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REVIEW

Role of autophagy in regulation of cancer cell death/apoptosis during anti-cancer therapy: focus on autophagy flux blockade

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Abstract Autophagy is a self-degradation process in which the cytoplasmic cargoes are delivered to the lysosomes for degradation. As the cargoes are degraded/recycled, the autophagy process maintains the cellular homeostasis. Anti-cancer therapies induce apoptosis and autophagy concomitantly, and the induced autophagy normally prevents stress responses that are being induced. In such cases, the inhibition of autophagy can be a reasonable strategy to enhance the efficacy of anti-cancer therapies. However, recent studies have shown that autophagy induced by anti-cancer drugs causes cell death/apoptosis induction, indicating a controversial role of autophagy in cancer cell survival or death/apoptosis. Therefore, in the present review, we aimed to assess the signaling mechanisms involved in autophagy and cell death/apoptosis induction during anti-cancer therapies. This review summarizes the process of autophagy, autophagy flux and its blockade, and measurement and interpretation of autophagy flux. Further, it describes the signaling pathways involved in the blockade of autophagy flux and the role of signaling molecules accumulated by autophagy blockade in cell death/apoptosis in various cancer cells during anti-cancer therapies. Altogether, it implies that factors such as types of cancer, drug therapies, and characteristics of autophagy should be evaluated before targeting autophagy for cancer treatment.

Keywords Autophagy · Autophagy flux · Apoptosis · Anti-cancer therapy · Lysosomal dysfunction

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Introduction

Autophagy is the process that involves the delivering of cytoplasmic cargoes to the lysosome for degradation (Eskelinen 2005). There are three types of autophagy processes: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA; Galluzzi et al. 2017; Yan et al. 2019). Microautophagy delivers cytoplasmic cargoes to the lysosome through direct invasion, whereas CMA selectively translocates the proteins containing KFERQ-like pentapeptide to the lysosome with the help of heat shock cognate protein (hsp70) (Kaushik and Cuervo 2012; Galluzzi et al. 2017).

Macroautophagy (hereafter referred as autophagy) involves the degradation of cellular components where the cytoplasmic components such as the portion of cytosol, endoplasmic reticulum (ER), Golgi apparatus, mitochondria, or other non-selective cargoes are first sequestered into a double-membrane vesicle called 'autophagosome', followed by its fusion with the lysosome and degradation by the action of lysosomal enzymes (Militello and Colombo 2011; Reggiori and Ungermann 2017). Autophagy is induced under the external or internal stress conditions, such as nutrient starvation, pathogen infection, ER stress, and so on (Mizushima 2007; Rashid et al. 2015; Park 2018; Yang et al. 2019a, b). The complete autophagy process leads to the production of macromolecules, such as amino acids and peptides, which can be later utilized by the cells for gluconeogenesis, energy production, protein synthesis, and nutrient mobilization to prevent starvation and hypoxia (Mizushima 2007). It is a dynamic process, which involves combination of the steps as follows: autophagosome formation, autophagosome–lysosome fusion, and cargoes degradation (Fig. 1).

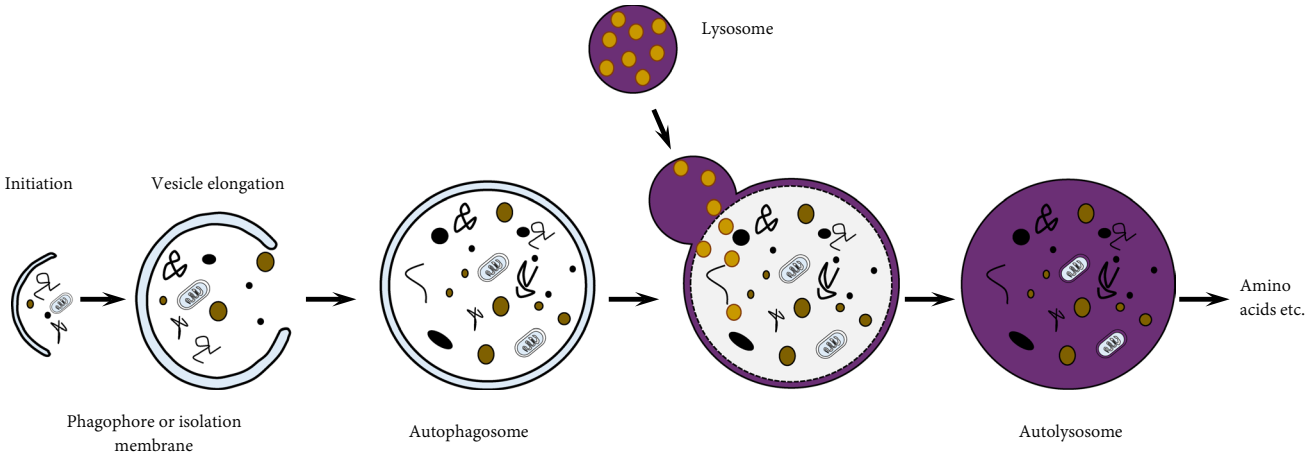

Fig. 1 Stepwise representation of the autophagy process. Autophagy begins with the formation of an isolation membrane (phagophore), which elongates into an autophagosome. During the elongation step, phagophore engulfs the intracellular cargo, such as portions of cytosol, endoplasmic reticulum, mitochondria, Golgi bodies, protein aggregates, lipid droplets, and also microorganisms invading the host cells forming a complete enclosed double-membrane autophagosome. The mature autophagosome fuses with the lysosome to form autophagolysosome, where the autophagolysosomal cargos are being lysed and degraded by lysosomal enzymes producing amino acids for example as the reusable byproducts

Formation of autophagosome

The first crucial step in the process of autophagy is the formation of autophagosome, which is initiated by the formation of a double-membrane vesicle, known as phagophore or isolation membrane. Phagophore is further elongated and closed by the action of different groups of the autophagy-related protein (ATGs) complexes (Mizushima et al. 2002). The first ATG protein complex, identified to initiate the phagophore elongation in yeast is Atg1 complex that consists of Atg1, Atg13, Atg17, Atg29, and Atg31, whereas, in mammals is Unc-51-like kinase (ULK) complex consisting of ULK1, Atg13, and retinoblastoma-inducible coiled-coil protein 1 (RB1CC1) (Wong et al. 2013). The main function of Atg1/ULK1 is to transduce signals and recruit other ATG proteins to the phagophore. It recruits the class III PI3K complex that consists of vacuolar protein sorting 34 (Vps34), Vps 15, Vps30 (Beclin1), or Atg14, and activates it to further produce phosphatidylinositol 3 phosphates (PtdIns3p), an important lipid required for the elongation of the phagophore membrane (Russell et al. 2013; Zhao and Zhang 2018). Further elongation and closure of phagophore have been shown to be mediated by two ubiquitin-like conjugation systems, namely Atg12-Atg5 complex and Atg8/LC3-phosphatidylethanolamine (PE) lipidation, resulting in complete formation of double membrane structure, known as autophagosome. The phosphatidylethanolamine lipidated LC3 (LC3II) is incorporated into the outer and inner membrane of the autophagosome as a final step. Thereafter, the intraluminal LC3II is degraded along with the cargoes, whereas, the cytosolic LC3II are de-lipidated and are returned back to the cytosol (Nakatogawa et al. 2009; Zhao and Zhang 2018).

Various sources of the phagophore/isolation membrane

The formation of phagophore/isolation membrane initiates from the small membranous portions that rupture from the organelles, such as plasma membrane, mitochondria, ER, Golgi bodies, recycling endosomes, etc. (Simonsen and Tooze 2009; Tooze and Yoshimori 2010; Militello and Colombo 2011) (Fig. 2). ER is subjected to changes under starvation leading to the release of a portion of ER enriched with phosphatidylinositol 3-phosphate (PI3P), known as omegasome. This structure protrudes from the ER and serves as a scaffold for the formation of autophagosome (Tooze and Yoshimori 2010; Militello and Colombo 2011). The association of mitochondrial outer membrane with Atg5 protein on the ER membrane assists in the delivery of mitochondrial lipid via mitofusin2 to the ER membrane to generate the autophagosomal compartments. Also, trans-Golgi bodies and a portion of the plasma membrane contribute to the membrane required for the generation of autophagosome (Tooze and Yoshimori 2010; Militello and Colombo 2011).

Autophagosome maturation/autophagy flux

The next step after the formation of autophagosome is its fusion with the lysosome and the degradation of the enclosed components or the cargoes. This is known as the

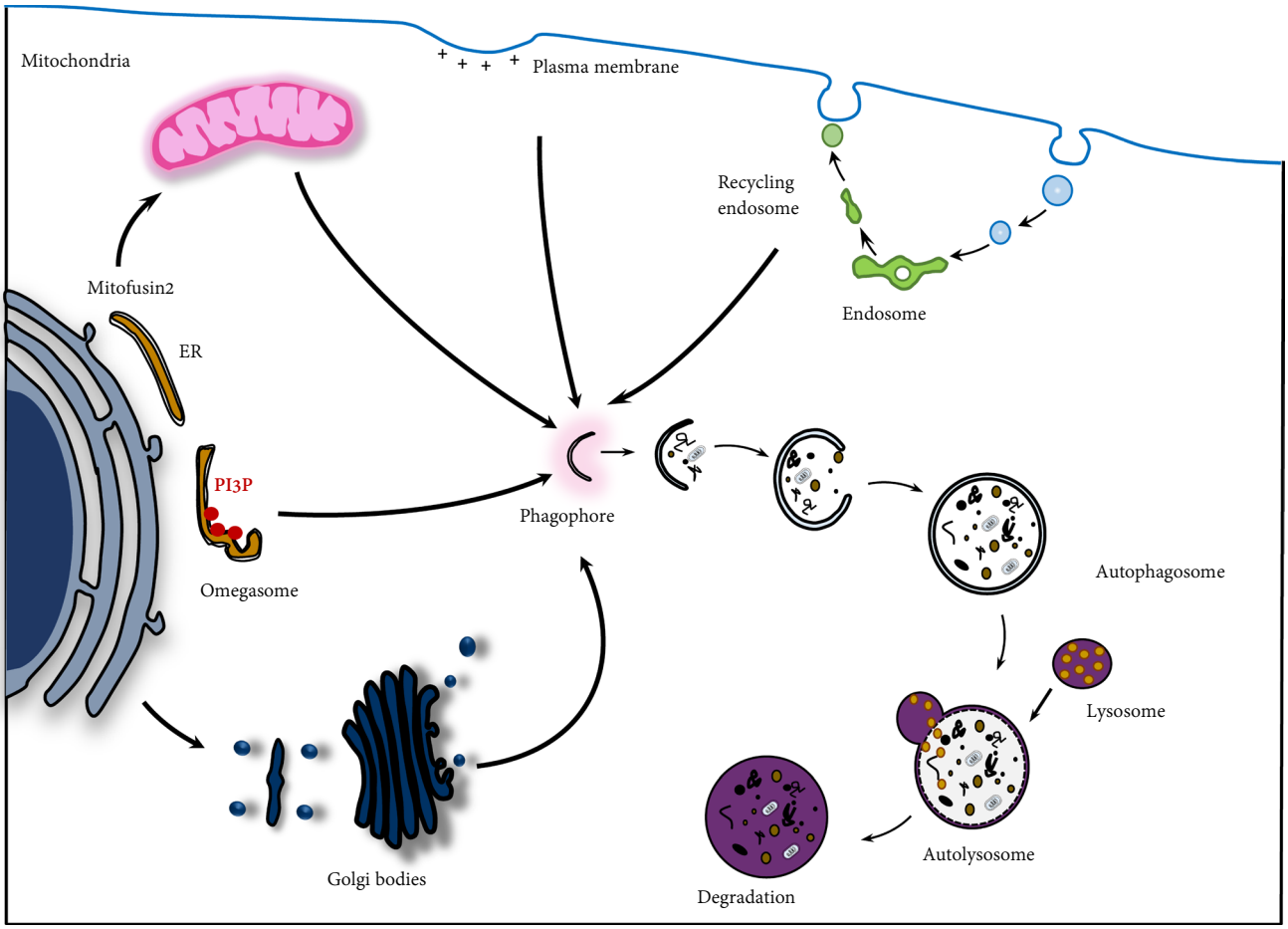

Fig. 2 Various sources of phagophore are depicted. Origination of phagophore membrane from different organelles

autophagosome maturation or autophagy flux. Not only the formation of autophagosome is important, but also its fusion with lysosome and the turnover is equally crucial. For the fusion of the autophagosome with the lysosome, there should be a match between the completion of the closure of the phagophore and activation of the fusion machinery. The formation of autophagosome occurs mainly in the cytosolic compartment whereas lysosomes are present predominantly perinuclear. Therefore, newly formed autophagosomes are required to reach the endosomes and lysosome. Microtubules and actin filaments have been shown to be associated with the autophagosomes to assist their transfer to the lysosome and to further facilitate their fusion, although they are not essential for the fusion step (Ganley 2013).

Three protein family complexes have been identified to be working together to facilitate the fusion of the autophagosome with the lysosome that are as follows: SNAP receptors complex (SNAREs), tethering factors, and Rab GTPases (Cai et al. 2007; Zhao and Zhang 2019) (Fig. 3).

SNARE complex consists of syntaxin 17 (STX17), SNAP29, and vesicle-associated membrane proteins 8 or 7 (VAMP8/7). STX17 is localized on the autophagosomal membrane; VAMP is localized on the endosomal/lysosomal membrane (Itakura et al. 2012; Takats et al. 2013); and SNAP-29 is localized in between STX17 and VAMP8 through binding (Guo et al. 2014; Zhao and Zhang 2019).

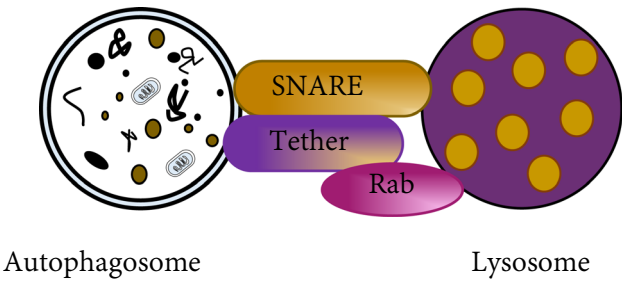

Fig. 3 Three classes of proteins, namely SNARE, tethers, and Rab act together to mediate the fusion between autophagosome and lysosome (Zhao and Zhang 2019)

The Rab GTPases, namely Rab2 and Rab21, are found to be located on the lysosomal membrane (Jean et al. 2015;

Lorincz et al. 2017). A genetic screen in the drosophila muscles has revealed that Rab2 binds to the homotypic fusion and protein sorting (HOPS) complex to facilitate the autophagosome fusion (Fujita et al. 2017), and further promotes autophagic- and endocytic-lysosomal degradation (Lorincz et al. 2017). Conversely, Rab21, has been shown to trigger the endolysosomal trafficking of VAMP8 (Jean et al. 2015).

Tethering factors acts as a bridge or connection between the intracellular trafficking vesicle and its target membrane (Yu and Hughson 2010). Multiple tethering proteins, such as Mon1-Ccz1, ectopic P granules protein 5 homolog (EPG5), HOPS, pleckstrin homology domain-containing family member 1 (PLEKHM1), Golgi reassembly-stacking protein of 55 kDa (GRASP55), BIR repeat containing ubiquitin-conjugating enzyme (BRUCE), Armus, etc., have been identified to be localized on the membrane of autophagosome and/or lysosome to promote fusion (Zhao and Zhang 2019). For example, HOPS interacts with STX17 to promote the autophagosome–lysosome fusion in mammalian cells and drosophila (Jiang et al. 2014; Takats et al. 2014). Interestingly, autophagy-related protein Atg4 also acts as a tethering factor to promote the autophagosome–endolysosome fusion (Diao et al. 2015). Moreover, PLEKM1 has been shown to regulate the autophagosome–lysosome fusion via HOPS complex and LC3/GABARAP protein (McEwan et al. 2015).

Approaches for assessing autophagy flux

Autophagy flux is usually defined as a measure of the autophagic system's degradation property, where the autophagic substrates are delivered to the lysosome for degradation, and its measure is crucial to estimate the functionality of the autophagy process. As LC3 is the only protein that specifically associates with autophagosomes and autolysosomes, its levels are correlated to the number of autophagosomes. The levels of LC3II can increase in two possible conditions:

(a) Increase in autophagosome synthesis (LC3II synthesis exceeds the number degraded).
(b) Block in LC3II turnover (the block can occur at any point after autophagosome formation, such as delay in the delivery of autophagosome to the lysosome, reduction in the fusion between both the compartments, and impaired lysosomal proteolytic activity).

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To elucidate the different possible interpretation of increased (or decreased) LC3II levels, one can measure LC3II levels by western blot in the presence of lysosomal protease inhibitors, such as bafilomycin A1, chloroquine (CQ), or lysosomotropic chemical, ammonium chloride (NH_4Cl) (Rubinsztein et al. 2009; Haspel et al. 2011) and the result can be interpreted as follows:

(a) Comparison of LC3II in between the chemical compound treated cells (in presence of bafilomycin A1) and cells treated with bafilomycin A1 alone.
    (i) If LC3II levels increase in the cells treated with specific chemical compound and bafilomycin A1 compared to those treated with bafilomycin A1 alone, it indicates that the level of LC3II (autophagosome formation) is increased by the chemical treatment.
    (ii) If LC3II levels decrease in cells treated with specific chemical compound and bafilomycin A1 compared to those treated with bafilomycin A1 alone, it indicates that the level of LC3II (autophagosome formation) is decreased by the chemical treatment.

(b) Comparison of LC3II in between chemical compound treated cells (in presence of bafilomycin A1) and cells treated with the compound alone.
    (i) If a specific chemical compound increase LC3II levels alone, and is not increased further in the presence of bafilomycin A1 treatment, it means that the chemical compound blocks LC3II degradation (blocks autophagy flux).
    (ii) If LC3II levels decrease (or no change) in the cells treated with chemical compound alone but it is further increased in the presence of bafilomycin A1, it means that the chemical compound increases both autophagosome synthesis and degradation.
    (iii) If LC3II levels increase in the cells treated with chemical compounds alone and are further increased in presence of bafilomycin A1, it means that the chemical compound increases LC3II synthesis simultaneously with a decrease in degradation or an increase in autophagy flux (Rubinsztein et al. 2009; Haspel et al. 2011).

However, this approach indicates only the increase or decrease in LC3II levels, but not the rate of autophagy flux, making this method insufficient to exactly interpret the autophagy flux. Therefore, the use of GFP (green fluorescence protein)-tagged LC3 (GFP-LC3) or mCherry (red fluorescence)-tagged LC3 (mCherry-LC3) plasmids have been introduced (Iwai-Kanai et al. 2008). Once GFP-LC3 or mCherry-LC3 plasmids are transferred into the cells, LC3 protein is processed into LC3I and then LC3II, and finally

recruited to the autophagosome membrane and the level of fluorescence punctate measured by microscopy denotes the number of autophagosomes/autolysosomes formed. Thus, the accumulation of GFP-LC3-labeled green fluorescence punctate represent the number of autophagosomes, whereas, the accumulation of mCherry-LC3-labeled red fluorescence punctate represents the number of autophagosomes and autolysosomes, as mCherry can be retained without degradation in the acidic environment of the lysosome (Iwai-Kanai et al. 2008).

Recently, a more advanced plasmid construct having both green and red fluorescence proteins tagged in the same plasmid, namely mRFP-GFP-LC3B (tfLC3B; tandem fluorescent-tagged LC3B) or mCherry-GFP-LC3B has been introduced, and thus, has been used to measure the autophagy flux from the same population of autophagy pool (Yang et al. 2018) (Fig. 4). However, the measurement of autophagy flux is still challenging because of its dynamic nature and the number of approaches that have currently been used to access the autophagy flux has limitations as well. Thus, further evaluation of the complete pool of autophagosomes over different time periods in the presence and absence of lysosomal protease inhibitors is required for further accuracy (Chen et al. 2010; du Toit et al. 2018).

Role of lysosomal biogenesis in autophagy flux

The final destination of autophagosome is the lysosome. Lysosome is a cytoplasmic organelle, responsible for the

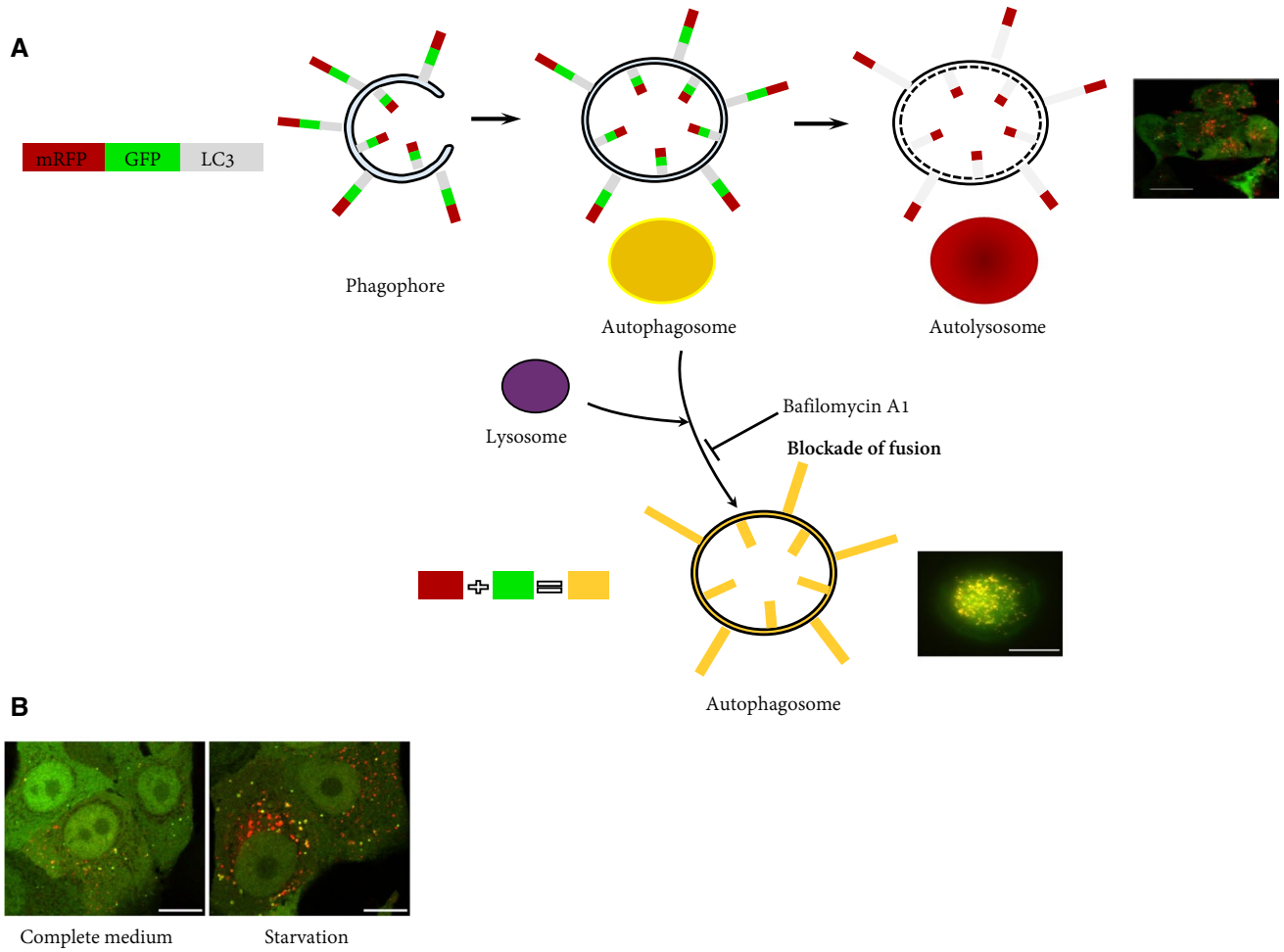

Fig. 4 Use of the recent advanced method utilizing the mRFP-GFP-LC3 plasmids to measure autophagy flux that has been shown here with examples. **a** Once the plasmid is introduced into the cells, LC3 is lapidated and located on the inner and outer membrane of the autophagosome showing LC3-dots. After the fusion of autophagosome with the lysosome, green fluorescence is quenched by the acid environment of the lysosome, leading to the appearance of only red fluorescence. This represents the occurrence of autophagy flux. However, if this pathway is blocked somehow, both green and red fluorescence produced from the plasmids leads to the appearance of yellow LC3-dots (du Toit et al. 2018). **b** Example of the measurement of autophagy flux by using the same plasmid in HeLa cells under starvation showing both autophagosomes (yellow dots) and autolysosomes (red dots) formation (Dupont et al. 2014). Bar indicates 10 μm

degradation of various biological macromolecules, such as proteins, lipids, nucleic acids, carbohydrates, etc. More than 60 acid hydrolases have been reported to be present in lysosome, which function together when the macromolecules reach the lysosome by various means, such as phagocytosis, endocytosis, autophagic pathway, etc. (Cabukusta and Neefjes 2018; Ballabio and Bonifacino 2020). Along with the luminal hydrolases, it also possesses specific sets of integral membrane proteins, lysosome-associated membrane proteins (LAMPs; Ballabio and Bonifacino 2020). The function of the lysosome is solely dependent on its acidic pH as it is crucial for the enhanced enzyme activity (optimal acid pH of 4.2–5.3) (Xiong and Zhu 2016; Colacurcio and Nixon 2016).

An increase in the luminal pH, and decrease in the LAMP1 expression, endopeptidase cathepsin B activity, and transcriptional activity of transcription factor EB (TFEB) are the consequences of lysosomal dysfunction (Yuan et al. 2019). For example, ER stress-mediated decrease in lysosomal function suppresses autolysosome formation (Nakashima et al. 2019).

Autophagy flux blockade and cancer cell death/apoptosis during anti-cancer drug therapy

Induction of autophagy process along with apoptosis has previously been shown to protect and impart resistance in cancer cells against anti-cancer drug treatments. Therefore, the inhibition of autophagy has been shown to further increase the efficacy of anti-cancer drug therapies (Yang et al. 2010; Luan et al. 2019; Niu et al. 2019). However, recent studies have demonstrated different roles of autophagy, where, it either augments the cancer cell death/apoptosis during anti-cancer therapies or the death/apoptosis is mediated via the autophagy process. In such cases, it is assumed that the different actions of autophagy might be due to the defects in the late stages of autophagy (autophagy flux) induced by anti-cancer therapies as described below in details.

Brain cancer

In temozolomide-resistant glioblastoma cells, lovastatin improves the anti-cancer effect of temozolomide via suppression of autophagy flux (Zhu et al. 2019). The autophagy flux blockade induced by lovastatin has been shown to be caused by the suppression of LAMP2 and dynein (two important proteins required for autophagosome–lysosome fusion) (Huynh et al. 2007; Nakamura and Yoshimori 2017). The supernatant derived from microglia (the brain macrophages) has been shown to induce death in glioma cells via inhibition of autophagy flux, especially by inhibiting the autolysosomal fusion leading to the unstable growth of autolysosomes, and permeabilization and release of lysosomal contents into the cytosol (Mora and Régnier-Vigouroux 2009).

Oral cancer

Tetrandrine, an alkaloid compound, is known to exhibit cytotoxic effect and induces both apoptosis and autophagy. Inhibition of cell viability and induction of apoptosis mediated by tetrandrine was abrogated by the treatment with autophagy inhibitors, such as bafilomycin A1 and 3-methyladenine (3-MA), and by genetic knockdown of the autophagy-related genes, including beclin1 and Atg5. This suggests a crucial role of autophagy process in the induction of apoptosis by tetrandrine in SAS human oral cancer cells (Huang et al. 2013). Also, interferon-alpha (IFNα) has been shown to induce apoptosis and autophagy in head and neck cancer cells, and the induction of apoptosis was attributed to the suppression of autophagy flux. It was also confirmed that the gene silencing of Atg5 leads to a decrease in autophagy flux induced by IFNα therapy leading to the induction of apoptosis. This implies that IFNα therapy promotes apoptosis by blocking autophagy flux in oral cancer cells (Yang et al. 2019a, b).

Lung cancer

The resistance of lung cancer during anti-cancer therapy can be overcome by blocking autophagy flux. For example, in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant lung cancers, candesartan and gingerol are found to be effective to reduce the resistance by blocking autophagy flux and thus, has improved the treatment of TRAIL-resistant lung cancers (Nazim et al. 2015; Rasheduzzaman and Park 2018). The antihypertensive drug candesartan, and a serotonin-norepinephrine reuptake inhibitor duloxetine, block autophagy flux leading to the accumulation of death receptor DR5, that later induces caspase-mediated apoptosis (Rasheduzzaman and Park 2018; Zinnah and PARK 2019). Gingerol, a major ginger component with anti-inflammatory and anti-tumorigenic activity, also inhibits autophagy flux, thereby enhances the cytotoxic effect of TRAIL in the treatment of TRAIL-resistant lung cancer cells (Nazim et al. 2015). Also, temozolomide–perillyl alcohol conjugate (TMZ–POH) has been shown to impair lysosomal acidification and maturation leading to the blockade of mitophagy flux and improve the susceptibility of non-small cell lung cancer (NSCLC) cells during radiation therapy (Chang et al. 2018). Graphite carbon nanofiber (GCNF) induces nanotoxicity in human lung cancer cells through autophagy flux blockade leading to the induction of apoptosis via generation of intracellular reactive oxygen species (ROS; Mittal et al. 2017).

Breast cancer

In breast cancers, gold nanocages (TANs) induce both types I (apoptosis) and type II (autophagic) cell death that are mediated by ROS production, an increase in mitochondrial membrane permeabilization (MMP), and cytochrome release from mitochondria. The autophagic cell death induced by gold nanocages is shown to be mediated by the lysosomal dysfunction leading to the autophagy flux blockade and the release of cathepsins (Raveendran et al. 2019). Combination treatment of histone deacetylase inhibitors (HDACi) and mevastatin increases the cytotoxicity and apoptosis which is attributed to the inhibition of Vps34/Beclin 1 complex and downregulation of Rab7, an active form of GTPase, which is necessary for autophagosome–lysosome fusion and leads to the inhibition of autophagy flux. As a result, cell cycle arrest occurs in the G2/M phase leading to the induction of apoptosis both in vitro and in vivo (Lin et al. 2017). In addition to the contribution of autophagy flux blockade in cell death/apoptosis, a recent study has suggested that seleno-purine molecule suppresses triple-negative breast cancer cells via cytostatic autophagy without autophagy flux blockade (Chang et al. 2019). This raises questions regarding the more complex nature of autophagy in breast cancer cell death/apoptosis undergoing various chemotherapies, making it more challenging to consider targeting autophagy during the treatment.

Liver cancer

In human hepatocellular carcinoma, aleuritolic acid (AA) has been shown to block autophagy flux and the inhibition of autophagy either by its inhibitors, such as 3-MA and Ly294002, or by Atg5 gene knockdown abrogated AA-mediated suppression of cell viability and induction of apoptosis, suggesting that the dysfunction in autophagy flux induced by AA leads to hepatocellular carcinoma cell death and apoptosis (Yi et al. 2018). Further, cationic liposomes (CLs) have been shown to induce necrosis in hepatocellular carcinoma via inhibition of autophagic flux and induction of lysosomal membrane permeabilization, further leading to the release of cathepsin, mitochondrial dysfunction, and ROS production (Yang et al. 2016).

Gastric cancer

Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, in combination with oxaliplatin enhances the cytotoxicity and apoptosis of gastric cancer cells, which is caused by an increase in lysosomal pH and blockade in autophagy flux induced by indomethacin (Vallecillo-Hernandez et al. 2018). The use of non-toxic concentration of nanoparticle (TiO_2) has been shown to improve the cytotoxic and apoptotic effect of 5-fluorouracil (5-FU) in gastric adenocarcinoma via ROS production, impairment of lysosomal function, and subsequently the autophagy flux blockade (Azimee et al. 2020). The plausible mechanism of autophagy flux blockade has been shown to be mediated by SP1, a zinc finger transcription factor, that induces p62 expression through its direct binding to the promoter of p62 in gastric cancer cells (Xu et al. 2018).

Pancreatic cancer

In human pancreatic cancer cells, the treatment with IMB-6G (*N*-substituted sophoridinic acid derivative having potent antitumor activity) decreases the cathepsin activity and inhibits the autophagy flux. Further, the inhibition of autophagosome elongation process through genetic knockdown of Atg5 has been shown to retract the lysosomal membrane permeability and the release of cytosolic cathepsin, suggesting a role of the autophagosomal–cathepsin axis in IMB-6G-mediated cell death and apoptosis in pancreatic cancer cells (Liu et al. 2017). In gemcitabine-resistant pancreatic cancer cells, LW6 (chemical inhibitor of hypoxia-inducible factor 1α) has been shown to improve the chemosensitivity along with autophagy flux inhibition (Zhang et al. 2019).

Colorectal cancer

Ganoderma lucidum polysaccharide (GLP) induces both autophagy and apoptosis along with a reduction in lysosomal acidity and cathepsin activity in colorectal cancer cells. Further, inhibition of autophagy by the action of inhibitors, such as 3-MA, in the early step reduces the GLP-induced poly (ADP-ribose) polymerase (PARP) cleavage, while the inhibition of the late step by another inhibitor, chloroquine further enhances it. The further enhancement of GLP-induced PARP cleavage by late step inhibition (CQ) might be due to the lysosomal dysfunction-mediated autophagy flux blockade in colorectal cancer cells (Pan et al. 2019). Accumulating evidences also suggest that ER stress induced by the accumulated ubiquitinated proteins triggers cell death and apoptosis in colorectal and renal cancer cells (Rossi et al. 2019; Lee et al. 2019). WIN, which is a synthetic cannabinoid, increases LC3II and p62 levels along with the suppression of cell viability and induction of apoptosis. The suppression of peroxisome proliferator-activated receptor gamma (PPARγ) as a result of autophagy flux blockade is one of the factor causing the induction of apoptosis and cell death in colon cancer cells (Pellerito et al. 2014).

Lymphoblastic leukemia

6-Cinnamamido-quinoline-4-carboxamide (CiQ) has been shown to induce apoptosis and blocks autophagy flux in human lymphoblastic leukemia, which is confirmed by the increase in LC3II and p62 levels in the presence or absence of bafilomycin A1 or CQ (inhibitors that block the fusion of autophagosome and lysosome). Inhibition of autophagy by genetic knockdown of Atg5 or beclin1 has also been shown to prevent CiQ-mediated cell death. Therefore, it suggests that cell death/apoptosis mediated by CiQ might be due to autophagy flux blockade in human lymphoblastic leukemia (Kuo et al. 2016).

Bladder cancer

Chloroquine (inhibitor that blocks the fusion of autophagosome and lysosome) itself has been shown to enhance the radiosensitivity of bladder cancer cells, and its effect is assumed to be due to autophagy flux blockade (Wang et al. 2018).

Cervical cancer

Anti-cancer drugs such as, menadione and NH_4Cl, have been shown to suppress the lysosomal function and autophagy flux along with the induction of apoptosis in cervical cancer cells. Its effect is known to be mediated by the accumulation of ubiquitinated proteins and ER stress due to improper autophagy flux (Yu et al. 2013). Recently, tubeimoside I (TBM), which is a traditional Chinese herb, is used as an anti-viral or anti-inflammatory herb, and has been shown to exhibit cytotoxic effect in cervical cancers via autophagy flux blockade (Feng et al. 2018a, b). The autophagy flux blockade mediated by TBM has been attributed to the lysosomal hydrophilic enzymes dysfunction rather than the changes in acid pH or lysosomal membrane proteins (Feng et al. 2018a, b).

Ovarian cancer

Temozolomide–perillyl alcohol conjugate (NEO212)-mediated inhibition of autophagy flux has been associated with the inhibition of cell cycle arrest at the G2/M phase, and the induction of mitochondrial fission, DNA damage, and apoptosis in ovarian cancer cells. The inhibition of autophagy flux mediated by NEO212 has also been associated with the inhibition of expression and activity of TFEB, an important transcription factor of genes-related to lysosomal biogenesis (Song et al. 2019). Ormeloxifene (ORM), which is a selective estrogen receptor modulator, does not inhibit autophagy flux, but has been shown to induce cell death and apoptosis in ovarian cancer cells, suggesting that autophagy is ultimately responsible for cell death (Bhattacharjee et al. 2018).

Other cancers

Autophagy flux blockade has been implicated in cell death/apoptosis in other cancer cells as well. For example, in myeloma cells, macrolide (an antibiotic) has been shown to block the autophagy flux, and its combination with Bortezomib (a proteasome inhibitor) has been shown to further enhance the cytotoxicity. Autophagy flux blockade-mediated ubiquitination of protein aggregates leads to ER stress, C/EBP homologous protein (CHOP) induction, and apoptosis, and has been shown to be involved in the induction of cytotoxicity during the combination treatment (Moriya et al. 2013). Physakengose G (PG) alters lysosome acidification, increases LAMP1 expression, and inhibits autophagy flux in bone cancers, and thus, it is assumed that autophagy flux blockade might contribute to cell death in this cancer type (Lin et al. 2018).

Biological signaling responsible for autophagy flux blockade

Any kind of means that block autophagy flux has been involved in cell death or apoptosis. A decrease on the levels of syntaxin 17 has been attributed to the autophagy flux blockade in adenocarcinoma (Tian et al. 2018). In TRAIL-resistant lung cancer cells, candesartan has been shown to block the autophagy flux via inhibition of AMPK activity, and it has been shown to further induce cell death and apoptosis (Rasheduzzaman and Park 2018). This is supported by another study in which the activation of AMPK was shown to induce autophagy flux to further support the survival and growth of epithelial cells, and also to develop resistance (Herrero-Martin et al. 2009).

Biological molecules responsible for autophagy flux blockade-mediated cell death/apoptosis

The accumulation of p62 protein caused by autophagy flux blockade leads to the formation of ubiquitin-protein aggregates by implementing the combination treatment of pulsatilla saponin D and camptothecin in breast cancer cells. Therefore, in this case, the aggregation of ubiquitin-proteins occur that further initiates the apoptotic cell death (Wang et al. 2019). Furthermore, another study has shown that the autophagy flux blockade-mediated p62 accumulation causes caspase 9-mediated apoptosis in graphene oxide (GO) treatment (Feng et al. 2018a, b).

Table 1 Molecular and cellular mechanism of autophagy flux blockade, lysosome dysfunction, and cell death/apoptosis

Cancers	Inducer	Signaling node leading to autophagy blockade	Expression of autophagy markers	Effect on lysosomal function	Effect on apoptosis	References
Lung	Temozolomide–perillyl alcohol conjugate	GTPase RAB7A via the mevalonate pathway	LC3II ↑, GFP-LC3 green dots ↑, mRFP-GFP-LC3 yellow dots ↑	LAMP ↑, Cathepsin D ↓, pH ↑	Dead cells ↑	Chang et al. (2018)
	Candesartan	AMPK ↓	LC3II ↑, p62 ↑	NM	DR5 ↑, c-Caspase-8 ↑, c-Caspase-3 ↑, c-FLIP ↓	Rasheduzzaman and Park (2018)
	Gingerol	NM	LC3II ↑, p62 ↑	NM	c-Caspase-3 ↑	Nazim et al. (2015)
Breast	Gold nanocages	ROS ↑, MMT ↑, Cytochrome *c* release ↑	Beclin1 ↑, LC3II ↑, p62 ↑	pH ↑, LMP ↑, membrane abrasion ↑	Cell viability ↓	Raveendran et al. (2019)
	Pulsatilla saponin D	Ubiquitinated protein aggregates ↑	LC3II ↑, p62 ↑, GFP-LC3 dots ↑	Cathepsin B ↓, Cathepsin D ↓, pH ↑	Cell viability ↓	Wang et al. (2019)
Liver	Aleuric acid	NM	LC3II ↑, p62 ↑, GFP-LC3 dots ↑, vacuole-like structures ↑, mCherry-GFP-LC3 yellow dots ↑	NM	c-Caspase-3 ↑, c-PARP ↑	Yi et al. (2018)
Gastric	Indomethacin	NM	LC3II ↑, p62 ↑	pH ↑, LAMP2 distribution from perinuclear to peripheral position ↑	Cell viability ↓	Vallecillo-Hernandez et al. (2018)
Pancreas	LW6	NM	LC3II ↑, p62 ↑, mRFP-GFP-LC3 yellow dots ↑	NM	c-Caspase-3 ↑, cell viability ↓	Zhang et al. (2019)
	IMB-6G	NM	LC3II ↑, p62 ↑, GFP-LC3 green dots ↑, mRFP-GFP-LC3 yellow dots ↑	Cathepsin ↓	c-Caspase-9 ↑, c-Caspase-3 ↑, c-PARP ↑	Liu et al. (2017)
Colon	*Ganoderma lucidum* polysaccharide (GLP)	p-mTOR ↑, p-MAPK/ERK ↑, p-AMPK ↓	LC3II ↑, p62 ↑, GFP-LC3 green dots ↑, mRFP-GFP-LC3 yellow dots ↑	pH ↑, cathepsin activity ↓	c-PARP ↑	Pan et al. (2019)
Lymphoblastic leukemia	6-cinnamido-quinolin-4-carboxamide (CiQ)	p-mTORC1 ↓, p-ERK ↑	LC3II ↑, p62 ↑, GFP-LC3 green dots ↑	LMP ↓	c-Caspase-9 ↑, c-PARP ↑	Kuo et al. (2016)
Bladder	Chloroquine	NM	LC3II ↑, p62 ↑, autophagosomes ↑	NM	c-Caspase-3 ↑, Bcl2 ↓	Wang et al.(2018)
Ovary	NEO212	p-AKT ↑, p-ERK ↑	Beclin1 (no change), LC3II ↑, p62 ↑, GFP-LC3 green dots ↑, mRFP-GFP-LC3 yellow dots ↑	LAMP1 ↓, LAMP2 ↓, TFEB nuclear translocation ↓	Bax ↑, c-Caspase-3 ↑, Cytochrome c ↑	Song et al. (2019)
Osteosarcoma	Physakengose	EGFR ↓, AKT ↓, mTOR ↓	LC3II ↑, p62 ↑, GFP-LC3 green dots ↑, mRFP-GFP-LC3 yellow dots ↑	LAMP1 ↑, pH ↑	Bax ↑, c-Caspase-3 ↑, c-Caspase-7 ↑, c-Caspase-8 ↑, c-Caspase-9 ↑, c-PARP ↑	Lin et al. (2018)

Table 1 (continued)

Cancers	Inducer	Signaling node leading to autophagy blockade	Expression of autophagy markers	Effect on lysosomal function	Effect on apoptosis	References
Adenocarcinoma	Menadione/ammonium chloride	Ubiquitinated protein accumulation and ER stress ↑	LC3II ↑, p62 ↑, GFP-LC3 green dots ↑	NM	c-Caspase-3 ↑	Yu et al. (2013)
	Coxsackievirus B	Syntaxin 17 (STX17) ↓	LC3II ↑, p62 (no change), GFP-LC3 green dots ↑, mRFP-GFP-LC3 yellow dots ↑	NM	Cell death ↑, c-Caspase-3 ↑	Tian et al. (2018)
Glioblastoma	Lovastatin	p-mTOR ↓, p-AKT ↓	LC3II ↑, p62 ↑, mRFP-GFP-LC3 yellow dots ↑	LAMP1 ↓, LAMP2 ↓, Dynein ↓	c-Caspase-3 ↑, c-PARP ↑, Bcl2 ↓	Zhu et al. (2019)

NM not measured, ↑: increase, ↓: decrease,

Additional mechanisms of apoptosis induction as a consequence of autophagy flux blockade have been further elaborated (Young et al. 2012; Iurlaro and Munoz-Pinedo 2016). Young and colleagues have demonstrated that the accumulation of p62 due to autophagy flux blockade causes caspase-8-mediated apoptotic induction in mouse embryonic fibroblasts (Young et al. 2012). In this model, Fas-associated death domain protein (FADD) is shown to integrate into the autophagosomal membrane through Atg5 binding. Thereafter, FADD recruits caspase-8 to the autophagosomal membrane, where p62 induces self-oligomerization of caspase-8 leading to its activation and initiation of caspase-mediated apoptosis (Young et al. 2012). The role of interaction of autophagy-related protein complex, Atg12-Atg5 with death receptor FADD to recruit caspase-8, activate and to induce apoptosis has been studied previously (Bell et al. 2008). Bortezomib, which is an inhibitor of proteasome, has been found to increase the interaction between Atg12-Atg5 complex and FADD that recruits caspase-8 to induce apoptosis in adenocarcinoma (Laussmann et al. 2011). Also, it increases the interaction between Atg12-Atg5 complex and FADD that further leads to the interaction of caspase-8 with ubiquitin-binding protein SQSTM1/p62 and the microtubule-associated protein light chain 3 (LC3) inducing caspase-8 and caspase-3 activation (Pan et al. 2011).

Restoration of lysosomal function and autophagy flux blockade protects cells from apoptosis

The mechanism underlying the role of autophagy flux blockade in contributing apoptosis induction in diverse cancer cells during anti-cancer drug therapy has not been clearly understood yet. Although autophagy flux blockade by anti-cancer therapies is proved to be beneficial to destroy the selected cancer cells, incomplete autophagy process can cause diverse range of disorders in normal cells. In such cases, the restoration of autophagy flux blockade is a beneficial strategy to block the apoptosis process. Apoptosis induced by ischemia–reperfusion (I/R) in myocardial tissues along with the blockade of autophagy flux and lysosomal dysfunction has been overcome by using sevoflurane postconditioning (SpostC), which helps in restoring the autophagy flux and lysosomal function (Zhang et al. 2014). Induction of apoptosis and inhibition of autophagy flux by the use of excessive antibiotics (erythromycin and clindamycin) in different human cell lines is replaced by overexpressing TFEB of lysosomal biogenesis (Prajapati et al. 2019). Overexpression of TFEB has been shown to increase the number of lysosomes leading to the restoration of autophagy flux and mitochondrial function (Prajapati et al. 2019). Further, rapamycin has been shown to restore the autophagy flux and to improve

the morphological abnormalities of muscles in cytochrome C oxidase (COX) knockout (KO) model (Civiletto et al. 2018). In this model, inhibition of mTORC1 activity led to an increase in the nuclear localization of TFEB, leading to an increase in the lysosomal biogenesis and further restoration of autophagy flux so that the dysfunctional mitochondria are dispersed (Civiletto et al. 2018). Re-acidification of the lysosome by the treatment with drugs (Folts et al. 2016), acidic nanoparticles (Colacurcio and Nixon 2016), or by mTORC1 inhibition (Nakadera et al. 2016) have improved the lysosomal function leading to the restoration of autophagy flux. Altogether, these results indicate the importance of optimal enzymatic function of the lysosome in the biogenesis of autophagy flux. Therefore, a decrease in the lysosomal activity leads to the inhibition of autophagosome–lysosome fusion, and further inhibition of the autophagy cargoes degradation (Table 1).

Concluding remarks and future perspective

The effect of diverse anti-cancer drugs on autophagy flux blockade and lysosomal dysfunction in different cancer types has been discussed here. Autophagy seems to play a role not only in cancer cell survival but also in death/apoptosis, which is believed to be mediated by the defect in the late stage of autophagy steps. Thus, the stages, levels, and nature of autophagy can be the triggering factor to decide whether the cancer cells would undergo survival or death/apoptosis under anti-cancer therapies. Cancer immunotherapy has become an interesting field in recent years as autophagy has been shown to potentiate processing and presentation of tumor antigens thereby stimulating anti-tumor immunity (Pan et al. 2016; Hu et al. 2017). Either the induction of autophagy or blockade of autophagy at its late-stage has been shown to exert anti-tumor activity by oncolytic viruses (OV) treatment (Hu et al. 2017) and this indicates the promising steps of immune system activation by autophagy, with the great hope in the field of cancer treatment.

To minimize the confusion for the role of autophagy in cancer cell survival and death during anti-cancer therapies, autophagy should be compared in different cancer types of the same origin both *in vitro* and *in vivo*. The complete process of autophagy (induction and autophagy flux) should be well studied so that anti-cancer therapies can be developed for clinical trials depending on the cancer types and action of autophagy.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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