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Dysfunctional processes in autophagy of cancer cells and therapeutic implications

Aurelian Udristioiu[1], Manole Cojocaru[2], Delia Nica- Badea[3], Viviana Roman[4]
[1], [2] Molecular Biology, Medicine Faculty, Titu Maiorescu University, Bucharest, Romania, Balkans
[3] Medicinal and Behavioral Sciences Faculty, Constantin Brâncuși University, Tg-Jiu, Romania, Balkans
[4] Center of Immunology, Institute Stefan S. Nicolau, Bucharest, Balkans

Abstract
Autophagy is a cellular process that maintains the homeostasis of the normal cell, but autophagic dysfunction is associated with human diseases, such as cancer. In cancer, the autophagy can be neutral, tumor-suppressive, or tumor-promoting in different contexts. Genomic analysis of human cancers indicates that the loss or mutation of core autophagy genes, (Atg) is uncommon, whereas oncogenic events that activate autophagy and lysosomal biogenesis have been identified. The initial signal to form auto-phagosomes is by the class III phosphatidyl-inositol (PI) 3 kinase complex consisting of sequence genes, Beclin1/Atg6 and class III PI3K (Vps34). This process is negatively regulated by binding of Bcl-2 family members such as Bcl-xL to Beclin1 preventing Beclin1 binding to the PI3K-III complex and thereby reducing autophagy. Optimal combination of inductors or inhibitors of autophagy with chemo or radiotherapy in a variety of tumor types in different phases can be successful approaches for improve the effect of anticancer therapies.

Keywords: autophagy, apoptosis, Bcl-2-protein, phosphatidyl-inositol (PI) 3 kinase complex, mammalian target of rapamycin (mTOR), Tensin-homologous phosphatase (PTEN)

1. Introduction
Each cell is "programmed" to "commit suicide," and this happens every day, without feeling any affection and without your life being jeopardized. The "suicide" of cells is part of the sustainability of life on Earth, so they die for the good of our body, they are the "heroes" that are found in each of usIn fact, in the absence of this mechanism, life would suffer.
Aging is not properly considered a disease; however, it is associated with different pathological conditions. In the last period of human life, cells undergo several changes, including DNA mutations, damages at several other molecules, and accumulation of protein aggregates. Several studies have demonstrated that autophagy activation protects from aging. In fact, not only autophagy levels decrease with age but also overexpression of autophagy-related genes (Atg) proteins contributes to improve life span in a human model of aging in vitro and in mouse models in vivo [1].

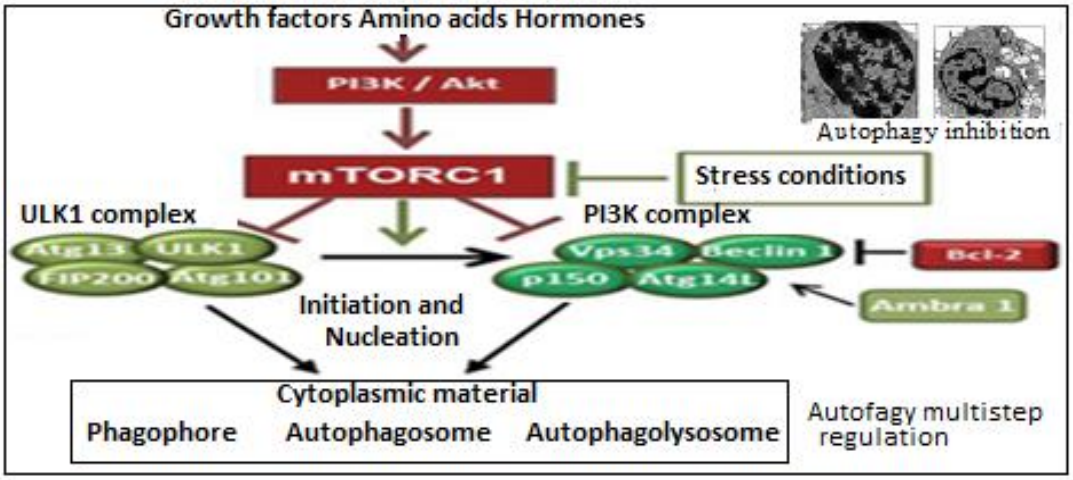

Fig 1

- Crucial role of autophagy in the maintenance of cellular homeostasis
- Signaling pathways involved in autophagy multistep regulation.
- P53 plays dual roles in regulating autophagy depending on its subcellular localization
- The PI3K/AKT/mTOR pathway in autophagy
- Autophagy inhibitors has therapeutic implications (therapeutic strategy)

2. Roles of autophagy in human pathology

As already mentioned, one of the most important functions of autophagy is the degradation of misfolded proteins, so in neurons, the failure of autophagy can contribute to neurodegeneration [2]. It can occur in Parkinson's disease, a neurodegenerative disorder characterized by α-synuclein accumulation in the brain. Recently, in a study, was demonstrated that autophagy inhibition by 3-methyladenine (3-MA) and by Atg5 knocking down in lymphocytes lead to a significant increase of α-synuclein levels [3]. The removal of mitochondria, source of reactive oxygen species (ROS), performed by autophagy, certainly protects cells from DNA mutations and prevents cellular transformation. It has also been demonstrated that deletion of the autophagic gene Beclin-1 may cause development of various malignancies in mouse models [4].

Furthermore, it has been demonstrated that autophagy-deficient tumors are more sensitive to several chemotherapeutic agents [5]. In this case, autophagy promotes the survival of cancer cells and protects them from the action of drugs that induce apoptosis. Although research in this field is just at the beginning, an encouraging number of works suggest that defects in the autophagy mechanism may be involved in the pathogenesis of autoimmune diseases.

In systemic lupus erythematosus (SLE), showed that factors present in the serum of SLE patients, probably antibodies, are able to induce autophagy in T lymphocytes from healthy donors, but not in T lymphocytes from patients with SLE. We speculated that chronic exposure to specific autoantibodies, as occurs in SLE, could lead to the selection of autophagy-resistant T lymphocytes [6, 7].

3. Physiological functions and molecular mechanism of autophagy

Autophagy is a degradation pathway characterized by the isolation of targeted cytoplasmic material in a typical double-membrane vesicle, known as autophagic vacuole or auto phagosome [8]. The subsequent fusion of the auto phagosome with the lysosome ensures the correct destruction of organelles, misfolded proteins, and microorganisms, carried inside the vesicle. Despite its emerging role in human pathology, autophagy is a physiological process involved in basal organelles turnover and in the removal of proteins aggregates [9], [Figure 2].

In response to the condition of cellular stress, such as growth factors and nutrients deprivation, intracellular components degraded by autophagy are recycled in order to generate ATP and sustain essential cell functions [10]. Autophagy is considered a pro-survival mechanism, allowing cells to respond to injury by degrading unnecessary and dysfunctional self-components; however, this ability may become a double-edged sword. Three types of autophagy can be distinguished: macro-autophagy, micro-autophagy, and chaperone-mediated autophagy. In this review, we will focus on macro-autophagy (hereafter referred to as autophagy), which is the most characterized type of autophagy.

Considering the crucial role of autophagy in the maintenance of cellular homeostasis, it is not surprising that several signaling-related molecules are involved in the perfect functioning of this process. Genetic screens in yeasts allowed the discovery of at least 37 autophagy-related genes (Atg) [11]. Many of these genes, encoding proteins involved in autophagy and its regulation, are evolutionarily conserved in humans.

The mammalian target of rapamycin (mTOR) complex 1 (mTORC1) regulates the activation of autophagy machinery, acting as a sensor of energy levels and integrating upstream signals deriving from other pathways, including the phosphoinositide 3-kinase (PI3K). In the presence of amino-acids and growth factors, mTORC1 represses autophagy by inhibition of Vps34 and ULK1 complexes. On the contrary, in low nutrients state, defined as starvation, the dissociation of mTORC1 from the induction complex triggers autophagy [12, 13, 14], [Figure 3].

The autophagosome derives from a double-membrane pre-autophagosome structure called phagophore, which seems to originate from different sources, including plasma membrane [15], endoplasmic reticulum [16] and Golgi complex, in mammalian cells [17]. Phagophore nucleation requires the activity of class III phosphatidylinositol 3-kinase (PI3K-III) complex containing Beclin-1 [18].

The initial signal to form auto-phagosomes is by the class III phosphatidyl-inositol (PI) 3 kinase complex consisting of sequence genes, Beclin1/Atg6, p150hVSp35, and class III PI3K (Vps34). The autophagy promoting function of Beclin-1 is influenced by the antiapoptotic protein Bcl-2; in fact, when Beclin-1 is bound to Bcl-2, autophagy is inhibited; instead, the dissociation from Bcl-2 allows Beclin-1 to interact with PI3K-III complex, and to activate autophagy [19]. On the contrary, Beclin-1 regulated autophagy protein 1 (AMBRA1) is a positive regulator of Beclin-1-dependent autophagy; thanks to its capacity to create a link between cytoskeletal motor proteins and class III PI3K complex [20, 21]. Two ubiquitin-like conjugation systems, Atg12–Atg5–Atg16L and microtubule-associated protein 1 light-chain 3 (LC3)–phosphatidylethanolamine (PE), mediate the second step of autophagy, which concerns the expansion and closure of the auto phagosome [22].

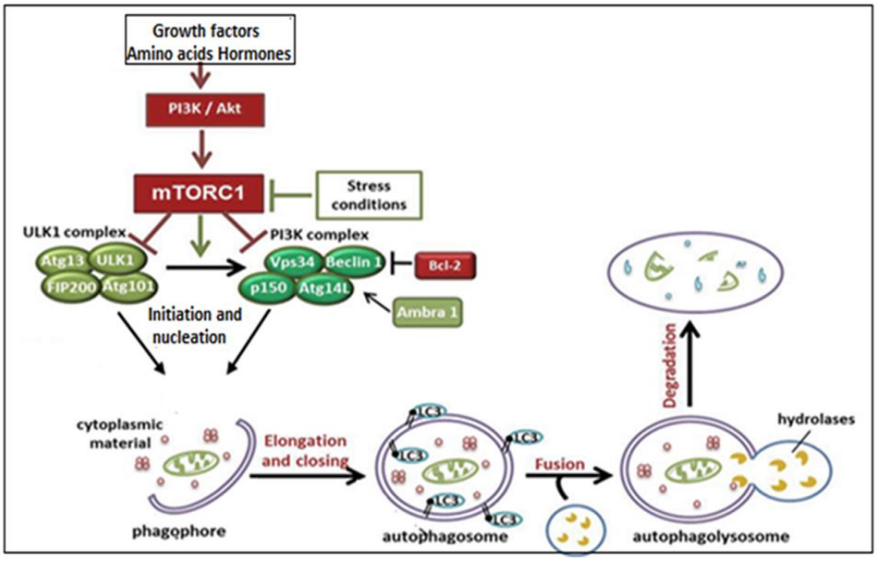

Fig 2: Schematic representation of signaling pathways involved in autophagy multistep regulation. In presence of growth factors and hormones, mTORC1 inhibits autophagy activation (Vomero M, Barbati C, Colasanti T, Perricone, C Novelli L, Ceccarelli F *et all*. Autophagy and Rheumatoid Arthritis: Current Knowledges and Future Perspectives. Front Immunol. 2018; 9: 1577.Published online 2018 Jul 18. doi: 10.3389/fimmu.2018.01577).

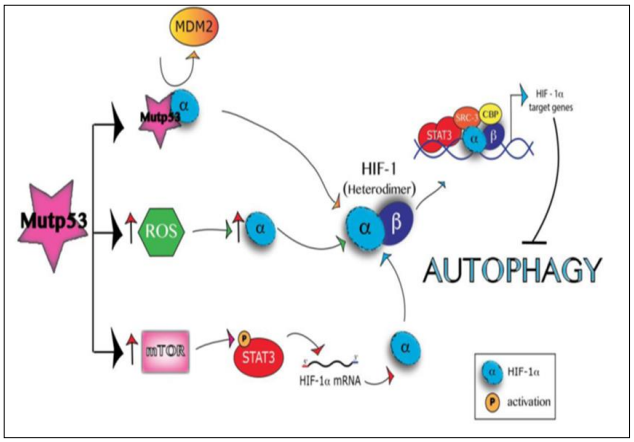

Fig 3: Schematic representation of the mechanisms at the basis of the stimulation of HIF-1 and HIF1- target genes by mutp53 resulting in autophagy inhibition. Regulation of HIF-1α pathway at different levels. (a) Growth factors related pathways; (b) pVHL related pathways; (c) FIH-1 pathway; (d) Mdm2-p53 mediated ubiquitination and proteasomal degradation pathway (. (Cordani M, Butera G, Pacchiana R, Donadelli M. Molecular interplay between mutant p53 proteins and autophagy in cancer cells. BBA - Reviews on Cancer 2016; 8: 1-34. doi:10.1016/j.bbcan.2016.11.003)

4. Protein p53-mediated autophagy: A prospective strategy for cancer therapy

The regulation of autophagy is quite intricate. It involves a series of signaling cascades including p53, known as the best-characterized tumor suppressor protein. Recent reports have indicated that p53 plays dual roles in regulating autophagy depending on its subcellular localization. Consistent with this role, p53 activity is compromised in a high proportion of all cancer types, either through mutation of the TP53 gene (encoding p53) or changes in the status of p53 modulators. Phosphorylation of the N-terminal end of p53 by the specific protein kinase disrupts Mdm2-binding and activates p-53 protein. Stimulation of HIF-1 and HIF1-target genes by mutant p53 protein results in autophagy inhibition. Thus, autophagy defects impair survival of apoptosis-defective tumor cells upon nutrient and oxygen limitation, leading to cell death by necrosis, which in turn is associated with inflammatory cell recruitment, cytokine production and nuclear factor κB (NFκB) activation, which has been linked to accelerated tumor growth [23], [Figure 4].

Integrity checkpoint, a molecular cascade that detects and responds to several forms of DNA damage caused by genotoxic stress. Oncogenes also stimulate p53 activation, mediated by the protein p14ARF, [24]. In carcinogenesis are involved a series of signaling cascades including p53, known as the best-characterized tumor suppressor protein**.** [25]. Recent reports have indicated that p53 plays dual roles in regulating autophagy depending on its subcellular localization. Nuclear p53 facilitates autophagy by trans activating its target genes, whereas cytoplasmic p53 mainly inhibit.

Many cancer cells contain inactivating mutations of p53, which explains why those cancer cells go on living [26]. The tumor suppressor p53 is a critical checkpoint protein in mammalian cells which is activated under genotoxic stress conditions, including DNA damage, hypoxia and oncogene activation, and responds by initiating tumor suppression mechanisms, such as cell cycle arrest, senescence and apoptosis. Under these conditions, p53 has been shown to trans activate autophagy-inducing genes and stimulate autophagy by inhibiting mTOR in an AMP-activated protein kinase (AMPK) [27]. Thus, positive regulators of apoptosis also induce autophagy, which is not very surprising given that both pathways are activated under similar stress conditions [28].

On the other hand, when autophagy is blocked, apoptosis is accelerated or apoptosis-defective cells undergo metabolic catastrophe and die by necrosis. Thus, cell fate in response to metabolic stress is determined by the functional status and the interaction between the stress-mitigating pathways of apoptosis and autophagy [29, 30]. Regarding cancer prevention, the function of autophagy as a protector of cellular homeostasis and genome integrity may be particularly important. Autophagy inhibition as a means to sensitize cancer cells to treatment has been validated in several studies*:* inhibition of autophagy by chloroquine, a lysosome-tropic agent that raises intra-lysosomal pH and

interferes with auto phagosome degradation within lysosomes, was shown to enhance the anticancer activity of the alkylating agent cyclophosphamide in a myc gene.Gene-induced lymphoma model and to induce p53-dependent cell death and tumor suppression in different myc-induced and ATM-deficiency lymphoma models [31].

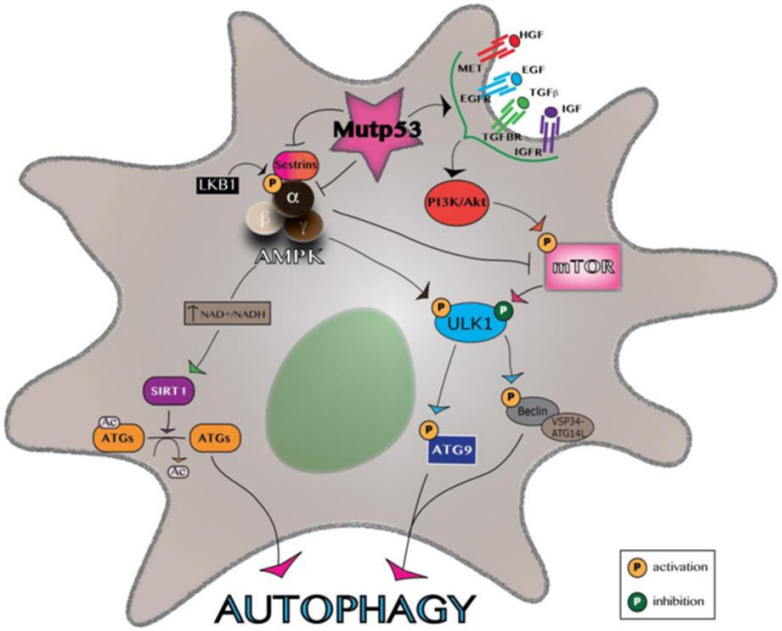

Fig 4*:* Schematic representation of molecular mechanisms by which p-53 mutant proteins inhibit AMPK and stimulate mTOR pathways that block autophagy. (Cordani M, Butera G, Pacchiana R, Donadelli M. Molecular interplay between mutant p53 proteins and autophagy in cancer cells. BBA - Reviews on Cancer 2016; 8: 1-34. doi:10.1016/j.bbcan.2016.11.003)

5. Role and regulation of autophagy in cancer

Increasing evidence reveals that autophagy dysfunction is associated with human diseases, such as cancer. Paradoxically, although autophagy is well recognized as a cell survival process that promotes tumor development, it can also participate in a caspase-independent form of programmed cell death. Induction of autophagy cell death by some anticancer agents highlights the potential of this process as a cancer treatment modality [32]. Dysfunctional autophagy contributes too many diseases. Large-scale genomic analysis of human cancers indicates that the loss or mutation of core autophagy genes (Atg) is uncommon, whereas oncogenic events that activate autophagy and lysosomal biogenesis have been identified. Thus, the role of autophagy in cancer is determined by nutrient availability, microenvironment stress, and the presence of an immune system [33].

Defective autophagy is implicated in tumorigenesis, as the essential autophagy regulator *B*eclin *1* is mono-allelically deleted in human breast, ovarian and prostate cancers, and *B*eclin $1^{+/-}$ mice are tumor-prone. Cell-autonomous mechanisms, involving protection of genome integrity and stability, and a non-cell-autonomous mechanism, involving suppression of necrosis and inflammation, have been discovered so far. Autophagy inhibition concurrently with chemotherapy or radiotherapy has emerged as a novel approach in cancer treatment, as autophagy-competent tumor cells depend on autophagy for survival under drug- and radiation-induced stress [34, 35, 36]. Lower Beclin 1 protein expression, as compared to Beclin 1 levels in normal adjacent breast tissue, was confirmed in a small series of human breast tumors but any correlation between allelic Beclin 1 loss, and thus defective autophagy, and clinical outcome in breast cancer remains to be investigated [37, 38, 39].

The mechanism by which autophagy defects lead to accelerated tumorigenesis is not readily apparent, especially given the well-documented pro-survival function of autophagy, which prolongs both normal and tumor cell survival under metabolic stress [40]. The protein kinases that are known to target this transcriptional activation domain of p53 can be roughly divided into two groups. A first group of protein kinases belongs to the MAPK family (JNK1-3, ERK1-2, p38 MAPK), which is known to respond to several types of stress, such as membrane damage, oxidative stress, osmotic shock, heat shock, etc. A second group of protein kinases (ATR, ATM, CHK1 and CHK2, DNA-PK, CAK, TP53RK) is implicated in the genome growth [41].

Regulators of apoptosis, such as anti-apoptosis protein Bcl-2/Bcl-xL and the pro-apoptosis protein family, BH3-only proteins, interact with Beclin 1 and can modulate autophagy. The anti-apoptotic protein Bcl-2 binds to Beclin 1 under non-stress conditions and inhibits autophagy in the ER, whereas the BH3-only protein Bad, BNIP3 and BH3 mimetics, such as ABT737 competitively inhibit the interaction between Beclin 1 and Bcl-2/Bcl-xL and stimulate autophagy. Constitutive activation of the PI3K/AKT/mTOR axis is a prototypic survival mechanism commonly encountered in human cancer [42].

Diverse cellular events, such as loss of the tumor suppressors phosphatase and tensin-homolog deleted on chromosome 10 (PTEN) and tuberous sclerosis complex (TSC) 1 and TSC2, amplification or mutation of class I PI3K, overexpression of AKT, constitutive activation of tyrosine kinase growth factor receptors and exposure to carcinogens, can all result in abnormal activation of this pathway and, ultimately, in autophagy suppression [43, 44], [Figure 5].

Two pathways are responsible for autophagy activation in response to starvation and ER stress: a) mediated by AMPK and Ca-MKK; b) involving p53 and damage-regulated

autophagy modulator (DRAM) activation. In this context, Ras exhibits an autophagy inhibitor (via class I PI3K activation) and an autophagy activator (via the RAF1/MEK1/2/ERK1/2 pathway) [45].

Several mutations or loss of PTEN function affect the activity of lipid phosphatase, which leads to the development of a variety of cancers. Of the three residues in the PTEN component, R-335 was found to be most important to interact with cellular membrane, in common with several other germline mutations, and was associated with inherited cancer [46], [Figure 6].

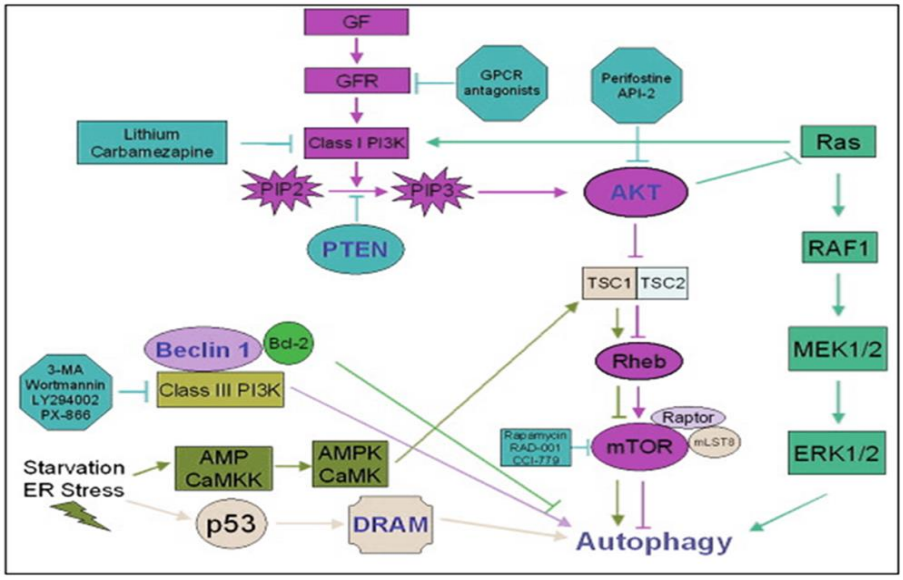

Fig 5: A first group of protein kinases belongs to the MAPK family (JNK1-3, ERK1-2, p38 MAPK), which is known to respond to several types of stress, such as membrane damage, oxidative stress, osmotic shock, heat shock, (Chen V, Karantza-Wadsworth V. Role and regulation of autophagy in cancer. Biochim. Biophys. Acta. 2009; 1793(9): 1516–23).

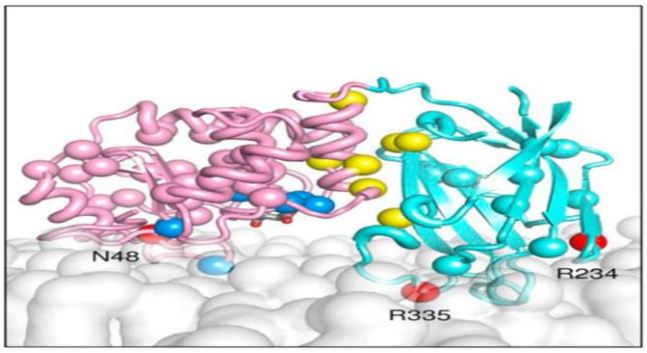

Fig 6*:* Fosfatase tensin-homolog (PTEN), in membrane location interacting in clinically important mutations (Lumb CN, Sansom MSP. HIF-1α pathway: role, regulation and intervention for cancer therapy. Defining the Membrane-Associated State of the PTEN Tumor Suppressor Protein. Acta Pharm Sin B. 2015 Sep; 5(5): 378-89).

6. Autophagy in Immunological Tolerance

Many studies demonstrated autophagy's contribution to the presentation of cytosolic antigens in association with MHC class II molecules, playing an important role not only in the acquired immune response but also in the maintenance of self-tolerance Mechanisms of central (in the primary lymphoid organs) and peripheral tolerance (in peripheral tissues) physiologically prevent immune responses to self-antigens [47]. During T cells development in the thymus, the recognition of peptide–MHC molecules on the surface of thymic epithelial cells (TECs) ensures that only thymocytes restricted to MHC molecules, and specific for non-self (foreign) antigens, will survive and continue their maturation. Emerging evidence indicates that autophagy contributes to the maintenance of the central tolerance mechanism [48].

Was recently revealed that there had been an alteration in the selection of the T cell receptor (TCR) restricted to MHC class II in mice transplanted with Atg5−/− thymus. Autophagy defects, in association with a consequent loss of self-tolerance, could be the reason of multiple signs of autoimmunity reported in these animals [49, 50]. These two opposite results probably depend on the different approach used to inhibit autophagy in the thymus, thus further investigations are necessary [51]. The involvement of autophagy in the presentation of self-antigens to immature T cells in the thymus was first analyzed by Kasai and colleagues, who showed a colocalization of LC3-II with the lysosomal compartment in which MHC–peptide complexes are formed. More recently, Aichinger *et al*. (2013) demonstrated that autophagy is essential for endogenous antigen-loading onto MHC class II of TECs for negative selection [52].

7. Autophagy in Lymphocytes Homeostasis

Peripheral immune cells play an important role in the perpetuation of autoimmunity by sustaining systemic inflammation status and by participating in the extension of joint destruction mechanisms. Many studies demonstrated that autophagy allows T and B lymphocytes to survive in conditions of nutrients deprivation or during stress stimuli [53]. Mice lacking Atg5 do not survive and have a reduction of peripheral T cells, showing how autophagy is essential for their survival. Since cytoplasmic calcium levels are essential for TCR-signaling pathways activation, autophagy-dependent calcium flux regulation could influence T lymphocytes activation. It has been demonstrated that CD4+ and CD8+ Atg5−/− cells are not able to properly proliferate following TCR stimulation [54]. Moreover, the inhibition of autophagy causes defects in T cell activation. In fact,

deletion of Atg7 results in decreased in IL-2 mRNA level and ATP generation, suggesting that autophagy is required to ensure appropriate energy level for T cell activation [55]. Similar data were obtained also on B lymphocytes, demonstrating that autophagy is essential for the maturation process and for the subsequent maintenance of B lymphocytes repertoire in the periphery [56, 57].

8. Autophagy inhibitors and therapeutic implications (therapeutic strategy)

According to multiple clinical trials, depending on the type of tumor and its stage of development, activation or inactivation of autophagy may contribute differently to tumorigenesis [58]. In this regard, if increased autophagy confers tumor resistance to death-inducing agents, its inhibition will allow an improved response to treatment [59]. There are two types of autophagy inhibitors: the early stage which blocks the formation of auto phagosomes (3-methyladenine-3 MA, wortmannin and LY294002) and late stage inhibitors present in the auto phagosome lysosome fusion and degradation phases (chloroquine-CQ, hydroxychloroquine- HCQ [60].

The study of preclinical models for the inhibition of pro-survival autophagy by genetic or pharmacological means has highlighted the possibility of tumor cell destruction and the onset of death of apoptotic cells [61], but also the fact that stress-induced autophagy in tumor cells can lead to resistance to treatment and tumor latency with eventual regeneration and tumor progression [62]. Several results from studies indicate that an optimal combination of inductors or inhibitors of autophagy (CO, HCO) with chemo or radiotherapy can be successful approaches, being already approved by the Food Dug Administration (FDA) [63, 64, 65].

Based on the fact that approximately 70% of clinical trials focus on the role of autophagy in cancer, it indicates that the potential for autophagy modulation in cancer treatment is promising. Clinical studies involving autophagy modulation in cancers have been designed to evaluate the effect of autophagy inhibition in combination with other conventional therapies. Only a small portion of the clinical studies on lung cancer, glioblastoma, pancreas, melanoma, breast and prostate cancer test CQ / HCQ as monotherapy. Current clinical studies (pancreatic adenocarcinoma) have shown that autophagy inhibition by HCQ as monotherapy is not sufficient [66]. Thus, the use of autophagy inhibitors in combination with chemotherapy can suppress tumor growth and trigger cell death in a higher percentage than both chemotherapy alone in vitro and *in vivo* Table 1, [67, 68].

The American Brain Tumor Consortium initiated a phase I / II trial for glioblastoma patients using HCQ, temozolomide and radiation highlighted the level of inhibition of HCQ dependent autophagy using an electron microscopy test on blood serum mononuclear cells [69]. Electronic micrographs of peripheral blood mononuclear cells from a glioma patient included in the Phase I/II study using temozolomide, radiation and hydroxychloroquine TO demonstrates the antitumor activity of this combination [Figure 7].

Induction of autophagy is another way that can help improve the effect of anticancer therapies when autophagy is cytotoxic by inducing cell death – apoptosis. They have been described in the literature and use a range of natural drugs / extracts to induce cell death mediated by autophagy in various cancer cells [70, 71, 72, 73, 74].

Some recent studies describe the important role of autophagy in regulating immune recognition and responding to tumor cell immunogenicity, involvement in tumor antigen, processing and subsequent activation of effector T cells [75]. In this direction, the adjuvant potential in stimulating the antitumoral immune response was experimentally demonstrated in mice with pulmonary carcinoma and melanoma [76].

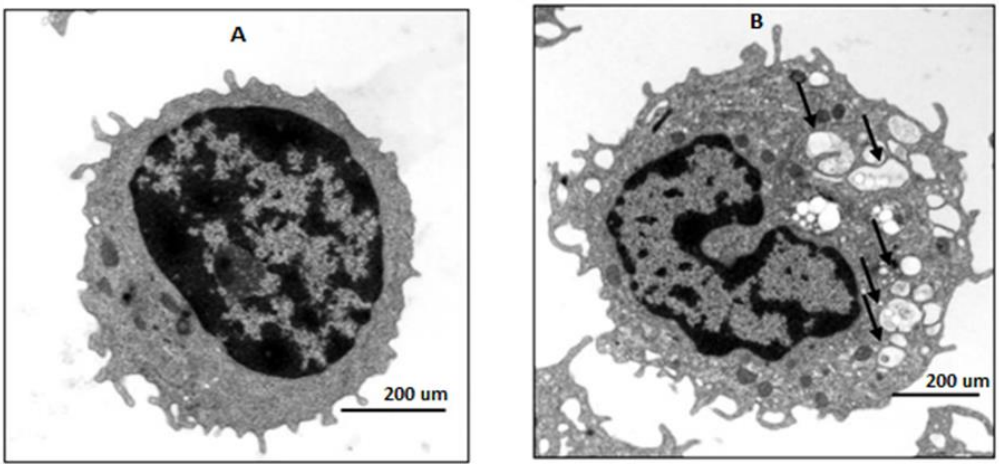

Fig 7: Pharmacodynamic assay for autophagy inhibition; Electron micrographs of peripheral blood mononuclear cells from a glioma patient enrolled on the phase I trial of temozolomide, radiation and hydroxychloroquine; (A) Pretreatment and (B) 3 weeks of combined therapy. Arrows: autophagic vesicles, scale bar 200 μm. (Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe T. Principles and Current Strategies for Targeting Autophagy for Cancer Treatment. Clin Cancer Res. 2011 Feb 15; 17(4): 654–66)

Table 1: Autophagy modulators apply in preclinical and clinical studies/ trials for cancer therapy. (Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe T. Principles and Current Strategies for Targeting Autophagy for Cancer Treatment. Clin Cancer Res. 2011; 17(4): 654–66).

Cancer type	Autophagy modulators	Model tested/clinical trial phase
Pancreas cancer		
Stage IIb or III pancreatic adenocarcinoma	HCQ + gemcitabine	Phase I/II
Advanced metastatic pancreatic adenocarcinoma	HCQ + gemcitabine/abraxane	Phase I/II

Metastatic pancreatic adenocarcinoma	HCQ; Gemcitabine, nab-paclitaxel	Phase II
Hepatocellular cancers HCC after liver transplantation	Sirolimus; RAD001; Sorafenib + HCQ	Phase II; Phase III; Phase II
Advanced HCC	Sirolimus; Sorafenib	Phase II/III,
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9. Concluding remarks

Autophagy defects are associated with susceptibility to metabolic stress, DNA damage, accumulation, genomic instability, and accelerated tumorigenicity, and these observations lead to the predictions that autophagy stimulation may preserve cellular fitness and genome integrity, and thus prevent cancer, and that tumors with chronic autophagy deficiency may be particularly sensitive to certain anticancer agents, such as DNA-damaging and anti-angiogenic drugs. Inhibition of autophagy concurrently with treatment may augment the antitumor activity, and thus the efficacy, of radiation and/or anticancer drugs. Ultimately, pharmacologic manipulation of autophagy for cancer prevention and treatment will depend on our ability to successfully recognize the functional status of autophagy in tumors and on the availability of specific autophagy modulators.

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11. Abbreviations

mTOR-mammalian target of rapamycin
mTORC1-mTOR complex 1
mTORC2-mTOR complex 2
Atg-Autophagy-related gene
PKB-protein kinase B
GAP1-general amino acid permease 1
AMPK 5'-AMP -activated protein kinase
hVps34-human ortholog of yeast vacuolar protein sorting 34
ULK1-UNC-51-like kinase 1
ULK2- UNC-51-like kinase 2
FIP200 focal adhesion kinase (FAK) family interacting protein of 200 kDa
GATE-16-Golgi-associated ATPase enhancer of 16 kDa
GABARAP-γ-aminobutyric acid (GABA) receptor associated protein
TGN-Trans-Golgi Network
ADM - adrenomedullin;
GLUTs -glucose transporter
HIF-1α, hypoxia-inducible factor-1α;
MAPK- mitogen-activated protein kinases;
Mdm2-mouse double minute 2 homolog;
MEK, -MAPK/ERK kinase;
MAP- kinase interacting kinase;
PI3K-phosphatidyl inositol-4, 5-bisphosphate-3 -kinase; TGF-α, transforming growth factor α; TGF-β3-transforming growth factor beta3
VEGF-vascular endothelial growth factor

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