) [14] and it is believed to be associated to specific subdomains of the endoplasmic reticulum (ER), termed omegasomes [15,16]. Among the key mediators initiating autophagosome formation, there is a set of evolutionarily conserved ATG gene products: the kinase-containing Ulk1/2 complex (ATG1 in yeast), the Class III phosphatidylinositol 3-kinase (PI3K) complex (composed by BECN1/ATG6-hVps34, hVps15 and ATG14L), the ubiquitin-like conjugation systems leading to the formation of the ATG5–ATG12–ATG16L1 complex, and the LC3/ATG8 phosphatidylethanolamine-conjugate (e.g., LC3-II) [17]. A second group of ATG proteins, which does not have orthologous group in yeast, has also recently emerged and appears to play a key role in regulating autophagy in high eukaryotes. One of these proteins is the transmembrane protein VMP1, whose expression triggers autophagy in mammalian cells even under nutrient-rich conditions [18,19]. Conversely, autophagy is completely blocked in the absence of VMP1 [18].

The autophagosome formation process is composed of isolation membrane nucleation, elongation, and completion steps. In mammals, the Class III PI3K plays an essential role in isolation membrane nucleation during autophagy [20]. The Class III PI3K is associated with BECN1/ATG6 and p150, the homolog of Vps15 (phosphoinositide-3- kinase regulatory subunit 4), to form the PI3K complex. This kinase catalyzes the generation of PI3P on the autophagosomal membrane, favoring the localization of other ATG proteins to the PAS during autophagosomal formation. The autophagosome nucleation system is ATG12-ATG5-ATG16L, which is a ubiquitin-like protein conjugation system essential for the formation of the PAS. ATG12 is conjugated to ATG5 [21]. E1-like ATG7 activates the carboxyl-terminal glycine residue of ATG12 through a high-energy thioester bond in an ATP-dependent manner. The ATG12-ATG5 conjugate further interacts with ATG16L1 to form a ~350 kDa multimeric ATG12-ATG5-ATG16L protein complex through the homo-oligomerization of ATG16L [22]. Another ubiquitin-like protein conjugation system is the modification of LC3 (a mammalian homolog of ATG8) by the phospholipid phosphatidylethanolamine (PE) [22], an essential process for the formation of autophagosomes. The cytosolic form of LC3 (LC3-I) is cleaved by cysteine protease ATG4 and then conjugated with PE by ATG7 and ATG3. This lipidated LC3 (LC3-II) then associates with newly forming autophagosome membranes. LC3-II remains on mature autophagosomes until its fusion with lysosomes [23, 24]. The conversion of LC3-I to LC3-II is thus well known as a marker of autophagy (Figure 2). However, the increase of LC3-II alone is not enough to show autophagy activation because the inhibition of LC3-II degradation in the lysosome by the impaired autophagy flux can also cause its accumulation.

While the origin of autophagic vacuoles remains disputable, several hypotheses have been proposed for the source of autophagosomal membrane during autophagosome formation. The first hypothesis is “de novo” formation of autophagosome by ATG9 reservoirs. In the second hypothesis, various organelles such as ER, mitochondria, and plasma membrane are used as

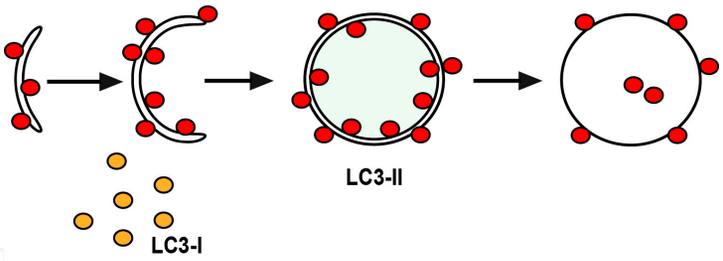

Figure 2. During autophagy the cytosolic form of LC3 (LC3-I) undergoes C-terminal proteolysis and lipidation (LC3-II) and translocates to the autophagosomal membrane. LC3 is currently used as a specific marker of autophagy.

an origin for the formation of the phagophore. Recently, cup-shaped structures called omegasome, a discrete region of the ER, were identified as a platform for autophagosome formation [25]. The ATG5 complex, LC3, and ULK1 have been shown to recruit into the omegasome after starvation, and ATG5- and LC3-positive membranes seem to emerge from the omegasome. It was also observed that omegasomes form in close proximity to the PI3K-containing vesicles which may synthesize the PI3P. This hypothesis is also supported by a notion of a physical association between the ER and early autophagic membranes [26].

2.3. Autophagy induction

Basal autophagy in unstressed cells is kept down by the action of the mammalian target of rapamycin complex 1 (mTORC1). Key upstream regulators of mTORC1 include the class I phosphoinositide 3-kinase-Akt pathway which keeps mTORC1 active in cells with sufficient growth factors and the AMP-activated protein kinase (AMPK) pathway that inhibits mTORC1 upon starvation and calcium signals [27,28].

Under stress conditions such as amino acid starvation, autophagy is strongly induced in many types of cultured cells. The effects of individual amino acids differ in their abilities to regulate autophagy. Amino acids including Leu, Tyr, Phe, Gln, Pro, His, Trp, Met, and Ala suppress autophagy in ex vivo perfused liver [29]. However, such profiles depend on cell types showing their different amino acid metabolisms in tissues. The questions on how cells sense amino acid concentration and physiological significance of autophagy regulation by amino acid starvation are not fully understood yet. It has been demonstrated that amino acid signaling pathways exist, which involve activation of mTORC1 and the subsequent regulation of the Class III PI3K. mTORC1 is involved in the control of multiple cell processes in response to changes in nutrient conditions [30]. Especially, mTORC1 requires Rag GTPase, Rheb, and Vps34 for its activation and subsequent inhibition of autophagy in response to amino acids [31, 32]. Energy levels are primarily sensed by AMPK, a key factor for cellular energy homeostasis. In low energy states, AMPK is activated and the activated AMPK then inactivates mTORC1 through TSC1/TSC2 and Rheb protein [33].

Thus, inactivation of mTORC1 is essential for the induction of autophagy and plays a central role in autophagy. In addition to amino acid signaling, hormones, growth factors, and many other factors including Bcl-2 [34] have also been reported to regulate autophagy. But, not all autophagy signals are transduced through mTOR signaling. A recent study showed that small-

molecule enhancers of the cytostatic effects of rapamycin (called SMERs) induce autophagy independently of mTOR [35]. Activities of the ULK1 are regulated by mTOR depending on nutrient conditions. Under growing and high-nutrient conditions, active mTORC1 interacts with ULK1 and phosphorylates ULK1 and mATG13 and, thus, inhibits the membrane targeting of the ULK1. During starvation condition, on the other hand, inactivated mTORC1 dissociates from ULK1 and results in the ULK1 complex formation (ULK1-mATG13-FIP200-ATG101) leading to autophagy induction [36].

2.4. The autophagy-related protein Beclin 1 (BECN1)

BECN1 (former Beclin 1), the mammalian ATG6, is a haploinsufficient tumor suppressor and an important effector of autophagy. BECN1 is a subunit of the PI3K complex, the action of which is antagonized by Bcl-2 [36,37]. BECN1 contains a BH3 domain that mediates its interaction with Bcl-2 [38,39]. The interaction between Bcl-2 and BECN1 leads to inhibition of autophagy by interfering with the formation and activity of the autophagy promoter complex, BECN1- PI3K [40] (Figure 3).

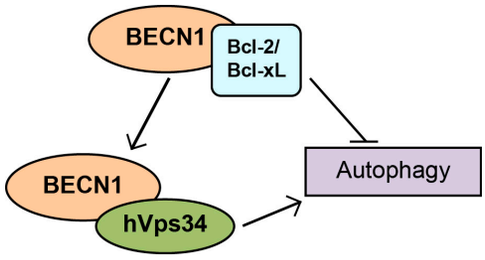

Figure 3. BECN1 is an effector of autophagy whose interaction with Bcl-2 inhibits its role as autophagy promoter. BECN1 and the PI3K complex, where hVP34 is the active subunit, are necessary for PI3P production over the autophagic structures and the consequent recruitment of additional ATG proteins.

2.5. The Vacuole Membrane Protein 1 (VMP1)

The pancreatitis-associated protein named VMP1 is a transmembrane protein with no known homologs in yeast. VMP1 was found searching for new molecules that were differentially expressed during acute pancreatitis [41]. VMP1 expression is induced by mutated K-Ras in pancreatic cancer cells [42] and by hyperstimulation of Gq-coupled cholecystokinin receptor (CCK-R) in pancreatic acinar cells during acute pancreatitis [43]. In the adult normal pancreas, VMP1 expression is not detectable but it is highly induced early during experimental acute pancreatitis and its expression levels correlate with morphological features resembling autophagy [44]. Moreover, VMP1 expression can be found in pancreatic acinar cells from rats developing spontaneous chronic pancreatitis (WBN/Kob rats) [45] and it is rapidly and highly expressed in experimental diabetes [45-47]. Finally, gemcitabine (2,2-difluorodeoxycytidine), the standard chemotherapy for the treatment of advanced pancreatic cancer, induces VMP1 expression in human pancreatic cancer cells [48-50]. We demonstrated that VMP1 expression triggers autophagosome formation in mammalian cells even under nutrient-replete conditions [18,19]. Remarkably, VMP1 pancreas-specific transgenic expression in mice promotes auto-

phagosome formation in acinar cells. Therefore, VMP1 expression may be involved in autophagy induction during acute pancreatitis, a disease defined as pancreas self-digestion. Furthermore, VMP1 is the only human-disease-inducible ATG-protein described so far.

VMP1 interacts with BECN1 through its hydrophilic C-terminal region (VMP1-ATGD) that is necessary for early steps of autophagosome formation. Hierarchical analyses in mammalian cells shows that VMP1 along with ULK1 localizes in the autophagosome formation site [13]. VMP1-BECN1 interaction is required for the formation of the PI3K complex acting in mammalian autophagy. This complex, which is composed of BECN1-hVps34-ATG14, promotes PI3P generation on autophagosomal membrane, favoring the localization of ATG16L1 and LC3 to the autophagosomal membrane during autophagosome formation [51,52]. The interaction between VMP1 and BECN1 requires the BECN1 domain that binds to Bcl-2 (BECN1-BH3 domain) [51]. During normal growth conditions, Bcl-2 binding to BECN1 is maximal, and when autophagy is induced, this interaction is strongly reduced [53]. VMP1 expression leads to the dissolution of the BECN1-Bcl-2 complex, indicating that VMP1 is involved in driving BECN1 into the autophagic process [38]. Thus, VMP1-BECN1 interaction through the VMP1-ATGD is required for the proper localization of PI3K activity on the autophagosomal membrane during mammalian autophagy, positioning VMP1 as a key regulator of the early steps of autophagosome formation possibly acting as a platform in the autophagosomal membrane.

2.6. Autophagy, a selective process

Early studies suggested that autophagy was a nonselective process in which cytoplasmic structures were randomly sequestered into autophagosomes before being delivered to the mammalian lysosome or the plant and yeast vacuole for degradation. Now, there is growing evidence that unwanted cellular structures can be selectively recognized and exclusively eliminated within cells. This is achieved through the action of specific autophagy receptors such as Nbr1 and p62, which is a ubiquitin-binding protein that interacts with LC3 [10,54]. Thus, excess or damaged organelles, including mitochondria, peroxisomes, lipid droplets, endoplasmic reticulum, and ribosomes, can be specifically sequestered by autophagosomes and targeted to the lysosome for degradation. Importantly, there is growing evidence that selective autophagy subtypes also have a wide range of physiological functions. Selective autophagic pathways target distinct cargoes to autophagosomes including mechanisms for the clearance of aggregated protein and for the removal of dysfunctional mitochondria (mitophagy). In pancreatic cells, autophagy has recently been shown to specifically turn over secretory granules damaged by acute pancreatitis as a protective cellular response [43].

2.7. Zymophagy, a novel selective type of autophagy

The pancreatic acinar cell activates VMP1-mediated autophagy early during acute pancreatitis [44]. Relevant data about the role of autophagy in pancreas were obtained using ElaI-VMP1 mice in which the pancreaatic acinar-cell-specific elastase promoter drives VMP1 expression in pancreas. Pancreases of these transgenic mice show numerous vesicles that stain for endogenous LC3, indicating that VMP1 induces the autophagosome formation and, therefore,

autophagy [18]. Interestingly, ElaI-VMP1 mice do not develop pancreatitis in normal conditions, confirming that autophagosome formation does not induce acute pancreatitis [18]. The immunomagnetic isolation of VMP1-autophagosomes containing zymogen granules from the ElaI-VMP1 transgenic mouse pancreas with acute pancreatitis allowed the discovery of a new type of selective autophagy named zymophagy that functions as an inducible cellular process that recognizes and degrades activated zymogen granules [43].

Zymophagy is characterized by the formation of autophagosomes containing zymogen granules. These organelles mediate the sequestration and degradation of pancreatitis-activated zymogen granules. CCK-R hyperstimulation with cerulein in wild-type animals, a classical model of acute pancreatitis, induced a markedly altered distribution pattern of the secretory granules such as fusion among zymogen granules as well as their fusion with condensing vacuoles. In addition, acinar cells lose their polarity, which results in the relocation of zymogen granules to the basolateral membrane. Surprisingly, ElaI-VMP1 mice subjected to CCK-R hyperstimulation reveal that acinar cells preserve their structure and polarity with negligible or no alteration in vesicular transport. Instead, pancreases from cerulein-treated ElaI-VMP1 mice presented autophagosomes containing zymogen granules displaying a distinct localization to the apical area of the acinar cell. This observation is confirmed using isolated mouse pancreas acini revealing that 15 min after cerulein treatment, zymophagy is detected [43]. The finding of different maturation levels of selective autophagic vesicles as well as the degradation of p62 provide evidence that autophagic flow remains primarily unchanged under CCK-R hyperstimulation [43].

Regarding the pathophysiological relevance of zymophagy during acute pancreatitis, it was demonstrated that zymophagy protects acinar cells from intracellular trypsinogen activation triggered in vivo by experimental pancreatitis induced by cerulein. Upon CCK-R hyperstimulation, wild-type mice developed acute pancreatitis with high amylase and lipase serum levels. On the contrary, enzymatic levels in cerulein-treated ElaI-VMP1 mice were significantly lower compared with wild-type mice. Consistently, ElaI-VMP1 mouse pancreas showed remarkably less macroscopic evidence of acute pancreatitis compared with wild-type animals that showed marked edema and hemorrhage. Histological analyses displayed a high degree of necrosis as well as inflammation in wild-type pancreas with acute pancreatitis. In contrast, neither necrosis nor significant inflammation was seen in cerulein-treated ElaI-VMP1 mice [41,43]. Thus, results obtained in the transgenic animal model showed that zymophagy functions as a protective pathophysiological mechanism against pancreatitis-associated injury.

Upon CCK-R hyperstimulation, acinar cells from wild-type mice showed early cytoplasmic trypsinogen activation, which is a hallmark of pancreatitis pathophysiology. Surprisingly, in acinar cells from ElaI-VMP1 mice, CCK hyperstimulation caused almost no activation of trypsinogen. Microscopic examinations using BZiPAR (rhodamine 110 bis-[CBZ-L-isoleucyl-L-prolyl-L-arginine amide] dihydrochloride), a cell permeable substrate that becomes fluorescent after the cleavage by the protease revealed only few activated granules that highly colocalize with VMP1, showing that zymophagy selectively sequesters the activated zymogen granules. Zymogen activation is an enzymatic chain reaction where initial zymogen granule alterations trigger rapid spread of active trypsin within the acinar cell. We think that the

degradation of early-activated zymogen granules by zymophagy prevents this deleterious event. Interestingly, the inhibition of autophagic flow markedly increased trypsin activity within acinar cells in ElaI-VMP1 mouse pancreases under CCK-R hyperstimulation confirming that zymophagy specifically degrades those zymogen granules that are initially activated by acute pancreatitis [43]. This function is confirmed in the in vivo animal model of acute pancreatitis where the ability of the ElaI-VMP1 mouse developing zymophagy clearly prevents the increment of enzymatic markers of pancreatic damage and morphological changes characteristic of acute pancreatitis.

Analysis of autophagosomes containing zymogen granules magnetically immunopurified from the pancreas of ElaI-VMP1 mice treated with cerulein revealed that, apart from zymogen granules, isolated vesicles contained LC3-II and, notably, strong signals of p62. Moreover, GFP-ubiquitin-transfected acinar cells subjected to CCK-R hyperstimulation showed colocalization between activated granules and ubiquitin aggregates but do not show colocalization between unaffected or normal zymogen granules and ubiquitin, indicating that the ubiquitin system serves as a targeting signal for activated zymogen granules during zymophagy. Therefore, activated zymogen granules are directly or indirectly ubiquitinated for their recognition by autophagic membranes in which ubiquitin acts as a label for selective engulfment. Nevertheless, activated zymogen granules were ubiquitinated upon acute pancreatitis and the VMP1-mediated selective autophagic pathway sequestered these ubiquitinated granules [43]. p62 may function as a cargo receptor during zymophagy. These data demonstrate for the first time that ubiquitin modifications may possess an additional function in acinar cells by promoting the degradation of highly harmful activated zymogen granules [55].

Zymophagy prevents pancreatic acinar cell death induced by CCK-R hyperstimulation [43]. Autophagosome formation inhibition with 3-methyladenine as well as autophagy flux interruption with vinblastine significantly reduced acinar cell survival in a cell model of acute pancreatitis. Moreover, VMP1 downregulation (shVMP1) also significantly decreases acinar cell survival under CCK-R hyperstimulation, showing that VMP1 expression and autophagy is required to prevent acinar cell death in acute pancreatitis. Therefore, VMP1 expression is activated in acinar cells to mediate zymophagy as a protective cellular response against cell death [55].

Furthermore, VMP1 expression and zymophagy are present in human pancreas affected by acute pancreatitis [43,55]. VMP1 is not detectable in human normal pancreas tissue but its expression is activated in human pancreatitis pancreatic specimens and highly colocalized with LC3 in autophagosomes. Moreover, autophagosomes markedly colocalized with zymogen granules. Remarkably, the finding of large autolysosomes without trypsin signal in pancreas of human pancreatitis suggests that affected zymogen granules are eventually degraded by zymophagy during human pancreatitis.

3. Autophagy in cell fate

Though there was controversy about whether autophagy could be at the same time a survival cell response and a programmed cell death pathway, there is none that autophagy has the

power to change the fate of a cell. From an energetic homeostasis point of view, autophagy is a major cellular response against starvation condition. Nevertheless, we have to keep in mind that autophagy plays key roles also in other cellular stress conditions such as oxidative damage [57,58], damaged organelles elimination [59-61], depletion of toxic proteins aggregates [62], host response to microorganisms [61], etc. In those cases, autophagy is not just a way to keep energy and cellular material but a specific cellular stress response. Therefore, it is not difficult to imagine that autophagy could be observed in several cellular life-threatening situations and, then, its pro-survival or pro-death role becomes diffuse. Solid and ineligible is the fact that, independently, its situation-specific role, a determined autophagy process, has a profound impact in the cellular fate.

3.1. Autophagy as nutritional stress response

We must begin our analysis from the most basic and evolutionarily conserved autophagy duty as nutrient stress response. In the starvation context, autophagy plays the first explored, and may be more obvious, task of autophagy, that is, the energy cellular support during nutrient-limiting conditions. Though the bulk degradation and recycling of cytoplasmic portions seem to be a simple event in cellular life, it has major consequences in cell fate and organismal adaptation. As it could be imagined, a proper autophagic flux during a challenging cellular nutritional status might be determinant for the cell survival. Furthermore, the importance of starvation-induced autophagy for cell survival can have beneficial or detrimental consequences to tissue in context dependence. For instance, autophagy could give a life opportunity to cells under urgent energy requirement such as organ starvation, ischemia, hypoxia, etc., being beneficial for a determinate tissue. On the other hand, the same mechanism gives to pancreatic cancer cells enough adaptation to survive in a highly hipoperfunded environment of the pancreatic tumor [63]. This dual behavior could also be observed with AMPK (5' AMP-activated protein kinase) which is one of the master regulators of the cellular energetic homeostasis and a direct autophagy trigger through ULK1 phosphorylation [64]. Similar to what is observed with autophagy, AMPK could be a tumor suppressor, stopping all anabolic process and activating all the catabolic ones including autophagy (probably contributing to the oncogene-induced senescence – see below) but, in other circumstance, it gives tumor high resistance to stress [65].

3.2. Autophagy and cell death

Connections between autophagy and disease have attracted an increasing amount of attention. By morphological studies, autophagy has been linked to a variety of pathological processes and autophagy was associated with cell death. Taking cell death as one major topic in cell fate, there are a large amount of data concerning the complex relationship between autophagy and apoptosis [65,66]. However, this relationship is far from being fully understood and, in many cases, it seems to be context-dependent. This autophagy–apoptosis relationship could be observed from the beginning since they share several inducing factors such as ROS [57], increased levels of cytosolic calcium concentrations [67], oncogenes, and p53 [68], among others (Figure 4A). Among those, the BH3-only proteins have a prominent role. These are pro-

apoptotic and different from the Bcl-2 family proteins which contain only one BH3 (Bcl-2 homology 3) domain [69]. BECN1 is a fundamental protein in autophagic mechanism (as mentioned above). This protein was one of the first described mammalian autophagy-related proteins [70]. BECN1 interacts with proteins of Bcl-2 family (Bcl-2, Bcl-XL and Mcl-1) [71]. Through interaction with the BECN1 BH3 domain, these proteins are able to inhibit BECN1-mediated autophagy [72, 73]. What is more, this interaction makes BECN1 not only a highly relevant autophagy actor but it is indeed also a haploinsufficient tumor suppressor [74]. On the other hand, BH3-only proteins are capable of disruption of Bcl-2-BECN1 interaction inducing apoptosis by Bcl-2 blockade and autophagy by releasing BECN1 [75].

Experimentally, impairment of autophagy in starving cells is able to induce apoptosis or at least a kind of cell death. This autophagy shortage impedes cell proper management of a metabolic stress or, in some cases, such as neurons, clearance of toxic cellular metabolic products. Moreover, in some way, this effect seems to be in both sides since inhibition of apoptosis could also be a potent trigger of the autophagy process. Transgenic depletion of pro-apoptotic molecules such as Bax or Bak or the use in vitro of the pan-caspase inhibitor Z-VAD-FMK were able to strongly induce autophagy and a cell death which is inhibited by autophagy inhibitors [76,77]. This seems to be the rule in most cases, a highly intricate autophagy-apotosis cross talk, where, despite their respective main duties, they act by inhibiting each other (Figure 4A). For instance, in one way, autophagy could reduce the cytosolic concentrations of pro-apoptotic proteins [78]. In the other way, activated caspases are able to cleave some autophagic proteins in which the fragmented products indeed acquired now pro-apoptotic properties [79-83]. Such is the case of ATG5, BECN1, and ATG4D [79-83].

Beyond the central role of ATG5 in autophagy machinery, this protein, in some situations, is able to induce apoptosis by two different ways. As mentioned above, a calpain-mediated cleaved ATG5 translocated to mitochondria, favoring its depolarization and triggering of apoptosis [84]. Moreover, ATG5 can also associate to FADD (Fas-associated death domain) enhancing apoptosis cell death [85].

The autophagic cell death is a programmed alternative to other sorts of cell demise. All of the comments above about the relationship of autophagy with cell death do not imply that autophagy could be by itself a cell death mechanism. Many researchers have suggested a number of times the existence of a really programmed autophagic cell death. Nevertheless, since autophagy is a cell survival pathway, there was concern about whether autophagy was a last cellular attempt to avoid apoptosis or if autophagy-related cell death only occurs in in vitro settings. These controversies were clarified with study of the degeneration of salivary glands during the D. melanogaster embryogenesis [86]. These glands suffered a cell death that is independent of caspases and completely dependent on autophagy [87].

All this intricate cross talk among apoptosis, autophagy, and autophagic cell death makes one rethink the definition of cell death types. Hence, the 2015 Nomenclature Committee on Cell Death made a switch from morphological- to biochemical-based definitions of cell death types [87]. They stated that autophagic cell death will be defined as processes of cell death that were

prevented by the use of pharmacological or genetic tools targeting at least two different components of autophagy machinery [87].

Beyond the molecular mechanism, autophagy is able to modulate the cell death response and, hence, cell fate indirectly. Mitophagy, the most studied form of selective autophagy, is aimed to eliminate damaged mitochondria contributing to its renewing and ROS homeostasis [88]. As mitochondrial impairment is one of most important apoptosis triggering events, elimination of damaged mitochondria could avoid the intrinsic apoptosis program (Figure 4B). Damaged mitochondria and subsequent decrease in the inner mitochondrial transmembrane potential ($\Delta \Psi$) led to leakage of several harmful substances such as ROS and intrinsic apoptotic-pathway-triggered leakage of others such as cytochrome c [88]. In response, damaged mitochondria may be fragmented and ubiquitinated by a PINK-Parkin-dependent mechanism [89]. Then, these damaged organelles are recognized and selectively eliminated by autophagy in order to allow the cell survival [64,83].

It is surprising that autophagy can also interfere with such an uncontrolled process as necrosis. This example was demonstrated to occur during acute pancreatitis with zymophagy [55]. In the cellular basis of this disease, there was a premature intracytoplasmic activation of digestive enzymes [41]. This last activity pushes pancreatic acinar cell to an inevitable necrosis and the catastrophe of the tissue in a sort of chain cascade [41]. Zymophagy, as selective type of autophagy, eliminates the granules where the dangerous zymogens were activated by a ubiquitin recognition mechanism [55]. This protective mechanism avoids the enzymatic autodegradation and subsequent necrosis of the acinar cell [55] (Figure 4B). This event, in fact, has consequences beyond cellular biology since it reduces gland inflammation and contributes to autolimitation of the disease.

3.3. Autophagy and cellular senescence

There exists another unexpected process related to cell fate which is influenced by autophagy: the cellular senescence [90]. This term is used to describe an irreversible (which differentiates it from quiescence) deep arrested status of the cell cycle. The senescence is a cellular alternative mechanism to apoptosis in response to certain stressors including oncogene overexpression. Then, the so-called oncogene-induced senescence is a potent tumor suppressor mechanism against cellular transformation [91-94] and it is the first option that cells have against oncogene activation. Autophagy participated in the transition phase of senescence establishment being part of the TOR-autophagy spatial coupling compartment (TASCC). The TASCC is a cytoplasmic sub-compartmentalization where mTOR is closely associated with lysosomes/autolysosomes fuelled by a constant autophagy flow outside of this area [95]. This activity resulted in an efficient synthesis-degradation coupling that seems to be crucial for senescence. Inhibition of autophagy impairs the senescent progression and it seems to be necessary to reach an intermediate state before the final setting of senescent phenotype. Finally, it was suggested that autophagy during senescence is triggered by the isoform ULK3 instead of ULK1 or ULK2; therefore, it is tempted to hypothethize that a specific type of autophagy may be related to senescence (Figure 4B).

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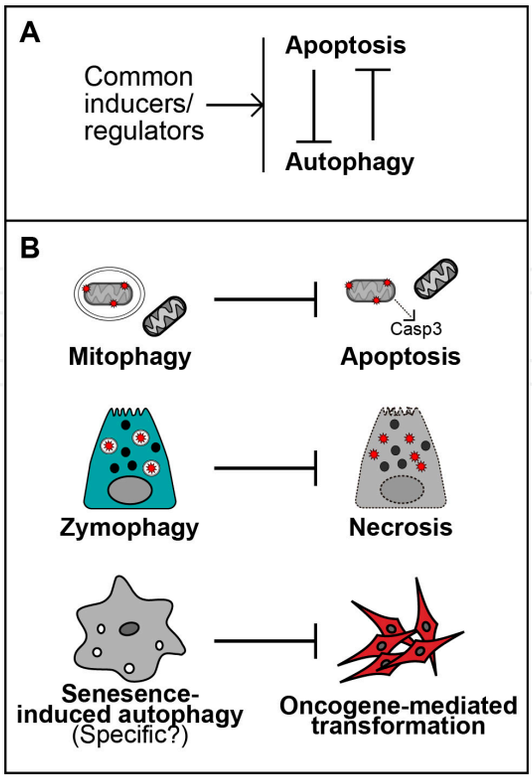

Figure 4. The implication of autophagy in cell fate. A) There is a deep crosstalk between autophagy and apoptosis. They share several common inducers and posses regulatory properties on each other. B) Autophagy is able to modify cell fate and some examples are depicted. Mitophagy eliminates the damaged mitochondria avoiding the apoptosis triggered by intrinsic pathway (upper panel). By the specific elimination of activated zymogen granules, zymophagy prevents the acinar cell damage and the necrosis in pancreas tissue (middle panel). Autophagy participates in the oncogene-induced senescence, a process capable of repress the oncogenic transformation (lower panel).

4. Autophagy in pancreatic diseases

Pathological processes such as pancreatitis and diabetes mellitus as well as cancer cell transformation and also cancer chemotherapy activate autophagy in human tissues and human tumor cells. While human normal pancreatic acinar cells do not have detectable autophagy levels, pancreatic diseases activate autophagy, confirming the relevant role of autophagy in human pancreatic disease. In addition, selective autophagy of pancreatic zymogen granules, zymophagy, has been discovered and characterized as a cell-protective process activated by the disease.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive human malignancies with 2-3% five-year survival rate. It remains a devastating and poorly understood malignancy. Its poor prognosis has been attributed to the inability to make a diagnosis while the tumor is still resectable and a propensity toward early vascular dissemination and spread to regional lymph nodes. Up to 60% of patients have advanced pancreatic cancer at the time of diagnosis and their median survival time is a dismal 36 months. This is due to both the aggressive nature

of the disease, the lack of specific symptoms and early detection tools, and its relatively refractory response to traditional cytotoxic agents and radiotherapy. Moreover, pancreatic cancer cells become more malignant or survive with an extremely poor blood supply. So far, little and contradictory data are available regarding the activity of autophagy and its regulation in pancreatic cancer cells. Experimental evidence pointed at autophagy as a pancreatic cancer cell mechanism to survive under adverse environmental conditions or as a defective programmed cell death mechanism that favors pancreatic cancer cell resistance to treatment.

4.1. Autophagy in cancer cell

Both downregulated and excessive autophagy have been implicated in the pathogenesis of diverse diseases such as certain type of neuronal degeneration, diabetes and its complications, and cancer [96]. Autophagy has also been implicated in cell death called autophagic or type II programmed cell death, which was originally described on the basis of morphological studies detecting autophagic vesicles during tissue involution [97].

Cancer cells in general tend to undergo less autophagy than their normal counterparts at least for some tumors [98,99]. The BECN1 autophagy gene is monoallelically deleted in 40-74% of cases of human sporadic breast, ovarian, and prostate cancer [99]. Heterozygous disruption of BECN1 increases the frequency of spontaneous malignancies and accelerates the development of virus-induced premalignant lesions [99] suggesting that defective regulation of autophagy promotes tumorigenesis. It has been proposed that autophagy suppresses carcinogenesis by a cell-autonomous mechanism involving protection of genome integrity and stability, and a nonautonomous mechanism involving suppression of inflammation and necrosis. On the other hand, autophagy may support the survival of rapidly growing cancer cells that have outgrown their vascular supply and are exposed to an inadequate oxygen supply or metabolic stress. By contrast, excessive levels of autophagy promote cell death [100]. Accordingly, it has been proposed that autophagy plays an important role both in tumor progression and in promotion of cancer cell death [101], although themolecular mechanisms responsible for this dual action of autophagy in cancer have not been elucidated.

It has been suggested that autophagy may be a cancer cell survival response to tumor-associated hypoxia. Tumor hypoxia has been used as a marker of poor prognosis [102]; however, how cancer cells become more malignant or survive with an extremely poor blood supply is poorly understood. When cancer cells are exposed to hypoxia, anaerobic glycolysis increases and provides energy for cell survival but as the glucose supply is also insufficient because of the poor blood supply, there must be an alternative metabolic pathway that provides energy when both oxygen and glucose are depleted [103,104]. Hypoxia in pancreatic cancer has been reported to increase its malignant potential [102]. Proliferating cancer cells require more nutrients than surrounding noncancerous cells do, though nutrition is supplied via functionally and structurally immature neovessels. Because autophagy-specific genes promote the survival of normal cells during nutrient starvation in all eukaryotic organisms, autophagy may react to the cancer microenvironment to favor the survival of rapidly growing cancer cells. LC3 expression in surgically resected pancreatic cancer tissue showed activated autophagy in the peripheral area, which included the invasive border, and concomitantly

shows enhanced expression of carbonic anhydrase [105]. This observation suggests that autophagy may promote cell viability in hypovascularized cancer tissue.

It has also been proposed that autophagy is a cancer cell survival response to tumor-associated inflammation [106]. Cancer-associated inflammation results in promotion of carcinogenesis and resistance to therapy. Several phenotypic alterations observed in cancer cells are a result of inflammatory signals found within the tumor microenvironment [106]. The receptor for advanced glycation end products (RAGE) is an induced inflammatory receptor constitutively expressed on many murine and human epithelial tumor cell lines [107,108] and the highest levels of RAGE expression were observed in murine and human pancreatic adenocarcinoma tumors. Genotoxic and/or metabolic stress lead to modest but reproducible increases in overall expression of RAGE on epithelial cell lines. RAGE expression correlates directly with the ability of both murine and human pancreatic tumor cell lines to survive cytotoxic insult. Targeted knockdown of RAGE significantly increased cell death, whereas forced overexpression promotes survival. Recently, it was reported that the enhanced sensitivity to cell death in the setting of RAGE knockdown is associated with increased apoptosis and decreased autophagy. In contrast, overexpression of RAGE is associated with enhanced autophagy, diminished apoptosis, and enhanced cancer cell viability. Knockdown of RAGE enhances mTOR phosphorylation in response to chemotherapy, thus preventing induction of a survival response. Inhibition of autophagy by means of silencing BECN1 expression in pancreatic cancer cells enhanced apoptosis and cell death [109]. These observations suggest that RAGE expression in cancer cells has a role in tumor cell response to environmental stress through the enhancement of autophagy. However, increased sensitivity to chemotherapeutic agents in RAGE-knockdown pancreatic cancer cells is dependent on ATG5 expression but independent of BECN1 expression [109]. These last findings suggested that the role of autophagy in the resistance to microenvironment insult or in the sensitivity to chemotherapeutic agent is the result of complex molecular pathways in the tumor cell.

On the other hand, repression of autophagy has been suggested as a cancer cell response to prolonged hypoxic conditions. Pancreatic cancer cell response to prolonged hypoxia may consist of inhibition of autophagic cell death. The short isoform of single-minded 2 (SIM2s) is a member of the basic helix-loop-helix family of transcriptional regulators [110] and is upregulated in pancreatic cancer. Microarray studies identified the pro-cell death gene BNIP3 as a target of SIM2s repression. Prolonged hypoxia induces cell death via an autophagic pathway involving the hypoxia-inducible factor 1 (HIF1)-mediated upregulation of BNIP3 [30,111]. Deregulation of both SIM2s and BNIP3 were associated with poor prognostic outcomes [112]. Decreased BNIP3 levels and poor prognosis clearly correlate with elevated SIM2s expression in pancreatic cancer. The loss of BNIP3, either by hypermethylation or by transcriptional repression, was correlated with inhibition of cell death [113, 114], whereas upregulation of BNIP3 sensitized pancreatic carcinoma cells to hypoxia-induced cell death [115]. SIM2s expression, concomitant with its repression of BNIP3, enhanced tumor cell survival under prolonged hypoxic conditions. Recent data linked increased SIM2s expression with enhanced cell survival during hypoxia-stress concomitantly with BNIP3 repression and the attenuation of hypoxia-induced autophagic processes.

Thus, inhibition of autophagic cell death by BNIP3 repression enhances tumor cell survival under prolonged hypoxic conditions [115].

Decreased autophagy in some cancer cells has been related to malignant stages of the disease. Cancer cells in general tend to undergo less autophagy than their normal counterparts supporting the contention that defective autophagic cell death plays a role in tumor progression. Studies of carcinogen-induced pancreatic cancer in animal models have shown that pancreatic adenocarcinoma cells have lower autophagic capacity than premalignant cells [116]. The WIPI protein family, which includes ATG18, the WIPI-1 homolog in S. cerevisiae, was genetically identified as a gene contributing to autophagy [116]. Human WIPI-1a is a member of a highly conserved WD-repeats protein family. hWIPI-1 is linked to starvation-induced autophagy in the mammalian system. Amino acid deprivation triggered an accumulation of endogenous hWIPI-1 protein to large vesicular and cup-shaped structures where it colocalizes with LC3. Starvation-induced hWIPI-1 formation was blocked by wortmannin, a principal inhibitor of PI-3 kinase-induced autophagosome formation [117]. Interestingly, WIPI proteins are linked pathologically to cellular transformation because all human WIPI genes are reportedly expressed aberrantly in a variety of matched human cancer samples. Strikingly, hWIPI-2 and hWIPI-4 mRNA expression is substantially decreased in 70% of matched kidney (10 patients) and 100% of pancreatic (seven patients) tumor samples. The majority of these samples were derived from advanced-stage tumors such as pancreatic adenocarcinomas stages I–IV. Hence, cancer-associated downregulation of hWIPI-2 and hWIPI-4 supports the possibility that decreased autophagic activity is necessary for the malignant stages of pancreatic cancer.

5. Perspectives

There is ample evidence supporting an active role for autophagy in cell physiology and disease. During the last decade, autophagy has turned from a morphological finding to a cellular process involving a membrane transport system and complex molecular machinery. Moreover, since the discovery of ATG genes, there have been many studies on the physiological and pathological roles of autophagy in a variety of autophagy knockout models. However, direct evidence of the connections between ATG gene dysfunction and human diseases has emerged only recently [56]. Here we have overviewed the physiological bases and molecular mechanisms of the autophagic process. We have introduced the reader to a novel autophagy-related transmembrane protein – VMP1 – whose expression triggers autophagy and its role in the cell response to disease. Elucidation of the specific extracellular and intracellular conditions that stimulate autophagy and the linkage of these conditions to either cell survival or cell injury and death in different cell types and during different pathological processes is a rapidly evolving and fruitful field of research. The development of therapies to take advantage of the potential cytoprotective effect of autophagy in several pathologies such as cancer or neurodegenerative diseases is a potentially promising avenue of investigation.

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References

[1] Cuervo AM. Autophagy: in sickness and in health. Trends Cell Biol 2004; 14: 70-77.

[2] Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 2004; 6: 463-477.

[3] Yang Z, Klionsky DJ. An overview of the molecular mechanism of autophagy. Curr Top Microbiol Immunol 2009; 335: 1-32.

[4] Hara T, Nakamura K, Matsui M et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 2006; 441: 885-889.

[5] Qu X, Zou Z, Sun Q et al. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 2007; 128: 931-946.

[6] Pattingre S, Levine B. Bcl-2 inhibition of autophagy: a new route to cancer? Cancer Res 2006; 66: 2885-2888.

[7] Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. Science 2004; 306: 990-995.

[8] Klionsky DJ. Autophagy. Curr Biol 2005; 15: R282-283.

[9] Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000; 290: 1717-1721.

[10] Klionsky DJ, Abdalla FC, Abeliovich H et al. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 2012; 8: 445-544.

[11] Sridhar S, Patel B, Aphkhazava D et al. The lipid kinase PI4KIIIbeta preserves lysosomal identity. EMBO J 2013; 32: 324-339.

[12] Mari M, Tooze SA, Reggiori F. The puzzling origin of the autophagosomal membrane. F1000 Biol Rep 2011; 3: 25.

[13] Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. Autophagy 2010; 6: 764-776.

[14] Xie Z, Klionsky DJ. Autophagosome formation: core machinery and adaptations. Nat Cell Biol 2007; 9: 1102-1109.

[15] Axe EL, Walker SA, Manifava M et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 2008; 182: 685-701.

[16] Hayashi-Nishino M, Fujita N, Noda T et al. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat Cell Biol 2009; 11: 1433-1437.

[17] Madeo F, Tavernarakis N, Kroemer G. Can autophagy promote longevity? Nat Cell Biol 2010; 12: 842-846.

[18] Ropolo A, Grasso D, Pardo R et al. The pancreatitis-induced vacuole membrane protein 1 triggers autophagy in mammalian cells. J Biol Chem 2007; 282: 37124-37133.

[19] Vaccaro MI, Ropolo A, Grasso D, Iovanna JL. A novel mammalian trans-membrane protein reveals an alternative initiation pathway for autophagy. Autophagy 2008; 4: 388-390.

[20] Kihara A, Noda T, Ishihara N, Ohsumi Y. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae. J Cell Biol 2001; 152: 519-530.

[21] He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet 2009; 43: 67-93.

[22] Karanasios E, Stapleton E, Manifava M et al. Dynamic association of the ULK1 complex with omegasomes during autophagy induction. J Cell Sci 2013; 126: 5224-5238.

[23] Ichimura Y, Kirisako T, Takao T et al. A ubiquitin-like system mediates protein lipidation. Nature 2000; 408: 488-492.

[24] Burman C, Ktistakis NT. Regulation of autophagy by phosphatidylinositol 3-phosphate. FEBS Lett 2010; 584: 1302-1312.

[25] Tooze SA, Yoshimori T. The origin of the autophagosomal membrane. Nat Cell Biol 2010; 12: 831-835.

[26] Musiwaro P, Smith M, Manifava M et al. Characteristics and requirements of basal autophagy in HEK 293 cells. Autophagy 2013; 9: 1407-1417.

[27] Hoyer-Hansen M, Jaattela M. AMP-activated protein kinase: a universal regulator of autophagy? Autophagy 2007; 3: 381-383.

[28] Zheng M, Wang YH, Wu XN et al. Inactivation of Rheb by PRAK-mediated phosphorylation is essential for energy-depletion-induced suppression of mTORC1. Nat Cell Biol 2011; 13: 263-272.

[29] Mortimore GE, Poso AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. Annu Rev Nutr 1987; 7: 539-564.

[30] Nobukuni T, Joaquin M, Roccio M et al. Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. Proc Natl Acad Sci U S A 2005; 102: 14238-14243.

[31] Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006; 124: 471-484.

[32] Sancak Y, Bar-Peled L, Zoncu R et al. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 2010; 141: 290-303.

[33] Gwinn DM, Shackelford DB, Egan DF et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 2008; 30: 214-226.

[34] Levine B, Sinha S, Kroemer G. Bcl-2 family members: dual regulators of apoptosis and autophagy. Autophagy 2008; 4: 600-606.

[35] Sarkar S, Perlstein EO, Imarisio S et al. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nat Chem Biol 2007; 3: 331-338.

[36] Mizushima N. The role of the Atg1/ULK1 complex in autophagy regulation. Curr Opin Cell Biol 2010; 22: 132-139.

[37] Liang XH, Jackson S, Seaman M et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999; 402: 672-676.

[38] Pattingre S, Tassa A, Qu X et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005; 122: 927-939.

[39] Feng W, Huang S, Wu H, Zhang M. Molecular basis of Bcl-xL's target recognition versatility revealed by the structure of Bcl-xL in complex with the BH3 domain of Beclin-1. J Mol Biol 2007; 372: 223-235.

[40] Oberstein A, Jeffrey PD, Shi Y. Crystal structure of the Bcl-XL-Beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein. J Biol Chem 2007; 282: 13123-13132.

[41] Vaccaro MI. Autophagy and pancreas disease. Pancreatology 2008; 8: 425-429.

[42] Lo Re AE, Fernandez-Barrena MG, Almada LL et al. Novel AKT1-GLI3-VMP1 pathway mediates KRAS oncogene-induced autophagy in cancer cells. J Biol Chem 2012; 287: 25325-25334.

[43] Grasso D, Ropolo A, Lo Re A et al. Zymophagy, a novel selective autophagy pathway mediated by VMP1-USP9x-p62, prevents pancreatic cell death. J Biol Chem 2011; 286: 8308-8324.

[44] Vaccaro MI, Grasso D, Ropolo A et al. VMP1 expression correlates with acinar cell cytoplasmic vacuolization in arginine-induced acute pancreatitis. Pancreatology 2003; 3: 69-74.

[45] Jiang PH, Motoo Y, Vaccaro MI et al. Expression of vacuole membrane protein 1 (VMP1) in spontaneous chronic pancreatitis in the WBN/Kob rat. Pancreas 2004; 29: 225-230.

[46] Grasso D, Sacchetti ML, Bruno L et al. Autophagy and VMP1 expression are early cellular events in experimental diabetes. Pancreatology 2009; 9: 81-88.

[47] Gonzalez CD, Lee MS, Marchetti P et al. The emerging role of autophagy in the pathophysiology of diabetes mellitus. Autophagy 2011; 7: 2-11.

[48] Pardo R, Lo Re A, Archange C et al. Gemcitabine induces the VMP1-mediated autophagy pathway to promote apoptotic death in human pancreatic cancer cells. Pancreatology 2010; 10: 19-26.

[49] Ropolo A, Bagnes CI, Molejon MI et al. Chemotherapy and autophagy-mediated cell death in pancreatic cancer cells. Pancreatology 2012; 12: 1-7.

[50] Gilabert M, Vaccaro MI, Fernandez-Zapico ME et al. Novel role of VMP1 as modifier of the pancreatic tumor cell response to chemotherapeutic drugs. J Cell Physiol 2013; 228: 1834-1843.

[51] Molejon MI, Ropolo A, Re AL et al. The VMP1-Beclin 1 interaction regulates autophagy induction. Sci Rep 2013; 3: 1055.

[52] Molejon MI, Ropolo A, Vaccaro MI. VMP1 is a new player in the regulation of the autophagy-specific phosphatidylinositol 3-kinase complex activation. Autophagy 2013; 9: 933-935.

[53] He C, Bassik MC, Moresi V et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature 2012; 481: 511-515.

[54] Reggiori F, Komatsu M, Finley K, Simonsen A. Selective types of autophagy. Int J Cell Biol 2012; 2012: 156272.

[55] Vaccaro MI. Zymophagy: selective autophagy of secretory granules. Int J Cell Biol 2012; 2012: 396705.

[56] Jiang P, Mizushima N. Autophagy and human diseases. Cell Res 2014; 24: 69-79.

[57] Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. Cell Death Differ 2015; 22: 377-388.

[58] Navarro-Yepes J, Burns M, Anandhan A et al. Oxidative stress, redox signaling, and autophagy: cell death versus survival. Antioxid Redox Signal 2014; 21: 66-85.

[59] Okamoto K. Organellophagy: eliminating cellular building blocks via selective autophagy. J Cell Biol 2014; 205: 435-445.

[60] Stolz A, Ernst A, Dikic I. Cargo recognition and trafficking in selective autophagy. Nat Cell Biol 2014; 16: 495-501.

[61] Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature 2011; 469: 323-335.

[62] Nikoletopoulou V, Papandreou ME, Tavernarakis N. Autophagy in the physiology and pathology of the central nervous system. Cell Death Differ 2015; 22: 398-407.

[63] Grasso D, Garcia MN, Iovanna JL. Autophagy in pancreatic cancer. Int J Cell Biol 2012; 2012: 760498.

[64] Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011; 13: 132-141.

[65] Zadra G, Batista JL, Loda M. Dissecting the Dual Role of AMPK in Cancer: From Experimental to Human Studies. Mol Cancer Res 2015.

[66] Marino G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 2014; 15: 81-94.

[67] Grotemeier A, Alers S, Pfisterer SG et al. AMPK-independent induction of autophagy by cytosolic Ca2+ increase. Cell Signal 2010; 22: 914-925.

[68] Maiuri MC, Tasdemir E, Criollo A et al. Control of autophagy by oncogenes and tumor suppressor genes. Cell Death Differ 2009; 16: 87-93.

[69] Doerflinger M, Glab JA, Puthalakath H. BH3-only proteins: a 20-year stock-take. FEBS J 2015; 282: 1006-1016.

[70] Aita VM, Liang XH, Murty VV et al. Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. Genomics 1999; 59: 59-65.

[71] Maiuri MC, Le Toumelin G, Criollo A et al. Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. EMBO J 2007; 26: 2527-2539.

[72] Malik SA, Orhon I, Morselli E et al. BH3 mimetics activate multiple pro-autophagic pathways. Oncogene 2011; 30: 3918-3929.

[73] Malik SA, Shen S, Marino G et al. BH3 mimetics reveal the network properties of autophagy-regulatory signaling cascades. Autophagy 2011; 7: 914-916.

[74] Elgendy M, Ciro M, Abdel-Aziz AK et al. Beclin 1 restrains tumorigenesis through Mcl-1 destabilization in an autophagy-independent reciprocal manner. Nat Commun 2014; 5: 5637.

[75] Andreu-Fernandez V, Genoves A, Messeguer A et al. BH3-mimetics- and cisplatin-induced cell death proceeds through different pathways depending on the availability of death-related cellular components. PLoS One 2013; 8: e56881.

[76] Shimizu S, Kanaseki T, Mizushima N et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004; 6: 1221-1228.

[77] Wu YT, Tan HL, Huang Q et al. Autophagy plays a protective role during zVAD-induced necrotic cell death. Autophagy 2008; 4: 457-466.

[78] Shaid S, Brandts CH, Serve H, Dikic I. Ubiquitination and selective autophagy. Cell Death Differ 2013; 20: 21-30.

[79] Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death Differ 2015; 22: 526-539.

[80] Wirawan E, Vande Walle L, Kersse K et al. Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. Cell Death Dis 2010; 1: e18.

[81] Betin VM, Lane JD. Caspase cleavage of Atg4D stimulates GABARAP-L1 processing and triggers mitochondrial targeting and apoptosis. J Cell Sci 2009; 122: 2554-2566.

[82] Cho DH, Jo YK, Hwang JJ et al. Caspase-mediated cleavage of ATG6/Beclin-1 links apoptosis to autophagy in HeLa cells. Cancer Lett 2009; 274: 95-100.

[83] Zhu Y, Zhao L, Liu L et al. Beclin 1 cleavage by caspase-3 inactivates autophagy and promotes apoptosis. Protein Cell 2010; 1: 468-477.

[84] Yousefi S, Perozzo R, Schmid I et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol 2006; 8: 1124-1132.

[85] Young MM, Takahashi Y, Khan O et al. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. J Biol Chem 2012; 287: 12455-12468.

[86] Berry DL, Baehrecke EH. Growth arrest and autophagy are required for salivary gland cell degradation in Drosophila. Cell 2007; 131: 1137-1148.

[87] Galluzzi L, Bravo-San Pedro JM, Vitale I et al. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ 2015; 22: 58-73.

[88] Youle RJ, Narendra DP. Mechanisms of mitophagy. Nat Rev Mol Cell Biol 2011; 12: 9-14.

[89] Kim I, Lemasters JJ. Mitophagy selectively degrades individual damaged mitochondria after photoirradiation. Antioxid Redox Signal 2011; 14: 1919-1928.

[90] Grasso D, Vaccaro MI. Macroautophagy and the oncogene-induced senescence. Front Endocrinol (Lausanne) 2014; 5: 157.

[91] Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. Nat Rev Cancer 2010; 10: 51-57.

[92] Nardella C, Clohessy JG, Alimonti A, Pandolfi PP. Pro-senescence therapy for cancer treatment. Nat Rev Cancer 2011; 11: 503-511.

[93] Serrano M, Lin AW, McCurrach ME et al. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997; 88: 593-602.

[94] Gorgoulis VG, Halazonetis TD. Oncogene-induced senescence: the bright and dark side of the response. Curr Opin Cell Biol 2010; 22: 816-827.

[95] Narita M, Young AR, Arakawa S et al. Spatial coupling of mTOR and autophagy augments secretory phenotypes. Science 2011; 332: 966-970.

[96] Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451: 1069-1075.

[97] Hoyer-Hansen M, Jaattela M. Autophagy: an emerging target for cancer therapy. Autophagy 2008; 4: 574-580.

[98] Toth S, Nagy K, Palfia Z, Rez G. Cellular autophagic capacity changes during azaserine-induced tumour progression in the rat pancreas. Up-regulation in all premalignant stages and down-regulation with loss of cycloheximide sensitivity of segregation along with malignant transformation. Cell Tissue Res 2002; 309: 409-416.

[99] Qu X, Yu J, Bhagat G et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003; 112: 1809-1820.

[100] Levine B. Cell biology: autophagy and cancer. Nature 2007; 446: 745-747.

[101] Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. Nat Rev Cancer 2007; 7: 961-967.

[102] Buchler P, Reber HA, Lavey RS et al. Tumor hypoxia correlates with metastatic tumor growth of pancreatic cancer in an orthotopic murine model. J Surg Res 2004; 120: 295-303.

[103] Izuishi K, Kato K, Ogura T et al. Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. Cancer Res 2000; 60: 6201-6207.

[104] Esumi H, Izuishi K, Kato K et al. Hypoxia and nitric oxide treatment confer tolerance to glucose starvation in a 5'-AMP-activated protein kinase-dependent manner. J Biol Chem 2002; 277: 32791-32798.

[105] Fujii S, Mitsunaga S, Yamazaki M et al. Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. Cancer Sci 2008; 99: 1813-1819.

[106] DeNardo DG, Johansson M, Coussens LM. Inflaming gastrointestinal oncogenic programming. Cancer Cell 2008; 14: 7-9.

[107] Abe R, Yamagishi S. AGE-RAGE system and carcinogenesis. Curr Pharm Des 2008; 14: 940-945.

[108] Arumugam T, Simeone DM, Van Golen K, Logsdon CD. S100P promotes pancreatic cancer growth, survival, and invasion. Clin Cancer Res 2005; 11: 5356-5364.

[109] Kang R, Tang D, Schapiro NE et al. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. Cell Death Differ 2010; 17: 666-676.

[110] Kewley RJ, Whitelaw ML, Chapman-Smith A. The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. Int J Biochem Cell Biol 2004; 36: 189-204.

[111] Azad MB, Chen Y, Henson ES et al. Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. Autophagy 2008; 4: 195-204.

[112] Burton TR, Gibson SB. The role of Bcl-2 family member BNIP3 in cell death and disease: NIPping at the heels of cell death. Cell Death Differ 2009; 16: 515-523.

[113] Okami J, Simeone DM, Logsdon CD. Silencing of the hypoxia-inducible cell death protein BNIP3 in pancreatic cancer. Cancer Res 2004; 64: 5338-5346.

[114] Mahon PC, Baril P, Bhakta V et al. S100A4 contributes to the suppression of BNIP3 expression, chemoresistance, and inhibition of apoptosis in pancreatic cancer. Cancer Res 2007; 67: 6786-6795.

[115] Abe T, Toyota M, Suzuki H et al. Upregulation of BNIP3 by 5-aza-2'-deoxycytidine sensitizes pancreatic cancer cells to hypoxia-mediated cell death. J Gastroenterol 2005; 40: 504-510.

[116] Guan J, Stromhaug PE, George MD et al. Cvt18/Gsa12 is required for cytoplasm-to-vacuole transport, pexophagy, and autophagy in Saccharomyces cerevisiae and Pichia pastoris. Mol Biol Cell 2001; 12: 3821-3838.

[117] Proikas-Cezanne T, Waddell S, Gaugel A et al. WIPI-1alpha (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy. Oncogene 2004; 23: 9314-9325.