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Review article

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BECLIN-1 AND ITS ROLE AS A TARGET FOR ANTICANCER THERAPY

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The Nomenclature Committee on Cell Death (NCCD, 2009) defines different types of cell death on the basis of morphological, enzymological, immunological and functional criteria. Four basic types of cell death are distinguished from the biochemical point of view: necrosis, apoptosis, autophagy and cornification. Autophagy (macroautophagy) is a highly conserved process by which defective organelles, non-functional proteins and lipids become sequestered within structures called autophagosomes, which fuse with lysosomes, and the engulfed components are then degraded by lysosomal enzymes. The role of autophagy is not only the elimination of components, it also serves as a dynamic recycling system that produces new materials and energy for cellular renovation and homeostasis. Beclin-1 is a protein that plays a central role in autophagy; it interacts with multiple cofactors (Atg14L, UVRAG, Bif-1, Rubicon, Ambra1, HMGB1, IP3R, PINK and survivin) to promote the formation of the Beclin-1-Vps34-Vps15 complex which triggers the autophagy protein cascade. Beclin-1 dysfunction may lead to immune disorders, liver and neurodegenerative diseases as well as cancer. A positive and negative correlation between the expression pattern and/or activity of Beclin-1 and carcinogenesis has been demonstrated. Here we describe recent advances in understanding the molecular dynamics and regulation of autophagy and we discuss Beclin-1's contribution to anticancer therapy.

Key words: *Beclin-1*, *autophagy, cancer, signal transduction*, *anticancer therapy*

INTRODUCTION

The biggest challenge in cancer treatment is the ability for cancer cells to proliferate indefinitely and uncontrollably. Rapid advances in the techniques and tools used for cell research over the last thirty years have greatly improved our understanding of carcinogenesis-related processes. A vast number of research projects have been focused on developing an effective method of damaging cancer cells while at the same time sparing healthy cells. In 2009, the Nomenclature Committee on Cell Death (NCCD) proposed a new definition for dead cells. A cell is considered dead when one of the following molecular or morphological criteria are met: (A) the cell has lost integrity of its plasma membrane, (B) the cell, including its nucleus, has undergone complete fragmentation into discrete bodies, and (C) its corpse (or its fragments) has/have been engulfed by adjacent cells *in vivo*. With regard to the above definition, the following types of cell death have been described: necrosis, apoptosis, autophagy, cornification and atypical cell death (mitotic catastrophe, anoikis, excitotoxicity) (1, 2).

Autophagy is universal to nearly all eukaryotic cells and probably evolved as early as one billion years ago as a means of cell survival during nutrient deprivation. This process is a multi-step, lysosomal degradation pathway that eliminates long-life proteins and/or converts damaged organelles into basic biomolecules which are then recycled back into the cytosol and provide nucleic, amino and free fatty acids for the synthesis of DNA/RNA, proteins and ATP. The word "autophagy" is derived from Greek and means "self-eating". The process was first described in 1963 by Christian de Duve (3). The first reports concerning phenotype-based identification of autophagy in *Saccharomyces cerevisiae* yeast appeared in 1992, and later that year a team led by Ohsumi (4) identified and characterised genes responsible for autophagy in yeast. Another step was a discovery by Mizushima *et al*., who in 1998 identified autophagy genes *ATG5* and *ATG12* in mammalian cells (5).

There are three main types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy (hereafter referred to as autophagy) is the best-characterised pathway of the three; the hallmark of this process is the formation of double-membrane vesicles (autophagosomes) that non-selectively sequester cytoplasmic components and deliver them to the lysosome or vacuole for degradation. Macroautophagy consists of a series of steps including initiation, elongation and expansion of the phagophore assembly site, formation and maturation of the autophagosomes, fusion with the lysosome and digestion (6, 7). The mechanism of individual stages of the process is very complex and is regulated by numerous factors, such as the mammalian target of rapamycin (mTOR), the "guardian of the genome" - protein 53 (p53), B-cell lymphoma 2 protein (Bcl-2), ribosomal protein S6 kinase (p70S6 kinase), class III phosphoinositide-3-kinase (PI3K-III), Beclin-1 and death-associated protein kinase (DAPK) (8-10). Microautophagy sequesters cytoplasmic components and delivers them for degradation by direct invagination, protrusion, and septation of the lysosomal membrane. In contrast to either macro- and microautophagy, delivery of cargo *via* CMA does not require the formation of intermediate vesicles or membrane

fusion. In this process only cytosolic proteins, but not complete organelles, containing a pentapeptide motif (KFERQ-consensus motif) bind to chaperone proteins (Hsc70). This complex translocates directly from the cytosol into the lysosomal lumen across the lysosome membrane *via* interaction with membrane protein 2A (LAMP-2A) (11-16) (*Fig. 1*). Moreover, many recent studies have revealed the existence of selective autophagy which leads to the removal of protein aggregates (aggrephagy), lipid droplets (lipophagy), secretory granules (zymophagy), the nucleus (nucleophagy), RNA (RNautophagy), microbes (xenophagy) or organelles, such as peroxisomes (pexophagy), mitochondria (mitophagy), surplus ER (reticulophagy) and ribosomes (ribophagy) (17).

At the molecular level, autophagy is controlled by AuTophaGy-related genes (ATG) and their respective Atg proteins. Thirty-four different proteins of this group (Atg1-Atg34) have been identified in yeast cells, having 34 orthologues in mammalian cells. Eighteen of them (Atg1-10, Atg12-14, Atg16-18, Atg29 and Atg31) are involved in all types of autophagy (18-20). These were divided into 6 functional groups. The first group is the ULK1 kinase complex (ULK-mAtg13-FIP200-Atg101), with mAtg13 being its major component that acts as an initiating trigger for autophagy. The second group is Atg9, a protein that promotes lipid transport and thus helps to assemble a phagophore. The third group is the class III PI3-kinase complex, composed of Vps34-Beclin-1-Vps15-mAtg14, and its role is to form a phagophore. The fourth group is PI3K-Atg2-Atg18, required for retrograde transport of Atg9 from the pre-autophagosomal structures (PAS), elongate the still immature phagophore membrane. The fifth group is the Atg12-Atg5-Atg16L complex that induces curvature formation in the growing phagophore. The last group are Atg8 proteins which, by binding with the C-terminus, induce phosphatidylethanolamine (PE) modifications which result in autophagosome formation (21-23).

BECLIN-1 - PROTEIN STRUCTURE, LOCATION AND BIOLOGICAL FUNCTIONS

Beclin-1 was discovered by Beth Levine (14), who in 1999 identified the *BECN1/ATG6* gene on chromosome 17q21. It encodes a protein with a linear sequence of 450 amino acids, with a molecular weight of approximately 60 kDa (*Fig. 2*). Beclin-1 is localised within the cytoplasmic structures, *i.e.* the endoplasmic reticulum, mitochondrion and nuclear membrane (24, 25).

Beclin-1 contains three main domains, each with a different function. The BH3 domain (Bcl-2 homology domain) localised at its N-terminus contains 125 amino acids and is able to bind members of the anti-apoptotic family of proteins, *e.g.* Bcl-XL, Bcl-2. The central coiled-coiled domain (CCD, amino acids 144-269) binds to the UV irradiation resistance-associated gene (UVRAG) and class III PI3 kinase. It regulates self-association and dimerisation both *in vivo* and *in vitro* (26-28). The third

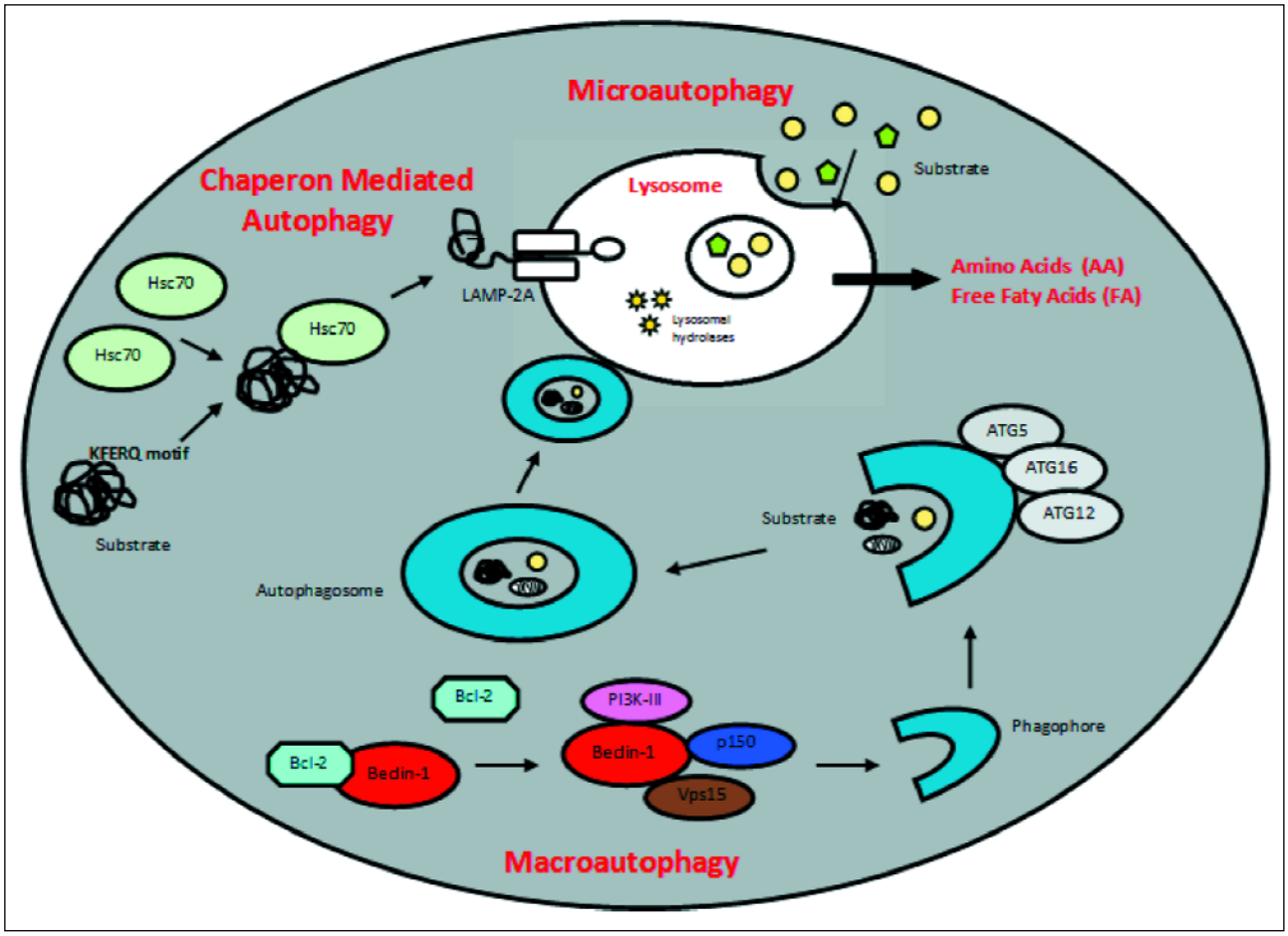

Fig. 1. Schematic representation of different types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy. Beclin-1 plays a central role in macroautophagy. It interacts with multiple cofactors to promote formation of the Beclin-1-PI3K-III-Vps15 complex which triggers the autophagy protein cascade.

domain, the evolutionarily conserved domain (ECD), binds to class III PI3 kinase and takes part in the binding of lipid membranes of cell organelles. Within this domain, three consecutive aromatic amino acids were identified (Phe359-Phe360-Trp361) that form a hydrophobic finger with a preference for lipid membranes enriched with cardiolipin (CL) and which occur, *e.g.* in the mitochondrion (29). Beclin-1 also has a short leucine-rich sequence of amino acids localised at the C-terminus that ensures an efficient nuclear export signal (NES). Mutations of *BECN1/ATG6* NES negatively affect its ability to induce autophagy in response to nutrient deprivation (30).

Beclin-1 is a specialised molecule which plays a multifunctional role in the cell. Beclin-1, by being directly involved in the initiation of autophagy, indirectly implicates an important role in numerous biological cellular processes, including development, endocytosis, adaptation to stress, aging and cell death. Several reports have shown a correlation between the expression pattern and/or activity of Beclin-1 and carcinogenesis (31-34). Beclin-1 dysfunction has been suggested in many diseases, *e.g.* in neurodegenerative diseases (Huntington's, Parkinson's or Alzheimer's) and in cardiomyopathy (35-37). Moreover, Beclin-1 is involved in the immune response to bacterial and viral infections by interacting with heat shock proteins, interferon-γ, TRAP and TBP protein; however, the exact mechanism of these processes still remains elusive (38).

THE ROLE OF BECLIN-1 IN AUTOPHAGY INITIATION

Beclin-1 is one of the key proteins regulating autophagy. It acts directly by triggering a cascade of proteins involved in autophagolysosome formation, as it is a component of the main signal-initiating complex (class III PI3 kinase, Beclin-1 and p150 protein) (23).

The role of Beclin-1 in autophagy initiation is dependent on the presence of unique proteins that are bound to its domains. Within CCD and ECD, the most important protein is class III phosphatidylinositol-3 kinase (PI3K-III), a mammalian homologue of vacuolar protein sorting (Vps34) that was first identified in *Saccharomyces cerevisiae* yeast (3). Mammalian cells have been shown to contain class I, II and III PI3 kinase. Class I and III PI3K kinases are involved in autophagy. The first is a component of the cell membrane and is a part of the signal transduction pathway to induce mTOR kinase. Class III PI3 kinase promotes cell proliferation and translocation of cytoskeleton elements within the cell (9, 39). Class III PI3 kinase can only act upon forming a complex with Beclin-1. The Beclin-1/PI3K-III complex is localised in the trans-Golgi apparatus and by stimulating increased production of phosphatidylinositol 3-phosphate (PIP3) it favours phagophore elongation and allows the recruitment of other Atg proteins to the phagophore (40). Blocking the activity of PI3 kinase with inhibitors such as wortmannin or 3-methyladenine suppresses autophagy, which confirms the strategic role of PI3K (41).

UV irradiation resistance-associated gene protein (UVRAG) binds to the CCD of Beclin-1. The two proteins form a complex that is crucial for phagophore maturation and autophagosome formation. Additionally, it has been demonstrated that the complex greatly enhances PI3K activity, thus inducing autophagy (42). Itakura *et al.* have shown that both UVRAG and Atg14 are necessary for class III PI3K-Beclin-1 complex stability, while knockdown of either of them results in a reduction of the Beclin-1 level (43). Bax-interacting factor-1 (Bif-1) is another positive regulator of Beclin-1 and interacts with Beclin-1 through UVRAG. Studies in mice have shown that knockout of Bif-1 suppresses the formation of autophagosomes and results in significantly higher rates of cancer in these animals (44).

Barkor/Atg14(L) protein also binds to the CCD. It is essential for triggering autophagy as has a role in targeting PI3 kinase to the early autophagosome (45, 46). Ambra1 is another protein that forms a complex with Beclin-1. The protein promotes the transport of Beclin-1 to the endoplasmic reticulum, where PI3K is expressed at the highest level. This explains the relationship between the cellular cytoskeleton and autophagosome formation (47, 48).

Vacuole membrane protein 1 (VMP1) binds to the BH3 domain of Beclin-1 to stimulate the formation of the class III PI3K-Beclin-1 complex. This factor is believed to positively influence autophagy because if it is absent in a cell, autophagy is completely blocked (49).

Anti-apoptotic protein family members bind to Beclin-1 through the BH3 domain. Binding or dissociation of the proteins results in the process being suppressed or activated. They function as regulators to prevent excessive expansion of the process and protect the cell from degradation while at the same time facilitating nutrient intake to ensure proliferation and growth (50). The interaction between Bcl-2 and Beclin-1 is unidirectional. Studies on HT-29 colorectal cancer cells confirm that upon binding to Beclin-1, Bcl-2 suppresses the formation of

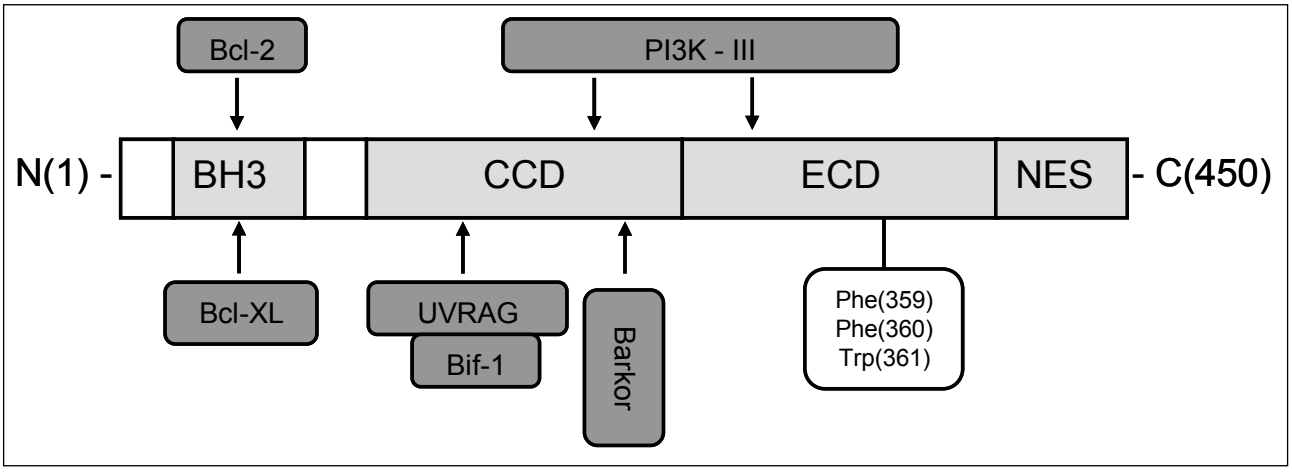

Fig. 2. Structure of the Beclin-1 protein and its positive and negative regulators. Beclin-1 contains three identifiable domains, such as a Bcl-2 homology motif (BH3), a coiled-coiled domain (CCD) and a conserved domain (ECD).

the Beclin-1/PI3K complex by reducing Beclin-1's affinity for PI3K (51). This effect is further enhanced by the presence of nutrient-deprivation autophagy factor-1 (NAF-1), which binds to Bcl-2 within the endoplasmic reticulum, thus enhancing the stability of the Bcl-2-Beclin-1 complex (52).

Phosphorylation of Bcl-2 by c-Jun N-terminal kinase 1 (JNK1) promotes dissociation of the Bcl-2-Beclin-1 complex. Wei *et al*. have demonstrated that in response to starvation conditions, phosphorylation of Bcl-2 on threonine 69, serine 70 and serine 87 is induced (50). Beclin-1-Bcl-2 complex dissociation is also mediated by extracellular signal-regulated kinase (ERK), which is regulated by high mobility group box-1 (HMGB1), a conserved nuclear protein maintaining chromatin architecture (53). Apart from its intranuclear role, HMGB1 also functions as an extracellular signal in case of inflammation, cell differentiation and migration, and cancer metastasis. Increased HMGB1 translocation from the nucleus to the cytosol in response to long-term cellular stress (*e.g.* starvation) induces autophagy (54).

The initiation of autophagy is not only the effect of dissociation of the Beclin-1-anti-apoptotic protein complex. The process may also involve Beclin-1 itself, which is phosphorylated on threonine 119 within its BH3 domain by a death-associated protein kinase (DAPK1), for which it is a substrate (55) (*Table 1*).

ROLE OF AUTOPHAGY IN TUMOURIGENESIS

Autophagy plays an important role in the regulation of survival and death signalling pathways in a variety of human diseases, including cancer. Numerous studies have established that autophagy has two faces in carcinogenesis. On the one hand, activation of autophagy functions as a tumour suppressor by degrading defective organelles and other cellular compounds. Animals with deletion of *Atgs* are at high risk of developing tumours (56). On the other hand, in cancer cells this pathway may act as a pro-survival mechanism to generate nutrients and energy during periods of starvation, hypoxia and stress induced by chemotherapy (15).

A common challenge to numerous research groups is determining whether autophagy protects cell survival or contributes to cell death. Bo Liu *et al*. proposed three hypotheses to explain the role of autophagy in cancer cell fate (57). One hypothesis proposes that the role of autophagy varies depending on the stage of tumour development; the second hypothesis suggests that autophagy can affect carcinogenesis in a cell- or tissue-specific manner; the third hypothesis proposes that autophagy plays a role at the molecular level by participating in the regulation of tumour suppressor genes or oncogenes. Key autophagic mediators, such as ATGs, mTOR, PI3K-III, p53, ROS and Beclin-1, have been shown to play a crucial role in modulating autophagic activity in cancer initiation and progression (58-60).

The connection between autophagy and cancer was first observed in 1999 when the *BECN1/ATG6* gene was found to stop tumour development (31). It was found that *BECN1* was monoallelically deleted in ovarian, breast and prostate cancers (61, 62). Decreased expression of *BECN1/ATG6* is observed in brain tumours (63) and cervical cancer (58). A lower level was also observed in MCF7 breast cancer cells when compared to normal breast cells. Studies have unequivocally demonstrated that lower expression of the *BECN1/ATG6* gene translates into higher proliferation potential and, conversely, a higher level of Beclin-1 means better prognosis (64). This theory is further confirmed by studies conducted in mouse models with monoallelic deletion of the Beclin-1-encoding gene (Beclin-1+/–), which have shown a significant increase in the number of spontaneous liver and lung cancers, leukemias and lymphomas when compared to animals having both alleles (Beclin-$1^{+/+}$) (65). As the UVRAG may bind to the CCD of Beclin-1, UVRAG-mediated activation of the Beclin-1/PI3K-III complex promotes autophagy while suppressing proliferation and inhibiting tumourigenesis of human colon cancer cells (66). Moreover, numerous studies suggest that Beclin-1 regulates two types of cell death, namely apoptosis and autophagy (67, 68). It has been demonstrated that overexpression of Beclin-1 in human gastric cancer (MKN28) enhances apoptosis triggered by cis-diamminedichloroplatinum (CDDP). On the other hand, Beclin-1 knockdown through the use of siRNA decreases CDDP cytotoxicity. This mechanism is connected with caspase-9 activity (69). A similar effect was observed in HepG2 liver cancer cells exposed to doxorubicin (70). Studies by Wirawan *et al*. have shown that caspase-3, -7 and -8 cleave Beclin-1 into Beclin-1-N and Beclin-1-C, thereby destroying its ability to induce autophagy while at the same time assigning it a new role in activating apoptosis by translocating Beclin-1-C to the mitochondrion, where it enhances cytochrome C (cyt C) release (71).

It was also demonstrated that Atgs, which are involved in the formation of autophagosomes, are linked to cancer initiation and progression. Silencing some crucial Atgs, such as Atg3, Atg4,

Table 1. Positive and negative cofactors of Beclin-1.

Positive regulators of Beclin-1	Negative regulators of Beclin-1
Ambra1	Bcl-2/Bcl-XL
UVRAG	Rubicon
Bif-1	NAF-1
HMGB1	Akt
Barkor/Atg14(L)	IP3R
Survivin	
PINK1	
JNK1	
ERK1/2	
DAPK1	
SLAM	
VMP1	

Atg10, and Atg12, causes sensitisation of tumour cells to a variety of stressful conditions (72). Deletion of Atg5 and Atg7 showed the development of benign liver adenomas, thus clearly suggesting a tumour-suppressive function of autophagy (15).

mTOR has been implicated in controlling the number of signalling pathways. This kinase functions as two multiprotein complexes, mTORC1 and mTORC2, and each has different sensitivity to rapamycin. mTORC1 consists of mTOR kinase, mLST8/GβL and the Raptor protein and is involved in the control of transcription, cell proliferation and autophagy, thus being a negative regulator in cancer cells (60).

Several studies have shown that the human tumour suppressor gene involved in genotoxic stress response and DNA damage repair, *i.e.* p53, contributes to the control and execution of autophagy (73, 74). The role of p53 in this pathway depends on its subcellular localisation. In the nucleus, p53 induces autophagy by AMPK activation to inhibit mTOR. In the cytoplasm, p53 inhibits autophagy by mTORC1 activation (75).

BECLIN-1 - A TARGET FOR ANTICANCER THERAPY

The major reason for the failure of many anticancer therapies is tumour resistance to apoptosis. In cells where apoptosis has been blocked, autophagy may be an alternative mechanism of eliminating cells from the organism. The induction of autophagy by the inhibition of anti-autophagic proteins, such as Bcl-2, PKCδ, and tissue transglutaminase 2 (TG2), leads to autophagic cell death in some apoptosis-resistant cancers (*i.e.*, breast and pancreatic cancers), thus suggesting a novel mechanism for the regulation of autophagic cell death (76). The relationship between autophagy and tumourigenesis has been reported in many studies (77). Numerous investigations confirm autophagy activation upon exposure to anticancer drugs, but this effect is dualistic; on the one hand, it can inhibit tumourigenicity leading to cancer cell death, but on the other hand it can play the role of a pro-survival mechanism in many human cancer cells (76). For example, antitumour properties were revealed in the low concentration of arsenic trioxide (As_2O_3), which triggers autophagy in human leukemic cells and gliomas. Furthermore, the combination of As_2O_3 with bafilomycin A1 autophagy inhibitor enhanced the antitumour effect of As_2O_3 through induction of apoptosis (78). Interestingly, pterostilbene (a naturally occurring structural analog of resveratrol) induced accumulation of autophagic vacuoles followed by cell death in HL60 human leukemia cells (79). A similar anticancer effect was demonstrated by pentagalloyl glucose, which induces significant accumulation of autophagosomes and lipid modification of the light-chain 3 protein in PC-3 prostate cancer cells (80). In turn, a pro-survival effect was revealed by 4-hydroxytamoxifen (4-OHT), which by induction of macroautophagy in ER^+ breast cancer cells (MCF7) leads to cell survival and facilitates the development of antiestrogen resistance (81). Earlier studies by Bursch *et al.* (82) identified the same result.

Beclin-1 has repeatedly been reported as a target for applied therapies and has been referred to as a gatekeeper gene whose role is to initiate or inhibit autophagy. The high incidence of spontaneous tumours in mice with monoallelic disruption of Beclin-1 has provided genetic evidence for autophagy-mediated suppression of tumourigenesis. In MCF-7 breast cancer cells, Hoyer-Hansen *et al.* identified two opposing roles for Beclin-1 in the control of tumour cell growth, *i.e.* sensitisation to autophagy and the requirement for cell proliferation. In cells exposed to EB1089, a chemotherapeutic vitamin D analogue, increased Beclin-1 expression was demonstrated to increase activation of autophagy and inhibition of tumourigenesis (83). A similar increase in the Beclin-1 level and inhibition of cancerogenesis by induction of autophagy was observed in MDA-MB-231 and T47D breast cancer cells treated with natural substances such as resveratrol and its synthetic derivative, aza-resveratrol (84). A well-known mTORC1 inhibitor, RAD001 (everolimus), is applied in treatment in the acute lymphoblastic leukemia (ALL) model in children. This drug reveals an antitumour effect by inducing autophagy with increased Beclin-1 expression and LC3-I conversion to LC3-II (85). Additionally, it was observed that in breast cancer stem cells (CSCs), the antitumour drug rottlerin induces autophagosome formation through increased expression of Atg5, Atg7, Atg12 and Beclin-1, whereas Atg7 and Beclin-1 exposure to siRNA blocks autophagy induced by this compound. Moreover, rottlerin-induced autophagy leads to the elimination of breast cancer stem cells by the apoptosis mechanism (86). A similar anticancer result was visible in a novel furanocoumarin, Feroniellin A (FERO), which by induction of autophagy causes apoptosis in human A549 lung cancer cells resistant to etoposide (A549RT-eto) (87). FERO has been involved in the formation of autophagosomes by conversion of LC3 I to LC3 II, enhancing expression of Beclin-1 and ATG5, and inactivation of mTOR. Furthermore, suppression of Beclin-1 by siRNA reduced FERO-induced apoptosis in A549RT-eto cells. These studies suggested that FERO can be a useful anticancer drug for multidrug-resistant lung cancer because it exerts a cytotoxic effect by inducing both autophagy and apoptosis.

The inhibition of autophagy may enhance efficacy of anticancer therapies by activating the apoptotic pathway. Studies by Yuan *at al.* (88) demonstrated the important role of Beclin-1 in activating autophagy in glioblastoma (GMB). They tested cucurbitacin I, a natural selective inhibitor of JAK2/STAT3, and showed that exposure of GMB cells to this compound upregulated Beclin-1 and triggered autophagosome formation and accumulation. Moreover, inhibition of autophagy by knockdown of Beclin-1 or treatment with chloroquine, an inhibitor of autophagy, markedly increased cucurbitacin I-induced apoptotic cell death. Also, Kim *et al.* (89) showed a relationship between autophagy and apoptosis in colorectal cancer cell using two chemotherapeutic agents - oxaliplatin and bortezomib. Treating cells with both substances effectively activated mitochondria-dependent apoptosis by activating the JNK-Bcl-xL-Bax pathway. Moreover, Beclin-1 is dissociated from Bcl-xL and initiated autophagy during treatment with oxaliplatin and bortezomib. These results suggested that combinatorial treatment significantly inhibited colorectal cancer tumour growth by Beclin-1/Bcl-xL and mediated crosstalk between apoptosis and autophagy. It has also been demonstrated that the widely used cytostatic drug methotrexate (MTX) induces apoptosis by suppressing autophagy in squamous cell carcinoma (SCC) cells showing overexpression of WW domain-containing oxidoreductase (WWOX). Autophagy inhibition in these cells involves downregulation of Beclin-1, Atg12, Atg5 and LC3-II (90).

Some studies have demonstrated that the inhibition of autophagy in cancer cells may be therapeutically beneficial in some circumstances, as it can sensitise cancer cells to different therapies. But sometimes autophagy protects cancer cells from death. In glioblastoma cells, Shen *at al.* showed that pro-apoptotic, low molecular weight inhibitor of vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) - $ZD6_{474}$ inhibits autophagy by decreasing the expression of Atg7 and Beclin-1, which protects these cells from the apoptotic effect of $ZD6_{474}$ which might, in turn, contribute to tumour resistance against this substance treatment (91). Furthermore, other studies showed that reduced expression of the autophagic gene Beclin-1 and increased expression of the

anti-apoptotic gene Bcl-xL have not only been shown to be associated with a malignant phenotype, but also with poor prognosis of cancer patients with hepatocellular carcinoma (HCC) (76). Therefore, in this case the induction of autophagy may help to reverse the malignant phenotype.

Beclin-1 expression is regulated not only by the application of anticancer compounds. Li Z *et al*. (92) showed that other than deletion in the gene copy number, DNA hypermethylation in the promoter and/or intron 2 may be a new mechanism responsible for downregulation of *BECN1* expression. Since Beclin-1 has important functions in apoptosis and autophagy, its epigenetic modification might provide new targets for cancer therapy (92).

SUMMARY

Cancer cells are characterised by their ability for unlimited proliferation, low differentiation profile and to escape from programmed cell death. At the core of initiation and progression of malignant transformation are disturbed signal transduction pathways that are relevant for the proper development and function of the cell. Scientific interest has been focused on the search for the molecular mechanisms limiting tumour development, among others, by inducing the death of cancer cells without damaging normal cells (93). In recent years, investigations into autophagy and the relationship between this process and apoptosis pointed to the importance of understanding both mechanisms in the promotion, progression and therapy of cancer. In accordance with Bursch (82), we believe that the intrinsic apoptosis pathway is frequently deregulated in human cancer cells, which eventually may give rise to cancer's resistance to radiation and chemotherapy, thus pharmacological concurrent targeting of autophagy- and apoptosis-signalling pathways in anti-cancer therapy might greatly improve their efficiency.

Many scientists have stated that there is no simple way of fighting cancer through autophagy. The different roles of autophagy in cancer cells seem to depend on tumour type, stage, and genetic context. It is now well established that autophagy can act as a tumour suppressor and tumour promoter. Autophagy is frequently viewed as a "double-edged sword" (94).

Extensive research into the programmed death of cancer cells with the use of various modulators can form the basis for new and efficient approaches to cancer therapy, such as induction of cancer cell autophagy. To ensure therapeutic success it is necessary to search for molecular targets for more effective treatments. The first link between autophagy and tumour growth consists in the participation of gene *BECN1*. It was observed that breast, prostate and ovary cancers appear more often when deletion of the region coding Beclin-1 is present. On the other hand, higher expression of this gene can provide an anti-cancer effect. It seems that the involvement of Beclin-1 in the autophagy machinery discussed in this paper confirms that this protein may become a new target for anticancer therapy.

Abbreviations: Ambra1 - activating molecule in Beclin-1-regulated autophagy; AMPK - AMP-activated protein kinase; Atg - autophagy-related gene; ATP - adenosine triphosphate; BECN 1 - Beclin-1; BCl-2 - B-cell lymphoma 2 protein; BH3 - Bcl-2 homology 3 domain; Bif-1 - endophilin B1/Bax-interacting factor 1; CCD - central coiled-coiled domain; CMA - chaperone-mediated autophagy; CL - cardiolipin; CSRS - cancer stem cells; cyt C - cytochrome C; DAPK - death-associated protein kinase 1; ECD - evolutionarily conserved domain; ERK - extracellular signal-regulated kinase; Hsc70 - heat shock cognate protein 70; HMGB1 - high mobility group box 1; IP_3R - inositol trisphosphate receptor; JNK1-c - Jun N- terminal kinase 1; LAMP-2A - lysosome-associated membrane protein 2A; mTOR - mammalian target of rapamycin; mLST8/GβL - mammalian lethal with SEC13 protein 8/G protein beta subunit-like; NAF-1 - nutrient deprivation autophagy factor-1; NES - nuclear export signal; NOD-like receptors - nucleotide-binding oligomerisation domain receptors; PAS - preautophagosomal structure; PDK-3 - phosphoinositide dependent kinase; PE - phosphatidylethanolamine; PINK1 - PTEN-induced putative kinase 1; PI3K-III - class III phosphoinositide-3-kinase; PIP3 - phosphatidylinositol triphosphate; PRR - pattern recognition receptor; p53 - tumour protein 53; p70S6 kinase - ribosomal protein S6 kinase; Rubikon - RUN domain protein as a Beclin-1 interacting and cysteine-rich containing protein; SLAM - signalling lymphocytic activation molecule, transmembrane protein; TBP - TATA-binding protein; TLP - TATA-binding protein-like protein; TLR - Toll-like receptor; TRAP - RNA-binding attenuation protein; UVRAG - UV irradiation resistance-associated gene; VMP1 - vacuole membrane protein 1.

Authors' contributions: ET and MR came up with the concept; ET, NL and PS collected the information; ET and NL prepared the manuscript and figures; ET and MR wrote the final manuscript.

All of the authors read and approved the final manuscript.

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