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Autophagy and cancer

Hacer Ezgi KARAKAŞ, Devrim GÖZÜAÇIK*
Molecular Biology, Genetics, and Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabancı University, İstanbul, Turkey

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Abstract: Autophagy is an evolutionary conserved intracellular degradation and stress response mechanism that is mainly responsible for the breakdown and recycling of cytoplasmic materials, including long-lived proteins, protein aggregates, and damaged organelles. In this way, autophagy provides the cell with building blocks and allows the maintenance of homeostasis under stress conditions such as growth factor deficiency, nutrient deprivation, hypoxia, and toxins. Consequently, abnormalities of autophagy contribute to a number of pathologies ranging from neurodegenerative diseases to cancer. Autophagy was reported to have a dual role in cancer. Depending on cancer stage, autophagy seems to act as tumor suppressor or as a mechanism supporting tumor growth and spread. In this review, we provide a summary of the relevant literature and discuss the role of autophagy in cancer formation and chemotherapy responses.

Key words: Autophagy, stress, cancer, tumor, oncogenes, tumor suppressors, metastasis, cell death, chemotherapy

1. Introduction

Autophagy is a basic cellular event conserved in all eukaryotes from yeast to man. It serves as an intracellular quality-control mechanism recycling long-lived or misfolded/aggregate-prone proteins and damaged organelles, such as mitochondria. Moreover, under stress conditions, autophagy is upregulated in order to generate building blocks that are necessary for cellular survival (Kuma and Mizushima, 2010; Rabinowitz and White, 2010). Therefore, autophagy mainly serves as a stress-adaptation and survival mechanism (Su et al., 2013). However, under certain conditions, autophagy was reported to contribute to programmed cell death either indirectly through crosstalk mechanisms with apoptosis (Gozuacik and Kimchi, 2004; Oral et al., 2012; Rubinstein and Kimchi, 2012) or directly through autophagic cell death (Gozuacik and Kimchi, 2007).

Abnormalities of autophagy were reported in various diseases, including neurodegenerative diseases, lysosomal storage diseases, infections, metabolic diseases, and ischemia/reperfusion injury (Schneider and Cuervo, 2014). Cancer is no exception. The roles of autophagy in cancer formation, growth, ischemia resistance, metabolic changes, neovascularization, and even metastasis and tumor dormancy were reported (Gewirtz, 2009; Guo et al., 2013b; Murrow and Debnath, 2013). Moreover, autophagic capacity was shown to significantly affect responses of cancer cells to anticancer agents and radiation (Eberhart et al., 2013). In this review we will mainly focus on the role of autophagy in cancer formation and cancer therapy.

2. Basic mechanisms of autophagy

There are 2 major catabolic mechanisms in mammalian cells: the ubiquitin-proteasome system and the autophagy-lysosomal system. Proteasomes degrade mainly short-lived and soluble proteins following their regulated ubiquitinylation (Hershko and Ciechanover, 1998). In contrast, lysosomal pathways rely on the delivery of cytosolic contents into the lumen of the organelle, an event that is followed by their degradation by specific hydrolases. Here, ingested cytoplasmic targets might be various. The list includes long-lived proteins, old and damaged organelles (e.g., mitochondria and peroxisomes); misfolded, mutant, and/or aggregated proteins; and pathogens such as bacteria, viruses, and parasites. So far, 3 subtypes of autophagy have been described in mammals: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy (Klionsky and Schulman, 2014).

Microautophagy proceeds through direct invagination of the vacuolar/lysosomal membrane and engulfment of nearby cytosolic materials (Uttenweiler et al., 2005). In addition to cargo digestion, microautophagic vacuoles also contribute to the maintenance of organelle size and membrane composition (Mijaljica and Devenish, 2011).

* Correspondence: dgozuacik@sabanciuniv.edu

CMA is an autophagic pathway allowing selective degradation of a subset of soluble proteins in lysosomes. During CMA, proteins containing the recognition and targeting motif KFERQ are selectively directed to lysosomes by a chaperone complex, consisting of HSC70 (heat shock cognate of the HSP70 family) and its co-chaperones. On lysosomal membranes, interaction with lysosome-associated membrane protein LAMP2A allows translocation into the lumen of the organelle (Massey et al., 2006). CMA plays a crucial role in the elimination of oxidized proteins and misfolded proteins, and it contributes to starvation responses (Kaushik and Cuervo, 2012).

Macroautophagy (autophagy herein) is the major cellular autophagy pathway. It involves the engulfment of cytosolic cargo by double-membrane structures called isolation membranes that eventually close to form vesicles called autophagosomes or autophagic vesicles. Fusion of the outer membrane of autophagosomes with lysosomal membranes results in the delivery of autophagy cargos into the lysosome lumen and formation of autolysosomes. Hydrolases residing there degrade cargo contents to their building blocks, allowing their recycling back to the cytosol (Klionsky, 2007).

There are also selective and nonselective types of autophagy. For example, while starvation-induced autophagy is generally considered as a nonselective form of autophagy, engulfment of organelles (mitophagy, pexophagy, ribophagy, ER-phagy), protein aggregates (aggrephagy), lipid droplets (lipophagy), and pathogenic invaders (xenophagy) occur in a selective way (Beau et al., 2008; van der Vaart et al., 2008; Kraft et al., 2009). Selective autophagy receptors include p62/SQSTM1 (sequestosome 1), NDP52 (nuclear domain 10 protein 52), NBR1 (neighbor of BRCA1 gene 1), BNIP3L (BCL2/adenovirus E1B 19-kD protein-interacting protein 3-like), and OPTN (optineurin) (Shaid et al., 2013).

Autophagy can be induced by several factors such as starvation, hypoxia, or pathogens (Levine and Kroemer, 2008; Mizushima and Levine, 2010; White et al., 2010; Wong and Cuervo, 2010). Autophagy proteins were initially identified in yeast during genetic studies, and at least 35 of them have been functionally analyzed (Nakatogawa et al., 2009). The basic autophagy system was shown to be preserved in mammals. Most of the autophagy-related (or ATG) genes play a role during autophagosome nucleation, expansion, vesicle closure, and finally fusion with lysosomes and degradation (Mizushima et al., 2011). We will now introduce major stages of autophagy and summarize important molecular events involved in their regulation.

2.1. Autophagosome nucleation

The mTORC1 protein kinase complex is the central nutrient sensing pathway in cells and a key regulator of autophagy (Ravikumar et al., 2004). Under nutrient-rich conditions, mTOR kinase regulates the phosphorylation of autophagy proteins ULK1/2 (UNC51-like kinase 1, mammalian homolog of yeast Atg1) and ATG13, and blocks autophagy (Figure 1). Stress conditions including hypoxia and growth factor or nutrient deprivation lead to the inactivation of the mTOR kinase, resulting in the activation of ULK1/2. This event is followed by a direct phosphorylation of ATG13 and FIP200 (FAK family kinase-interacting protein, 200 kDa) by ULK1/2 and stimulation of autophagy. The active ULK1/2 complex (ULK1/2-ATG13-FIP200-ATG101) then translocates to autophagosome nucleation centers and triggers consecutive stages.

Another key autophagy complex is the phosphatidylinositol 3-kinase (PI3K) complex. In the mammalian system, this complex is composed of ATG14L (also known as ATG14 and Barkor), BECN1 (Beclin 1, yeast Atg6 ortholog), hVPS34 (vacuolar protein sorting protein 34-VPS34-homolog), and hVPS15 (Figure 1) (Itakura et al., 2008; Sun et al., 2008; Zhong et al., 2009; Matsunaga et al., 2010). In the PI3K complex, hVPS34 is the kinase and its activity and localization is controlled by other components of the complex. For example, ATG14L was shown to recruit the complex onto the outer leaflet of the endoplasmic reticulum (ER) membranes. BECN1 is an important regulator of hVPS34 activity. In fact, PI3K complex activity is responsible for the accumulation of autophagy-related lipid molecules, namely phosphatidylinositol 3-phosphate (PI3P), in autophagosome initiation/nucleation sites. PI3P islands that form on the outer membrane leaflet of the ER serve as landing pads for the recruitment of autophagy effectors containing phosphoinositide-binding domains, such as FYVE finger domains or PH domains (for example, WIPI/Atg18 proteins). Additionally, AMBRA1 (activating molecule in BECN1-regulated autophagy), UVRAG (UV irradiation resistance-associated gene), and BIF1 (endophilin B1) were shown to regulate the complex, whereas RUBICON (run domain- and cysteine-rich domain-containing BECN1-interacting protein) protein and antiapoptotic BCL2 (B-cell leukemia 2) proteins including BCL2, BCLXL/BCL2L1, and MCL1 were reported as inhibitors (Funderburk et al., 2010). Activation of ULK1/2 was reported to be important for the localization of the PI3K complex to autophagic membranes, placing the ULK1/2 complex upstream of the PI3K (Matsunaga et al., 2010). Additionally, AMBRA1 was shown to release a fraction of the PI3K complex from its association with dynein and allow its localization to autophagosomal sites in a ULK1/2-dependent manner (Mizushima et al., 2011).

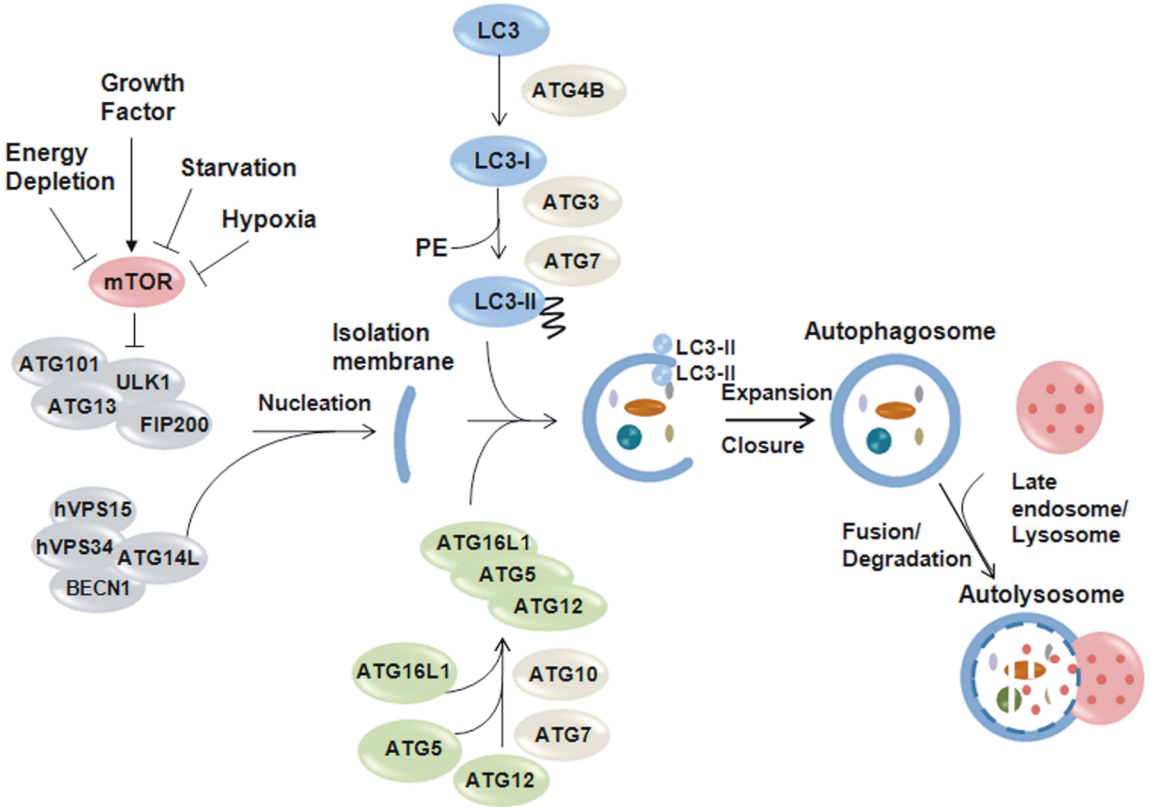

Figure 1. Key morphological steps of autophagy in mammals. After mTOR inhibition (for example, with energy depletion, starvation, or hypoxia), the autophagy process begins with the nucleation of the isolation membrane. The 2 ubiquitin-like conjugation systems ATG16L1-ATG5-ATG12 and LC3-II participate after their activation in the expansion of the double membrane and the closure of the isolation membrane. Once it is completed, the structure is called an autophagosome. In mammalian systems, the autophagosome may fuse with a late endosome to form an amphisome, or it may fuse directly with a lysosome to form an autolysosome. Once the cargos are degraded, the products are released to the cytosol to be reused by the cell.

2.2. Autophagosome expansion and closure

Two ubiquitylation-like systems are known to mediate autophagosome expansion: the Atg12-Atg5 conjugation system, and the microtubule-associated protein 1 light chain 3 (Atg8 in yeast and MAP1LC3 or shortly LC3 in mammals) lipidation system (Figure 1) (Kirisako et al., 2000; Mizushima et al., 2001). In the first system, Atg12 is activated by Atg7 (E1-like enzyme), then transferred to Atg10 (E2-like enzyme). Finally, Atg12 is covalently conjugated to the Atg5 protein (Mizushima et al., 2001). Subsequently, the Atg12-Atg5 conjugate interacts with Atg16 (ATG16L1 in mammals) to form a complex of higher molecular weight (Figure 1) (Fujita et al., 2008). In the yeast system, the Atg12-Atg5-Atg16 complex (ATG12-ATG5-ATG16L1 complex in mammals) is mainly localized to the surface of autophagosomes, and it dissociates from membranes following autophagic vesicle completion (Mizushima et al., 2001; Itakura and Mizushima, 2010).

In the second system, in order to expose a glycine residue at its C terminal, LC3 protein is cleaved by ATG4B proteins soon after its synthesis. Processed protein stays in the cytosolic free form called LC3-I. Subsequently, LC3-I is activated by ATG7 (E1-like enzyme), transferred to ATG3 (E2-like enzyme), and covalently linked to a lipid molecule, namely phosphatidylethanolamine (PE). The lipid-conjugated LC3 form is called LC3-II and it is localized both on isolation membranes and autophagosomes (Satoo et al., 2009). Hence, demonstrations of LC3 lipidation and LC3-I to LC3-II conversion, as well as recruitment of cytosolic LC3 to autophagosomes, are widely used as reliable markers of autophagic activity. The ATG12-ATG5-ATG16L1 complex is thought to act as an E3-like enzyme for LC3 lipidation, creating a direct link and interdependency between the 2 systems (Otomo et al., 2013). LC3 lipidation is necessary for the elongation and completion of autophagic vesicles. Moreover, LC3 proteins

that are located in the inner membrane of autophagosomes serve as anchors for selective autophagy receptors, such as p62/SQSTM1, helping the recruitment of cargo proteins. GABARAP (GABA-A receptor-associated protein), GABARAPL1 (GABA-A receptor-associated protein-like protein 1), and GABARAPL2/GATE16 (GABA-A receptor-associated protein-like protein 2) are other homologs of LC3 in mammals, and specific roles of these proteins and functional overlap between them are under investigation.

2.3. Fusion with lysosomes and degradation

In order to recycle cargo including long-lived proteins or damaged organelles, and to provide the cell with nutrients under stress conditions, autophagosomes fuse with late endosomes or lysosomes and lead to the "digestion" of structural constituents into their building blocks (for example, protein macromolecules are digested into amino acid building blocks) (Figure 1). Vesicular structures that are formed by autophagosome-lysosome fusion are called autolysosomes. Several proteins play a role during fusion events, including lysosomal membrane proteins such as the V-ATPase complex and LAMPs (lysosomal-associated membrane proteins) (Eskelinen et al., 2003) and small GTPases such as RAB7 (Ras-associated protein 7), RAB22, and RAB24 (Bucci et al., 2000; Kauppi et al., 2002; Munafo and Colombo, 2002). Additionally, ATG binding proteins such as UVRAG and cytoskeletal components, especially microtubules that direct autophagosomes towards lysosomes, were shown to be important for fusion (Jahreiss et al., 2008). SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins also play an important role (Fader et al., 2009). Upon induction of autophagy, Stx17 (syntaxin17) is recruited to completed autophagosomes and interacts with cytosolic Qbc-SNARE SNAP (synaptosomal-associated protein)-29 and the SNARE proteins VAMP7 (vesicle-associated membrane protein 7) or VAMP8, which are located on lysosomal membranes. This process drives the fusion between the outer membrane of autophagosomes with lysosome membranes (Itakura et al., 2012). Lysosomal lumen that is packed with lysosomal hydrolytic enzymes, including cathepsin proteases and lipases, degrades both autophagosome inner membranes and cargos, a process that is followed by recycling of digested constituents back to the cytosol.

3. Regulation of autophagy

3.1. Regulation of autophagy by signaling pathways

3.1.1. mTOR pathway

Various signals including growth factor signals, amino acids, and energy status are integrated by mTOR complexes (Ravikumar et al., 2004). mTOR kinase takes part in 2 functional complexes, namely mTORC1 and mTORC2. The former complex is a major regulator of autophagy, and the latter mainly regulates cytoskeleton reorganization and migration. The mTORC1 complex is sensitive to the drug rapamycin, and it consists of the mTOR protein kinase, RAPTOR (regulatory associated protein of mTOR), mLST8/GβL, and PRAS40 (proline-rich Akt substrate of 40 kDa) (Kim et al., 2003).

As explained above, mTORC1 controls the formation of autophagy-related proteins complexes containing ULK1/2 and ATG13 (Jung et al., 2009). Indeed, inhibition of mTORC1 leads to an increase in ULK1/2 kinase activity, leads to the phosphorylation of ATG13 and FIP200, and triggers activation of autophagy (Hosokawa et al., 2009).

3.1.2. AKT/PKB pathway

Binding of growth factors or hormones such as insulin to receptors on the cell surface activates class I PI3K proteins. Activated PI3K proteins convert phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3) and recruit AKT/PKB to the plasma membrane. Active AKT/PKB contributes to cell growth by enhancing protein translation through mTOR-related phosphorylation and activation of downstream ribosomal protein S6 kinase, 70 kDa (RPS6KB/P70S6K), and phosphorylation of translation initiation factor 4EBP1 (EIF4EBP1) and others (Klionsky et al., 2005). AKT/PKB-mediated stimulation of mTORC1 activity results in autophagy inhibition.

A well-studied connection between AKT/PKB and mTOR is dependent on the TSC1/2 proteins. The TSC1/TSC2 complex negatively regulates mTORC1 through inactivation of the mTOR-activator RHEB proteins (Guertin and Sabatini, 2007). AKT/PKB was shown to directly phosphorylate TSC2 and induce inactivation of the TSC1/TSC2 complex, relieving mTOR from RHEB-dependent repression.

Recently, an AKT/PKB-mediated phosphorylation of the autophagy protein BECN1 was reported to inhibit autophagy through sequestration of the protein by a 14-3-3/vimentin intermediate filament complex (Wang et al., 2012).

3.1.3. AMPK (AMP-activated kinase)

The LKB1-AMPK pathway links metabolic status to cell growth control by sensing intracellular energy levels in cells (Shackelford and Shaw, 2009). In mammalian cells, AMPK activity is important for stress responses, including starvation responses (Meley et al., 2006). Increased AMP/ATP ratio reflecting a deficit in cellular energy status is a major signal that activates AMPK (Corradetti et al., 2004). Active AMPK inhibits mTORC1 signaling by interfering with the activity of the GTPase RHEB (Meijer and Codogno, 2004) and by activating eEF2-kinase, which controls protein translation (Wu et al., 2006). RHEB inactivation was shown to be a result of

TSC2 phosphorylation by AMPK, an event that stimulates TSC2 activity and that turns off mTORC1, activating autophagy (Inoki et al., 2003). AMPK can also stimulate autophagosome formation by phosphorylating and activating ULK1 (Egan et al., 2011; Kim et al., 2011).

3.1.4. BECN1 and the PI3K complex

BECN1 is an important regulator of autophagy. It is a component of the PI3K complex that plays a role during the initiation/nucleation phase of autophagosome formation. In fact, as mentioned previously, BECN1 regulates hVPS34 PI3K activity and PIP3P levels in autophagosome nucleation sites. Strikingly, antiapoptotic proteins of the BCL2 family, including BCL2, $BCLX_L$, and MCL1, were shown to bind BECN1 through its BH3 (BCL2 homology domain 3) domain and to inhibit autophagy (Pattingre et al., 2005). BECN1-BCL2 protein interaction may be interrupted as a result of the phosphorylation of BCL2 proteins by the stress kinase JNK-1 (c-Jun N-terminal kinase 1) (Wei et al., 2008). Inversely, DAPK (death-associated protein kinase) can phosphorylate BECN1, reducing its BCL2 protein binding affinity (Zalckvar et al., 2009). Similarly, proapoptotic proteins BAD and BAX, and BH3 mimetics, led to the dissociation of BECN1-BCL2 complexes (Maiuri et al., 2007; Luo and Rubinsztein, 2010).

3.2. Regulation of autophagy through transcriptional and posttranscriptional mechanisms

3.2.1. NFKB

Nuclear factor kappa-B (NFKB) is a family of transcription factors that are involved in a variety of biological responses ranging from immune responses and inflammation to cancer (Karin, 2006). NFKB was shown to have a dual role in autophagy regulation. The promoter region of *BECN1* was reported to contain an NFKB binding site (Copetti et al., 2009), and p65/RelA from the NFKB family upregulated *BECN1* expression and induced autophagy in HEK293 and U2OS cells (Copetti et al., 2009). In contrast, NFKB activation reduced autophagy by inhibiting BECN1 and ATG5 expression in macrophages (Schlottmann et al., 2008). Moreover, inhibition of NFKB was shown to potentiate starvation-induced autophagy in myelodysplastic syndrome cells (Fabre et al., 2007). Hence, the NFKB system seems to activate cell-type-dependent signals that lead to opposing outcomes in autophagy activation.

3.2.2. HIF1

The transcription factor HIF1 (hypoxia-inducible factor 1) is a master regulator of gene expression under hypoxic conditions (Manalo et al., 2005). Hypoxia-mediated activation of HIF1 induced autophagy-related BH3 domain proteins BNIP3 (BCL2/adenovirus E1B 19-kDa protein-interacting protein 3) and BNIP3L (BCL2/adenovirus E1B 19-kDa protein-interacting protein 3-like) proteins (Bellot et al., 2009). BNIP3 and BNIP3L could bind to BCL2 and disturb BECN1-BCL2 protein interaction. BCL2-free BECN1 was then able to activate autophagy.

3.2.3. FOXO proteins

In response to growth and insulin stimulation, 3 proteins of the FOXO (Forkhead box O) family of transcription factors, namely FOXO1, FOXO3, and FOXO4, are regulated by AKT phosphorylation. If AKT is blocked, FOXO proteins are translocated into the nucleus and transactivate their target genes (Salih and Brunet, 2008). FOXO3 can especially induce the expression of several autophagy genes, such as *LC3*, *GABARAPL1 BNIP3*, and *BNIP3L* (Mammucari et al., 2007).

3.2.4. p53

The p53 transcription factor is defined as the guardian of genome, and it is one of the key tumor suppressor proteins. p53 prevents oncogenic formation by orchestrating pathways including DNA damage responses, cell cycle, apoptosis, and oncogene-related proliferative pathways (Vousden, 2009). Recent studies implicated p53 in autophagy regulation. Under stress conditions, p53, accumulated in the nucleus, transactivated several autophagy modulating genes such as *DRAM* (damaged-regulated autophagy modulator) and *TIGAR* (TP53-induced glycolysis and apoptosis regulator). *DRAM* was shown to stimulate accumulation of autophagosomes (Crighton et al., 2006) while, *TIGAR* inhibited autophagy through modulation of the glycolytic pathway and intracellular reactive oxygen species (ROS) levels (Bensaad et al., 2009). p53 also has a cytosolic fraction, and, interestingly, the cytoplasmic form of p53 had an inhibitory effect on autophagy (Tasdemir et al., 2008).

3.2.5. ER-stress-related responses

Imbalances of cellular calcium metabolism, transfer of mutant and/or abnormal proteins into the lumen, glycosylation problems, hypoxia, and so on cause accumulation of unfolded proteins in the ER and stimulate ER-specific stress pathways called the unfolded protein response mechanism or the ER-stress response. Among these stress response mechanisms, phosphorylation of the translation initiation factor eIF2α by PERK is a key event that turns off cap-dependent protein translation. However, stress-specific transcriptions factors such as ATF4 (activating transcription factor 4), ATF6 (activating transcription factor 6), XBP1 (X box-binding protein 1), and CHOP (CCAAT-enhancer-binding protein homologous protein) are activated under these conditions. ER-stress response-related transcription factors including ATF4 and CHOP were involved in the upregulation of autophagy proteins such as LC3 and ATG5 and helped sustain autophagy responses under continuous stress

conditions (Kouroku et al., 2007; Rouschop et al., 2010). Additionally, calcium release from the ER during stress was reported to activate autophagy activation by the death-inducing kinase DAPK (Gozuacik et al., 2008).

3.2.6. Posttranslational modifications of autophagy proteins

Autophagy proteins undergo various posttranslational modifications including phosphorylation, oxidation, acetylation, and proteolytic cleavage. For example, during starvation-induced autophagy, ATG4A and ATG4B were oxidized from their cysteine residue (Scherz-Shouval et al., 2007). ATG5, ATG7, LC3, and ATG12 were deacetylated (Lee and Finkel, 2009) and ATG5, ATG7, and BECN1 were shown to be cleaved by calpain (Yousefi et al., 2006; Kim KW et al., 2008; Xia et al., 2010). Moreover, Atg4D, ATG3, and BECN1 were substrates for caspases (Betin and Lane, 2009; Luo et al., 2010; Oral et al., 2012). The majority of these modifications were shown to modulate autophagic responses of cells.

3.2.7. microRNA (miRNA) regulation of autophagy

miRNAs are small, endogenous noncoding RNA molecules involved in the posttranscriptional regulation of mRNAs, regulating protein levels. miRNA can bind specific response elements found in noncoding regions of at least dozens of mRNAs. In this way, miRNAs control stability and translation mRNAs, reshape protein expression profiles, and modulate and coordinate a number of important biological events, including cell proliferation, differentiation, and death. Recent studies underlined the importance of miRNA-related control of autophagy. miRNAs including MIR30A, the MIR376 family, and MIR181A were shown to target key autophagy proteins in various stages of autophagosome formation and control autophagic responses under various stress conditions (Zhu et al., 2009; Korkmaz et al., 2012; Korkmaz et al., 2013; Tekirdag et al., 2013). The list of autophagy-related targets of miRNAs is growing and it includes important autophagy proteins ATG4, ATG5, and BECN1 (Tekirdag et al., 2014).

4. Role of autophagy in cancer

Accumulating evidence in the literature indicates that abnormalities of autophagy play a role in cancer formation and development. However, the exact nature of this link between autophagy and cancer seems to be context-dependent. Figure 2 summarizes the role of autophagy in the early versus late stages of cancer. In early stages involving cancer formation, autophagy plays a tumor-suppressor role through degradation of dysfunctional organelles such as mitochondria, limitation of ROS production and prevention of damaged DNA accumulation, and prevention of genomic instability (Mathew et al., 2009b). However, at later stages, and especially in some contexts (e.g., oncogenic K-RAS pathway activation), autophagy acts as a survival pathway supporting tumor growth under unfavorable metabolic conditions (scarce nutrient supply, hypoxia, and high energy/oxygen demand), in spite of abnormal and insufficient tumor vascularization (Levine, 2007). Additionally, autophagy provides resistance to anoikis, a special type of apoptosis that cells activate following detachment from the basal lamina, a property that facilitates tumor cell evasion from primary sites, invasion, and spread (Fung et al., 2008). Cancer cell dormancy and chemotherapy resistance were also linked to the autophagic capacity of cells (Gewirtz, 2009). The link between cancer and autophagy is further supported by the fact that some autophagy-related proteins also function as tumor suppressors or oncogenes (Gozuacik and Kimchi, 2004; Morselli et al., 2009). In the following sections, we will discuss relevant literature and provide examples about the dual role of autophagy in cancer.

4.1. Tumor suppressor role of autophagy

Defective autophagy was linked to several diseases, including cancer. Manipulation of autophagy genes in mice provided important clues as to the role of autophagy in cancer. For example, *Atg4C*-deficient mice not only have reduced starvation-related autophagy responses but also have increased susceptibility to the development of chemical carcinogen-induced fibrosarcomas (Marino et al., 2007). Mice with heterozygous *Atg5* deletion, as well as mice with homozygous *Atg7* deletion, developed benign liver adenomas (Takamura et al., 2011). Mice with a heterozygous disruption of the *Beclin 1* gene (mouse ortholog of human *BECN1*) were generated by 2 independent laboratories (Qu et al., 2003; Yue et al., 2003). In both lines of genetically modified mice, *Beclin 1* haploinsufficiency resulted in the formation of spontaneous tumors, including lung adenocarcinomas, hepatocellular carcinomas (HCCs), and lymphomas. The tendency to develop HCCs was increased when these mice were crossed with liver cancer-prone mice (Yue et al., 2003). Similarly, *Uvrag* or *Bif1* heterozygous deletion also enhanced cancer susceptibility in mice (Liang et al., 2006; Takahashi et al., 2007). These findings underline that tumor suppression is a shared property of different components of the autophagic pathway, rather than being a phenotype unique to the disruption of an individual autophagy gene. In line with these findings, mutations or deletions of several autophagy genes were reported in human cancers (see below).

Accumulating data in the literature provide clues about how suppression of autophagy may lead to cancer. It is now well established that autophagy-defective cells cannot recycle organelles, cytoplasmic long-lived proteins, and protein aggregates. As a result, these cells accumulate damaged mitochondria, and their cytoplasms

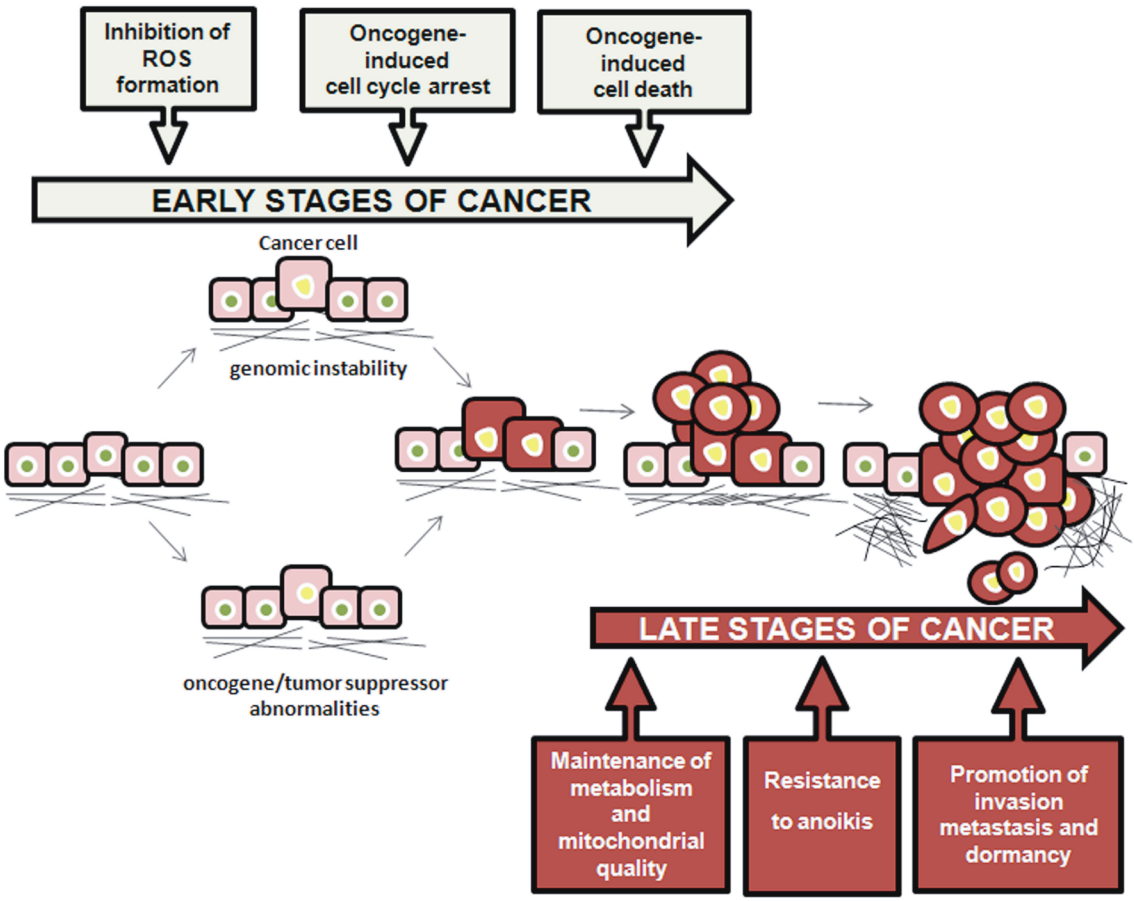

Figure 2. Dual role of autophagy in cancer. Due to genomic instability or abnormalities in oncogene/tumor suppressors, some cells may change their character and start to grow abnormally. These genetic instabilities may occur due to dysfunctional mitochondria and increased production of ROS in the cell. At early stages of the cancer, cells can eliminate the dysfunctional mitochondria by a selective type of autophagy called mitophagy. Oncogene-induced senescence may also be another mechanism to restrict cell growth and proliferation during oncogenic stress. Moreover, autophagy-related cell death can prevent cancer cell growth. On the other hand, at later stages of cancer, autophagy can act as a survival pathway to support tumor growth under metabolic stresses such as hypoxia or nutrient deprivation and maintain mitochondria function. Moreover, autophagy provides resistance to anoikis (detachment-induced apoptosis) and facilitates invasion, metastasis, and cancer cell dormancy.

are packed with deposits of p62 protein (and possibly other proteins). Hence, together with energy problems and metabolic stress, autophagy-defective cells show a remarkable increase in ROS levels, making them prone to DNA double-strand breaks, gene amplifications, and chromosome rearrangements (Mathew et al., 2007, 2009a). These results were confirmed both in 3D culture assays and in vivo in mice (Karantza-Wadsworth et al., 2007; Mathew et al., 2009a). Moreover, there is evidence that autophagy deficiency and p62 deregulation might be associated with the suppression of the canonical NFKB pathway and accelerated oncogenesis through the noncanonical pathway (Mathew et al., 2009a). Thus, autophagy acts as a protection mechanisms against proteotoxicity and genotoxic stress, and it helps maintain genome integrity and proper cellular signaling.

During cancer formation, cells generally inactivate apoptosis pathways and become less sensitive to apoptotic stimuli. In a context where apoptosis pathways were blocked, autophagy incompetent cells were reported to be more prone to undergo necrosis during metabolic stress, a cell death that results in the extracellular release of cellular contents and that triggers inflammatory responses. For example, activation of autophagy-suppressor AKT pathway or loss of a *Beclin 1* allele in combination with the overexpression of BCL2 made cells more susceptible to necrosis during metabolic stress and resulted in inflammatory cell infiltration to tissues in vivo (Degenhardt et al., 2006). In fact, necrosis and inflammation correlate with enhanced tumorigenic potential in solid tumors. Hence, limitation of metabolic stress and prevention of necrotic cell death and inflammation may also be

factors contributing to the tumor-suppressor function of autophagy in vivo.

Senescence is the status of cell cycle arrest with an active metabolism. Together with apoptosis induction, senescence is 1 of the 2 major anticancer mechanisms that are activated in normal cells in response to oncogene activation. In an interesting study, Young et al. (2009) showed that autophagy was activated in human diploid fibroblast cells during oncogene-induced senescence. Indeed, expression of oncogenic Ras rapidly activated autophagy in this system. Autophagy was required for the establishment of senescence arrest since RNAi-mediated depletion of *Atg5* or *Atg7* facilitated the ability of cells to escape from senescence and delayed senescence-associated cytokine production (Young et al., 2009). In line with these results, in an in vivo model, senescence marker-positive regions of tumor tissues contained more LC3-positive puncta than proliferative regions, indicating that autophagic activity was higher in senescent cells (Young et al., 2009). These results indicate that oncogene-induced senescence may be another autophagy-related tumor-suppressor mechanism that restricts normal cell growth and proliferation during oncogenic stress, preventing further genomic impairments.

In contrast with its role as a stress response mechanism for cell survival, under certain in vitro contexts and in vivo conditions, autophagy might lead to cell death (Gozuacik and Kimchi, 2007). Several tumor suppressor and death-related proteins, including DAPK, DRP1, ZIP, and a p19ARF form, triggered nonapoptotic and autophagy-dependent cell death (Inbal et al., 2002; Shani et al., 2004; Reef et al., 2006; Gozuacik et al., 2008). In analogy with apoptosis, it was proposed that a defect in "autophagy-related cell death" or "autophagic cell death" could contribute to cancer formation (Gozuacik and Kimchi, 2007). For example, introduction of oncogenic H-Ras protein to nontransformed cells was shown to activate nonapoptotic cell death with autophagic characteristics (Elgendy et al., 2011). In this way, autophagy was shown to block clonogenic survival of transformed cells and prevent tumor formation. H-Ras-induced autophagic cell death was blocked by silencing the BH3-only protein NOXA or autophagy protein BECN1. BCL2 proteins that sequester BECN1 were also shown to block the effect (Elgendy et al., 2011).

4.2. Tumor-promoting role of autophagy

In early stages of carcinogenesis, autophagy seems to mainly exert a tumor-suppressor function. However, in established tumors, autophagy might act as a mechanism supporting the survival of cancer cells**.** Cancer cells face a number of stress factors, including hypoxia, pH changes, growth factor deprivation, nutrient insufficiency and irregular nutrient support, and inappropriate extracellular matrix signals. To cope with these harsh and unfavorable conditions, cancer cells activate autophagy, allowing them to maintain cellular biosynthesis and ATP levels and survive. Indeed, cancer cells in the interior parts of solid tumors, where the above-mentioned conditions are more dramatic, had especially elevated levels of autophagy compared with those at tumor margins (Degenhardt et al., 2006). In in vivo models of cancer, autophagy helps sustain tumor metabolism, maintains mitochondrial quality and mitochondria function, controls reactive oxygen production, and allows nutrient recycling (Guo et al., 2013a). For example, autophagic activity was elevated in pancreas cancer cells and in tumor specimens, and suppression of autophagy resulted in tumor regression and extended lifespan in pancreas cancer xenografts and genetic mouse models (Yang et al., 2011). In K-Ras-driven transgenic cancer models, autophagy supported tumor survival and growth by preserving mitochondrial energy production and metabolism (Guo et al., 2011). Interestingly, in lung tumors developing on a mutant K-Ras background, progression of tumors from adenomas to adenocarcinomas was blocked, and knockout of the autophagy gene *Atg7* in this context led to the formation of benign oncocytomas instead (Guo et al., 2013a). Similarly, autophagy-deficiency impaired tumor growth in B-Raf-driven lung tumors (Strohecker et al., 2013). In a mammary-specific *Palb2* gene knock-out mouse model of breast cancer, autophagy impairment by allelic loss of *Beclin 1* delayed tumor development in a p53-dependent manner (Huo et al., 2013).

Moreover, autophagy was reported to promote tumor invasion. In epithelial cells transformed with oncogenic Ras, depletion of autophagy genes resulted in a decrease in motility and invasion capacity in 3-dimensional cultures and reduced pulmonary metastases in vivo (Lock et al., 2014). Autophagic activity was also important for the survival of dormant cancer cells in vivo (Lu et al., 2008).

All these data underline the importance of autophagy in the maintenance of tumor metabolism and progression to malignancy, and even metastasis, in different biological scenarios.

4.3. Cancer-related changes in autophagy genes/proteins

Accumulating data in the literature provides evidence that genes and proteins of the autophagy machinery and other autophagy regulators might be mutated and/or dysregulated in cancer (Table). In line with the experimental data, studies with human tumor specimens and comparisons with normal tissues point to a context of the tumor-grade- and tumor-type-dependent nature of the autophagy/cancer relation. Most of these studies are correlative; nevertheless, autophagy gene mRNA and/or protein expression changes and mutations were observed in different tumor series.

Table. Cancer-related changes in core autophagy genes and proteins.

Gene/protein	Cancer-related changes	References
ULK1 (Atg1)	Increased expression in esophageal squamous cell carcinomas and hepatocellular carcinomas Reduced expression in breast cancers	(Jiang et al., 2011; Tang et al., 2012; Xu et al., 2013; Jiang et al., 2014)
ATG2B	Frameshift mutations in gastric cancers and colorectal cancers	(Kang et al., 2009)
ATG3	Reduced expression in myelodysplastic syndrome patients with leukemic evolution	(Ma et al., 2013a)
ATG4B	Increased expression in CD34(+) chronic myeloid leukemia cells	(Rothe et al., 2014)
ATG5	Low-frequency frameshift mutations in gastric cancers and colorectal cancers Genetic variations may correlate with thyroid carcinoma susceptibility Reduced expression in natural killer cells and in gastric, colorectal, and hepatocellular carcinomas Increased expression in CD34(+) chronic myeloid leukemia cells	(Iqbal et al., 2009; Kang et al., 2009; An et al., 2011; Cho et al., 2012; Plantinga et al., 2014; Rao et al., 2014; Rothe et al., 2014)
BECN1 (Atg6)	Reduced expression in breast cancers, nonsmall cell lung cancers, renal clear cell carcinomas, brain tumors, cervical squamous cell carcinomas, hepatocellular carcinomas, ovarian cancers, osteosarcomas, melanomas, and glioblastomas Mutations in ovarian cancers, human breast cancers, prostate cancers Reduced expression due to gene methylation in breast cancers Increased expression in CD34(+) chronic myeloid leukemia cells, colon cancers (stage IIIB), non-Hodgkin lymphomas, cholangiocarcinomas	(Russell et al., 1990; Futreal et al., 1992; Cliby et al., 1993; Saito et al., 1993; Tangir et al., 1996; Aita et al., 1999; Liang et al., 1999; Qu et al., 2003; Wang et al., 2006; Lee et al., 2007; Miracco et al., 2007 ; Ding et al., 2008; Liu et al., 2008; Shen et al., 2008 ; Li et al., 2009; Zhang et al., 2009; Huang JJ et al., 2010; Huang X et al., 2010; Koukourakis et al., 2010; Lazova et al., 2010; Li Z et al., 2010; Miracco et al., 2010; Nicotra et al., 2010; Dong et al., 2011; Liu et al., 2011; Sivridis et al., 2011; Jiang et al., 2012; Choi et al., 2013; Deng et al., 2013; Dong et al., 2013; Laddha et al., 2014; Rothe et al., 2014)
LC3 (Atg8)	Increased expression in triple-negative breast cancers, colorectal cancers, gastrointestinal cancers, pancreatic cancers, and non-Hodgkin lymphomas Reduced expression in lung cancers and nonsmall cell lung cancers, melanomas, and glioblastomas	(Sato et al., 2007; Fujii et al., 2008; Liu et al., 2008; Yoshioka et al., 2008; Othman et al., 2009; Huang X et al., 2010; Miracco et al., 2010; Nicotra et al., 2010; Sivridis et al., 2011; Jiang et al., 2012; Choi et al., 2013)
ATG9B	Frameshift mutations in gastric cancers and colorectal cancers	(Kang et al., 2009)
ATG10	Increased expression in colorectal cancers is associated with metastasis Genetic variations may correlate with breast cancer susceptibility	(Jo et al., 2012; Qin et al., 2013)
ATG12	Frameshift mutations in gastric cancers and colorectal cancers	(Kang et al., 2009)
ATG16L1	Genetic variations may correlate with colorectal cancers and thyroid carcinoma susceptibility Increased expression in oral squamous cell carcinomas	(Nomura et al., 2009; Huijbers et al., 2012; Nicoli et al., 2014)
UVRAG	Mutations in colorectal cancers and gastric cancers	(Ionov et al., 2004; Liang et al., 2006; Kim MS et al., 2008; Knaevelsrud et al., 2010)
BIF1	Reduced expression in colorectal cancers, pancreatic ductal adenocarcinomas, gastric cancers, urinary bladder cancers, gallbladder cancers, and prostate cancers	(Lee et al., 2006; Takahashi et al., 2007; Coppola et al., 2008a; Coppola et al., 2008b; Kim SY et al., 2008; Coppola et al., 2011; Ko et al., 2013; Takahashi et al., 2013)
PIK3C3/hVPS34	Differentially expressed between the TEL/AML1-positive and negative acute lymphocytic leukemia groups SNP associated with esophageal squamous cell carcinomas	(Hu et al., 2005; Gandemer et al., 2007)
GABARAP	Increased expression in colorectal carcinomas and benign and malignant thyroid tumors Reduced expression in neuroblastomas and breast cancers	(Roberts et al., 2004; Klebig et al., 2005; Roberts et al., 2009; Miao et al., 2010)
GABARAPL1	Reduced expression in lymph node-positive high-grade breast cancers, acute myelocytic leukemias, and hepatocellular carcinomas	(Berthier et al., 2010; Brigger et al., 2013; Liu et al., 2014)
GABARAPL2 / GATE16	Reduced expression in acute myelocytic leukemias	(Brigger et al., 2013)

A direct functional link between autophagy and cancer was shown for the first time by the identification of *BECN1* as a haploinsufficient tumor suppressor, a gene that is monoallelically deleted in a large population of ovarian, breast, and prostate cancers (Liang et al., 1999; Qu et al., 2003). For example, out of 17 pairs of matched normal breast and breast carcinoma tissues, 15 sample pairs had higher levels of BECN1 in normal than in tumor breast tissue. Furthermore, in an overlapping tumor series, analysis by immunohistochemistry revealed a significant decrease in BECN1 protein levels in 18 of 32 breast carcinoma cells compared with normal breast lobular of ductal epithelial cells (Liang et al., 1999). In line with these results, expression of *BECN1* was reported to be reduced in breast cancers, nonsmall cell lung cancers, renal clear cell carcinomas, brain tumors, cervical squamous cell carcinomas, hepatocellular carcinomas, ovarian cancers, osteosarcomas, melanomas, and glioblastomas (Russell et al., 1990; Futreal et al., 1992; Cliby et al., 1993; Saito et al., 1993; Tangir et al., 1996; Aita et al., 1999; Liang et al., 1999; Qu et al., 2003; Wang et al., 2006; Lee et al., 2007; Miracco et al., 2007; Ding et al., 2008; Liu et al., 2008; Shen et al., 2008; Li et al., 2009; Zhang et al., 2009; Huang JJ et al., 2010; Huang X et al., 2010; Koukourakis et al., 2010; Lazova et al., 2010; Li Z et al., 2010; Miracco et al., 2010; Nicotra et al., 2010; Dong et al., 2011; Liu et al., 2011; Sivridis et al., 2011; Jiang et al., 2012; Choi et al., 2013; Deng et al., 2013; Dong et al., 2013; Laddha et al., 2014; Rothe et al., 2014).

The key component of BECN1 protein complexes, the PI3-kinase hVPS34/PIK3C3, was differentially expressed between the TEL/AML1-positive and -negative acute lymphocytic leukemia groups (Gandemer et al., 2007). Moreover, a single nucleotide polymorphism (SNP) in the *hVPS34/PIK3C3* gene was found to be associated with esophageal squamous cell carcinomas (Hu et al., 2005).

UVRAG, a regulator of BECN1 complexes, was monoallelically deleted in human colon cancers (Liang et al., 2006) and gastric carcinomas (Kim MS et al., 2008). Additionally, a polyadenine tract in the *UVRAG* gene (A10 in exon 8) was reported as a target of frameshift mutations, leading to a decrease in autophagic capacity of colon and gastric cancers with microsatellite instability (Ionov et al., 2004; Knaevelsrud et al., 2010).

Expression of another autophagy gene, *ULK1*, was downregulated in breast cancers and this event was closely related to the progression of the disease (Tang et al., 2012). In contrast, *ULK1* was overexpressed in hepatocellular carcinomas (Xu et al., 2013) and in esophageal squamous carcinomas (Jiang et al., 2011, 2014). Another component of the ULK1 complex, FIP200, was also analyzed in tumor samples. Loss of heterozygosity of *FIP200* was associated with breast cancer in 20% of cases (Chano et al., 2002).

Other autophagy-related genes and proteins were also reported to be subject to cancer-related changes. For example, frameshift mutations of the key autophagy gene *ATG5* were observed in gastric cancer and colorectal cancer samples (Kang et al., 2009). Similarly, a SNP in the *ATG5* gene was associated with increased susceptibility to nonmedullary thyroid carcinoma (Plantinga et al., 2014). Loss of *ATG5* expression was observed in gastric, colorectal, and hepatocellular carcinomas (An et al., 2011; Cho et al., 2012). Moreover, in natural killer cell malignancies, deletion of the 6q21 region that also includes the *ATG5* gene was shown to lead to a decrease in gene expression (Iqbal et al., 2009). However, *ATG5* expression was increased in CD34(+) chronic myeloid leukemia cells (Rothe et al., 2014).

LC3 proteins that play a critical role in autophagosome formation and elongation were also analyzed in tumors. For example, while the expression of *GABARAP* in colorectal and thyroid carcinomas was upregulated, it was downregulated in neuroblastomas and breast cancers (Roberts et al., 2004; Klebig et al., 2005; Roberts et al., 2009; Miao et al., 2010). Moreover, *GABARAPL1* was found to be downregulated in acute myelocytic leukemias, hepatocellular carcinomas, and lymph node-positive high-grade breast cancers (Berthier et al., 2010; Brigger et al., 2013; Liu et al., 2014). Likewise, the expression of *GABARAPL2/GATE16* was decreased in acute myelocytic leukemia cells (Brigger et al., 2013).

The above-mentioned analyses and others indicate a strong correlation between cancer formation and autophagy-related changes. Strikingly, in many cases, a downregulation of an autophagy gene was reported in tumor samples rather than an amplification and an upregulation (Table). For example, *ULK1*, *BECN1*, and *GABARAPL1* expression levels were reported to be decreased in independent studies performed with breast cancer specimens (Liang et al., 1999; Wang et al., 2006; Miracco et al., 2007; Ding et al., 2008; Liu et al., 2008; Shen et al., 2008; Berthier et al., 2010; Huang X et al., 2010; Li Z et al., 2010; Miracco et al., 2010; Jiang et al., 2011; Tang et al., 2012; Brigger et al., 2013; Liu et al., 2014). Moreover, reduced expression of several autophagy genes/proteins were observed in gastric, colorectal, renal, pancreas, bladder, and prostate cancers and hepatocellular carcinomas, neuroblastomas, some leukemias, etc. Surprisingly, besides long-range chromosomal abnormalities, autophagy gene-specific mutations and rearrangements were not frequently encountered, leading some groups to question the functional relevance of these observations (Laddha et al., 2014). However, several SNP polymorphisms were found in ATG genes and they correlated with cancer susceptibility. These results are in line with the tumor-suppressor role of autophagy in early stages of cancer formation. In light of experimental

preclinical studies, it is highly probable that the described changes in autophagy-related genes and proteins directly contribute to tumor formation through dysregulation of autophagic activities.

5. Autophagy and anticancer therapies

Autophagy upregulation was observed in response to anticancer treatments, i.e. radiation therapy, chemotherapy, and even molecular targeted therapies (Eberhart et al., 2013). Both cytoprotective and death-inducing properties of autophagy were reported during chemotherapy or radiotherapy of cancer, and autophagic capacity of cancer cells seems to affect their response to anticancer treatments. In this section we will briefly summarize the role of autophagy in determining response to chemotherapy. In this context, modulation of autophagy offers the possibility of altering the course of treatments and affecting therapeutic outcomes. Depending on the tumor type and the treatment approach, autophagy inhibition or activation might reestablish sensitivity to therapeutic agents and overcome treatment resistance.

Autophagy inhibitors that are commonly used in experimental studies fall into 2 major categories: early-stage inhibitors targeting the hVPS34-containing PI3K complex and interfering with autophagosome formation include, and 3-methyladenine (3-MA), wortmannin, and LY294002. Late-stage inhibitors, including antimalarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ) and late-endosomal/lysosomal H+ pump inhibitor bafilomycin A1, block autolysosome formation and lysosomal degradation. Commonly used autophagy activators include rapamycin and its derivatives.

5.1. Autophagy inhibition in anticancer therapy

There are several studies showing that combining pharmacologic inhibition of autophagy with current anticancer therapies might improve the clinical efficacy of chemotherapeutic agents (Livesey et al., 2009).

For example, 3-MA was shown to potentiate cell death induced by SAHA (suberoylanilide hydroxamic acid, a histone deacetylase inhibitor) in chronic myelogenous leukemia (CML) cells (Carew et al., 2007). In colon cancer xenografts, autophagy inhibition by 3-MA was shown to increase apoptosis induction by 5-fluorouracil and led to tumor regression (Li J et al., 2010). Celastrol, a quinone methide triterpenoid, could induce apoptosis in pancreatic cancer cells, and when the therapy was combined with 3-MA, the therapeutic effect of celastrol was improved both in vitro and in vivo (Zhao et al., 2014).

On the other hand, late-stage inhibitors CQ and HCQ increased cytotoxic effects of p53 and alkylating agents in a Myc-induced mouse model of lymphoma (Amaravadi et al., 2007). When CQ was combined with vorinostat, an HDAC inhibitor, a significant reduction in tumor burden and an increase in apoptosis was observed in a colon cancer xenograft model (Carew et al., 2010). Similarly, therapeutic efficacy of the Src inhibitor saracatinib was increased when combined with CQ in a prostate cancer xenograft mouse model (Wu et al., 2010). Tyrosine kinase inhibitor imatinib-mediated death of CML cells was increased following inhibition of autophagy using CQ (Bellodi et al., 2009). Moreover, CQ enhanced the effect of SAHA in both imatinib-sensitive and imatinib-resistant lines of CML (Carew et al., 2007). Similarly, autophagy inhibition by HCQ enhanced therapeutic responses of breast cancer cells to anthracycline epirubicin both in vitro and in vivo (Chittaranjan et al., 2014). Moreover, bafilomycin A1 was found to enhance antitumor effects of irradiation, arsenic trioxide, or Akt inhibitors (Paglin et al., 2001; Kanzawa et al., 2003; Degtyarev et al., 2008).

All of these studies and others underline the potential of autophagy inhibition as a treatment strategy.

5.2. Autophagy activation in anticancer therapy

In contrast with the above-mentioned examples, induction of autophagy by anticancer agents or autophagy-inducing compounds was reported to improve cancer therapy in certain contexts. Autophagy induction by drugs alone or in combination with autophagy inducers such as rapamycin derivatives or general mTOR inhibitors was shown to potentiate anticancer effects in these studies.

Rapamycin and its analogs (rapalogs), temsirolimus (CCI-779), everolimus (RAD-001), and deforolimus (AP-23573), selectively target the mTORC1 complex and stimulate autophagy. Rapalogs were tested as anticancer agents alone and in combination with chemotherapy or radiotherapy. For example, RAD001 induced autophagy and sensitized papillary thyroid cancer cells to doxorubicin treatment and radiotherapy (Lin et al., 2010). In line with this, rapamycin and its derivatives showed additive or synergistic effects when used in combination with paclitaxel, carboplatin, cisplatin, vinorelbine, doxorubicin, and camptothecin (Geoerger et al., 2001; Grunwald et al., 2002; Shi et al., 2002; Mondesire et al., 2004; Pandya et al., 2007; Steelman et al., 2008).

mTORC2, the other complex including mTOR kinase and that is resistant to rapamycin and its derivatives, also contributes to autophagy regulation, and inhibitors targeting both mTOR complexes (mTORC1 and mTORC2), including Torin1, PP242, AZD8055, and WYE-125132, were also used to improve the efficacy of cancer therapy. For example, AZD8055 is an orally available ATP-competitive inhibitor of mTOR kinase activity. AZD8055 showed significant antitumor activity and led to tumor growth reduction in glioma, prostate, colon, and uterus human tumor xenografts (Chresta et al., 2010). Another potent mTOR inhibitor, WYE-125132, was shown to exhibit strong antitumor activity against breast, glioma,

lung, and renal cancer cells in vitro and against breast and lung tumors in vivo (Yu et al., 2010).

Several other anticancer molecules were shown to activate autophagy and cell death in cancer cells. Strikingly, cell death could be blocked by chemical or genetic inhibition of autophagy in many cases (Eberhart et al., 2013). For example, treatment of breast cancer cells with antimetabolite pemetrexed killed them, and cell death was blocked by 3-MA or by the knockdown of *BECN1* (Bareford et al., 2011). Combination of pemetrexed with rapamycin potentiated pemetrexed toxicity in multiple tumor cell types. In another example, autophagy inhibition by the knockdown of *ATG5* or *BECN1* significantly blocked the cytotoxicity of an ERBB1/ERBB2 inhibitor (lapatinib) and BH3 domain antagonist obatoclax combination (Martin et al., 2009). Similarly, EGFR inhibitor cetuximab activated autophagy and cell death in combination with rapamycin (Li X et al., 2010). Similarly, BCR-ABL inhibitor imatinib induced cytotoxicity that could be attenuated by the inhibition of autophagy (Shingu et al., 2009).

All of these studies indicate that overactivation of autophagy in cancer may also show cytotoxic effects depending on cancer type and context.

6. Conclusion

Autophagy is one of the most important and basic cellular mechanisms. Not surprisingly, the basic machinery of autophagy has been preserved from yeast to man with little variation. Being a central event in the preservation of cellular and organismal homeostasis, abnormalities of autophagy result in the disturbance of key cellular events, including catabolism/metabolism balance, energy equilibrium, ROS control, and organelle recycling. Consequently, autophagy abnormalities cause or exacerbate pathologies.

Autophagy plays a central role in cellular responses to various types of stress, including exposure to toxins and carcinogens, genotoxicity and DNA damage, inflammation-related cytokines and lymphokines, viruses, and even oncogene activation, and it helps the cell to preserve proper protein function, mitochondrial function, and genomic health. Under these conditions, autophagy might contribute to critical "cell proliferation versus cycle arrest" and "cell survival versus cell death" decisions. Additionally, autophagy was implicated in events that modulate immune surveillance through its role in antigen presentation, T and B cell maturation, and inflammation (Munz, 2009; Carneiro and Travassos, 2013; Ma et al., 2013b; Viry et al., 2014). All of these functions are in line with a tumor-suppressor role of autophagy during early stages of cancer formation (Figure 2).

On the other hand, in established cancers and especially solid tumors, autophagy may act as a major metabolic stress response, supporting the survival of cancerous cells facing harsh conditions such as nutrient and growth factor deprivation, hypoxia, ROS accumulation, and acidosis. Rapidly dividing tumor cells from animal models of cancer might even become "addicted to autophagy" and their metabolism and survival might heavily rely on it (White, 2013). These conditions might even be exacerbated following detachment from primary tumor sites during invasion, metastasis, and dormancy. Thus, during later stages of cancer, autophagy seems work in favor of tumor growth and spread (Figure 2). Strikingly, results of studies analyzing cancer cells and tissues from patients mainly showed a decrease in autophagy-related gene/protein expression, even though the opposite was observed for some tumor types (Table). A general decrease in the autophagic capacity of cells was reported in transformed cells (Gozuacik and Kimchi, 2004). It is thus possible that cancer-prone cells acquire autophagy-dampening changes to overcome autophagy-related tumor suppression in the early phases of transformation, while preserving the capacity to upregulate stress-induced autophagy when faced with harsh conditions found in the tumor environment.

Autophagy has important implications for cancer treatment. Most cancer treatment strategies (chemotherapy, radiotherapy, and new-line targeted molecules) were shown to activate autophagy in target cells. In line with the prosurvival role of autophagy in established cancers, autophagy inhibitor drugs, alone or in combination with cancer therapy, were shown to improve the efficacy of treatment approaches in many cases. However, in some other cases, activation of excessive autophagy was shown to potentiate the anticancer properties of the treatments. In some studies, autophagy activator rapalogs and general mTOR inhibitors were shown to increase the efficacy of chemotherapy. It should also be mentioned that autophagy inhibition in the context of tumor treatment was mainly achieved using lysosomotropic agents such as CQ and HCQ. In addition to autophagy inhibition, these agents were shown to disrupt lysosomal integrity and activate apoptosis or nonapoptotic cell death (Eberhart et al., 2013 and references therein). It is thus possible that additive or synergistic effects of anticancer regimens might not only depend on autophagy modulation effects of the above-mentioned drugs. Another concern is related to the tumor-suppressor role of autophagy in normal tissues: to avoid side effects in normal cells and tissues following systemic administration of autophagy drugs, the effects of these treatment modalities on normal, noncancerous tissues of cancer subjects should be thoroughly documented during the development of more specific autophagy-inhibitor or -activator anticancer drugs.

The autophagy field gained momentum with discoveries involving this basic biological event in human

diseases, including cancer. However, we are just beginning to appreciate the relevance and importance of autophagy in cancer biology, and further studies are required. Data from several independent groups indicate that autophagy modulation has big potential as a novel cancer treatment approach. Therefore, advances in the field will surely have implications beyond the lab bench and will very probably have an impact in the near future on the way malignancies will be handled by clinicians.

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