Review Article

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Cellular Self-Digestion Unveiled: Autophagy's Impact on Cancer

* Chowdhury ARK[1], Naser IB[2]

Abstract

Autophagy is a strictly controlled process in which cells break down and recycle their own components by transporting them to lysosomes. Multiple studies have shown that autophagy has a diverse range of physiological and pathological functions in cells. Autophagy in cancer has contradictory functions, serving as both a suppressor and a driver of tumor growth. Specifically, it may exhibit several roles in relation to cancer treatment, either leading to cancer resistance or enhancing susceptibility to radiation and chemotherapy. Hence, autophagy has the potential to augment the efficacy of anticancer medications and radiation treatment.

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Introduction

Autophagy is a dynamic cellular process where proteins and organelles are enclosed inside intracellular membrane structures for the purpose of degradation and turnover. This mechanism is evolutionarily conserved and takes place in all eukaryotic cells, ranging from yeast to humans.[1–3] During the initiation of autophagy, segments of the cytoplasm and intracellular organelles are enclosed inside double-membrane structures called Autophagosomes (Fig. 1). Afterwards, the autophagosomes merge with lysosomes to create autolysosomes, where the enclosed substances are broken down by lysosomal hydrolases and then reused. Autophagy serves not only as a fundamental process for the degradation of proteins and organelles, but also plays a crucial part in other physiological processes.[1–3] When cells are exposed to environmental stresses such as lack of nutrients and infection by pathogens, autophagy occurs, leading to either adaptation and survival, or death. Autophagy is often detected in the presence of diseased circumstances, such as neurodegenerative disorders and inherited myopathies.[4,5] Neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases are examples of such conditions. Nevertheless, the question of whether autophagy provides protection against or contributes to the development of certain illnesses remains a subject of debate. Mounting research highlights the significance of autophagy in cancer.[6,7] Our comprehension of the function of autophagy in cancer is now in its nascent phase, and even the most basic inquiries like whether autophagy induces the death of cancer cells or shields them from unfavourable circumstances still remain unresolved. This review explores the role of autophagy in cancer and examines the possibility

1. ** Ashiqur Rahman Khan Chowdhury, Graduate Student, Bioinformatics and Biotechnology, Department of Life Sciences, Independent University Bangladesh (IUB), Bashundhara, Dhaka-1229.*
2. *Dr. Iftekhar Bin Naser, Associate Professor, Department of Mathematics and Natural Sciences, BRAC University, Mohakhali, Dhaka-1212.*

Address of Correspondence:

Email: *khan.chowdhury21@gmail.com*

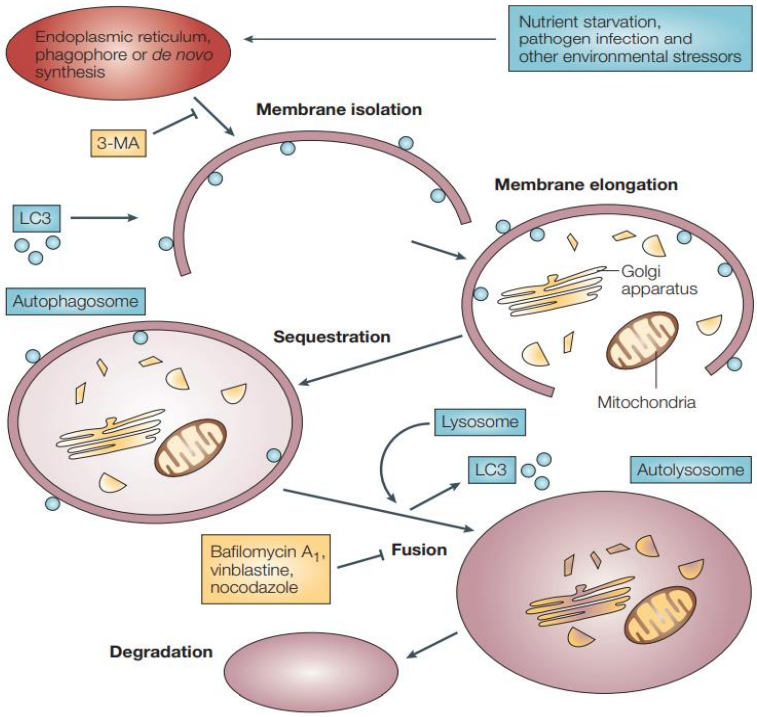

Fig. 1. Biological process of autophagy (Autophagy may be triggered by conditions such as food deprivation, pathogen invasion, and other environmental stresses. Autophagy begins by sequestering double-membrane-bound components inside a fully intact cell).[8]

of autophagic mechanisms as a new strategy for treating cancer.

Autophagy Mechanism

Autophagy is a mechanism that is exceptionally conserved throughout evolution. It helps to fulfil metabolic needs and maintain homeostasis by using an intracellular recycling system or self-degradation.[9] Autophagy is triggered in several cellular stress situations, including hunger, cellular injury, and the accumulation of faulty proteins.[10] Autophagy may be categorized into three types: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy has a role in segregating cytoplasmic cargo into phagophores, leading to the creation of autophagosomes that consist of double membrane vesicles. Subsequently, autophagosomes combine with lysosomes to create autolysosomes, which then proceed to perform the processes of degradation and recycling.[11]

Microautophagy is a kind of autophagy where cytosolic components are directly engulfed by the lysosomal membrane via the capture of cargo.[12] Chaperone-mediated autophagy is a kind of autophagy that selectively targets specific cargo. This process involves the recognition and transport of cargo, which is bound to chaperone proteins like HSC70, across the lysosomal lumen. The transport is facilitated by a receptor on the lysosomal membrane called lysosomal-associated membrane 2A (LAMP-2A)[13] Different autophagic pathways that might be present in cancer cells are described in Fig. 2.

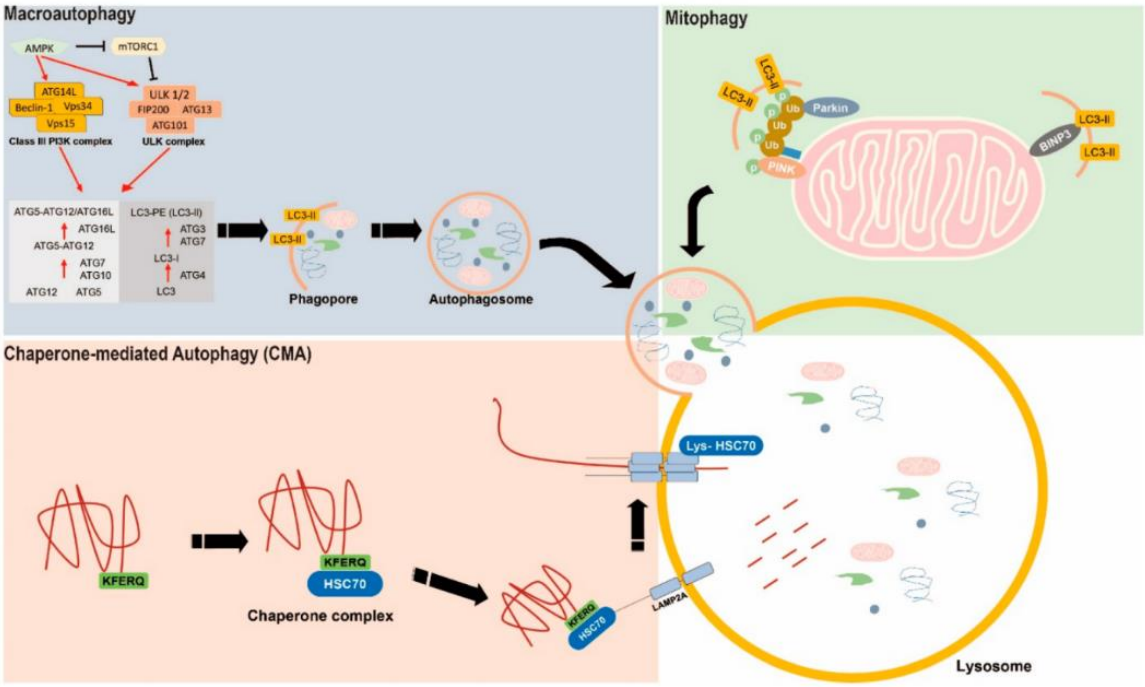

Fig. 2. Different autophagic pathways present in cancer cells (Macroautophagy is a widely occurring autophagic pathway, mitophagy is a specialized form of autophagy that specifically targets mitochondria, and chaperone-mediated autophagy is a selective form of autophagy with a distinct mechanism. The autophagic route is shown by black arrows. The autophagic signal channel is shown by red arrows).[14]

Macroautophagy

The macroautophagic process involves many distinct stages, including initiation, nucleation, and maturation. During the initial stage, the production of phagopores involves the participation of many autophagy-related genes (ATGs), which are derived from mitochondria, endoplasmic reticula, and plasma membranes.[15]

Mitophagy

Mitochondria are vital cellular organelles involved in metabolism, serving a crucial function in energy generation, cellular transcription, regulation of cell death, and maintenance of homeostasis.[16] Mitophagy, a process of autophagocytosis, is responsible for the removal of aging or damaged mitochondria.[17]

Mitochondria that experience impaired function are destroyed via the process of mitophagy. This ensures the generation of new mitochondria and prevents harm to cellular architecture and function.[18] The signalling pathways that govern mitophagy may be categorized into the following two groups: PTEN-induced putative kinase 1 (*PINK1*) Parkin-mediated and non-mediated processes.[16]

Chaperone-mediated autophagy

Chaperone-mediated autophagy is a specific kind of autophagy that has distinct mechanisms for identifying and moving cargo into the lysosomal membrane in mammalian cells.[13] CMA focuses on proteins that are specifically targeted by heat shock 70 kDa protein 8 (HSC70). HSC70 is

recognized and is attached to the pentapeptide motif (KFERQ) in the substrate protein.[19] HSC70-associated substrate proteins translocate to the lysosomal membrane and interact with the monomer of the cytosolic tail of LAMP2A, promoting the formation of multiple units of LAMP2A.[20,21] The substrate proteins undergo unfolding and are translocated into the lysosomal lumen through mediation, where they undergo rapid degradation.

Cancer and Autophagy

Autophagy was first linked to cancer, making it one of the earliest illnesses to be related with this cellular process.[22–26] However, the precise molecular pathways and the function of autophagy in cancer cells have not yet been definitively determined, and in fact, they may be contradictory. During the initial phases, autophagy typically functions as a mechanism to prevent tumor growth by enabling cells to eliminate damaged cellular components, thereby reducing reactive oxygen species and DNA damage. However, in later stages of tumor development, autophagy can support the survival of cancer cells in environments with limited oxygen and nutrients, thereby promoting tumor growth.[27,28] The reliance of tumor cells on autophagy exhibits significant variability. Although certain tumor models, such as pancreatic cancer, exhibit elevated levels of autophagy even in nutrient-rich conditions, indicating that autophagy contributes to tumor growth maintenance.[29] Autophagy levels in tumor cells and non-tumor cells vary across different tumor models (see comprehensive analysis done by Ogier-Denis & Codogno).[30] Significantly, autophagy also contributes to the response of cancer to therapy, since cancer treatments primarily subject cells to stress and damage in order to cause cell death.[31] The effects of therapy-induced autophagy in cancer cells may have both positive and negative consequences, depending on the specific kind of cancer, the stage of disease development, and the type and duration of autophagy.[31–34] Indeed, several research have shown that enhanced autophagy results in resistance to both radiation and chemotherapy. Conversely, several additional investigations have shown that numerous anticancer medicines trigger autophagy-related cell death in cancer cells.[35,36] The description of several clinically approved anticancer strategies as inducing autophagy highlights the importance of understanding the functional role of autophagy in specific cancer contexts. This understanding could potentially lead to the development of new methods to enhance the effectiveness of antitumor drugs and radiation therapy.

Apoptosis and Autophagy

The question of whether autophagy finally eradicates cancer cells is still a subject of debate. The observation of autophagic vacuoles in cancer cells undergoing therapy suggests that they experience autophagy-mediated cell death. However, it is important to note that the relationship between autophagy and cell death is not necessarily a direct cause-and-effect correlation. Anticancer therapies may cause harm to the organelles inside cancer cells, leading to the activation of autophagy as an early response. Autophagy serves to safeguard the cells by isolating and breaking down the damaged organelles. Nevertheless, when a certain threshold of damage inside cells is surpassed,

autophagy may be activated to eliminate the damaged cells from a tissue by the induction of cell death.[37] In autophagic cell death, caspases remain inactive and there is no observable DNA degradation or nuclear fragmentation, which may be evaluated using DNA laddering techniques.[8] Autophagic cell death, in contrast, is distinguished by the breakdown of the Golgi apparatus, polyribosomes, and endoplasmic reticulum prior to nuclear disintegration. These organelles, on the other hand, are conserved in apoptosis. Thus, the presence of caspase-independent cell death, together with a higher quantity of autophagic vesicles, might potentially serve as a distinguishing characteristic of autophagic cell death.[38-40] To accurately identify cells undergoing autophagic death, it is necessary to combine autophagy detection tests with other morphological and biochemical investigations, since there is no straightforward and singular approach available for this purpose. Despite the fact that tumor cells have been seen to experience both apoptosis and autophagic cell death in response to treatment, there is little understanding of the relationship between these two processes.[8] Apoptosis and autophagy may exhibit crosstalk, indicating that they are not necessarily distinct processes. Apoptosis is often induced by inhibiting autophagy, whereas inhibiting apoptosis leads to the activation of autophagy.[41,42] These data suggest a potential relationship between apoptosis and autophagy. Nevertheless, when tamoxifen was administered to breast cancer cells, electron microscope research revealed that some cells were undergoing apoptosis, others were undergoing autophagy, and others exhibited indications of both processes.[43] However, further research is required to ascertain the different pathways that may govern the initiation of autophagy by anticancer treatments.

Immunotherapy and Autophagy

The significance of immune response in cancer treatment has been increasingly attracting attention. Autophagy has recently been identified as a significant factor in the control of immunological recognition and response.[44] Research has shown that autophagy enhances the immunogenicity of tumor cells by participating in the processing of tumor antigens and the subsequent activation of effector T-lymphocytes. Hence, implementing techniques that target autophagy induction might be used as an adjunct to enhance the stimulation of the anticancer immune response. For instance, the use of vaccines made from tumor autophagosomes has been shown to stimulate the production of cytotoxic immune cells and, as a result, trigger antitumor responses in mice that have lung carcinoma and melanoma cell lines.[45] Recent research indicates that inhibiting autophagy enhances the antitumor immune response in immunotherapeutic approaches, such as T-cell transfer, vaccines, and the administration of antibodies or recombinant cytokines. This is because heightened autophagy levels in cancer cells tend to suppress the antitumor immune response.[46] According to published research, blocking autophagy may enhance the ability of activated effector T and NK cells to kill tumor cells. Administering large doses of IL-2 in conjunction with chloroquine resulted in improved long-term survival, reduced toxicity related to vascular leakage, and higher proliferation and infiltration of immune cells in the liver and

spleen.[47] Autophagy also has a crucial function in enhancing the immunogenicity of tumor cells. It is involved in antigen processing and the subsequent activation of effector T-cells. Inducing autophagy might be used as an additional technique to activate the immune response against tumors.[45,48]

Cancer Therapy and Autophagy

While autophagy has historically been seen as a system that promotes cell survival and protection, many investigations have shown that it may lead to alternative consequences. Presently, there are at least four different functional types of autophagy that have been delineated.[49,50] Firstly, cytoprotective refers to the situation where cells die or stop functioning if autophagy is prevented. Secondly, cytotoxic refers to the situation where autophagy activation leads to cell death and blocking it allows cells to survive. Thirdly, cytostatic refers to the situation where autophagy activation causes cells to stop growing. Lastly, nonprotective refers to the situation where autophagy does not impact cell growth when it is blocked.[49,50] These forms are differentiated only based on their functional properties, which may have comparable morphological, biochemical, or molecular profiles.[49]

Autophagy as Anti-cancer Therapy

As previously mentioned, the various functional types of autophagy impact the cellular response to anticancer treatments. Determining whether autophagy is cytoprotective or cytotoxic/cytostatic is crucial for developing techniques to modulate it, either by decreasing or increasing its activity, in order to impact cellular susceptibility to treatment. Multiple clinical studies have focused on targeting cytoprotective autophagy.[49] Undoubtedly, if the augmentation of autophagy provides tumor cells with resistance to death-inducing drugs, the suppression of autophagy will enable a more potent response to therapy.[51] Multiple autophagy inhibitors have been found and classed as either early-stage inhibitors, which hinder the production of autophagosomes, or late-stage inhibitors, which target the fusion of autophagosomes with lysosomes and subsequent destruction. Experiments using both pharmacological autophagy inhibitors and genetic methods to silence or reduce autophagy-associated genes have shown that tumor cells become more sensitive to autophagy-inducing stimuli, often leading to an increase in apoptosis.[49,51]

Multiple clinical studies have been assessing the use of autophagy inhibitors in conjunction with radiation and chemotherapy to enhance their overall effectiveness.[52,53] An investigation conducted in individuals with melanoma demonstrated enhanced median progression-free survival and an elevated incidence of stable disease among patients.[52,54] In addition, investigations conducted in individuals with myeloma showed a greater incidence of partial response and stable illness.[53,55,56] While clinical studies have shown the feasibility of inhibiting autophagy in patients with these drugs, there is still scope for further enhancement. Furthermore, these particular drugs, although having previously received approval from the FDA, need to be given at larger doses in order to suppress autophagy and remain in the patients' bodies for extended durations, up to five years.[53,57] Alternatively, the stimulation of autophagy may enhance the efficacy of anticancer treatments in cases when autophagy itself is harmful to cells,

either by directly causing cell death or by triggering other mechanisms of cell death, such as apoptosis.[50,58,59] Various medicines and natural extracts, including those currently used in clinical settings, have been shown to trigger autophagy-mediated cell death in various types of cancer cells.[36,60-66]

Challenges and Future Directions

Research suggests that the regulation of autophagy plays a crucial role in the development of tumors, therefore presenting a potential target for therapeutic intervention. Firstly, in cancer cells with impaired autophagy, which makes them resistant to cell death, introducing autophagy-inducing signals such as upregulating or activating BECN1 or PTEN may induce cell death or hinder cell growth. While the introduction of BECN1 into breast cancer cells using stable transfection enhances autophagic activity and decreases tumorigenic ability[67], there is currently no research documenting the antitumour impact of BECN1 expression in experimentally developed tumours. Secondly, medicines like rapamycin have the potential to trigger autophagic cell death in cancer cells that are already capable of undergoing autophagy. Evaluating the initiation of autophagy in tumor samples collected from patients has aided in determining the tumor cells' ability to undertake autophagy.[68-71] Autophagy was seen in 7 out of the 12 tumor types examined using transmission electron microscopy, including breast and lung cancer.[72] Although the sample size of tumors was modest, the authors hypothesized that autophagy is present in a wide range of tumors. Thirdly, in tumor cells where the activation of autophagy is responsible for treatment resistance, the use of autophagy inhibitors like bafilomycin A1 might enhance the sensitivity of cancer cells to therapeutic drugs by shifting the autophagic process towards an apoptotic phase. Assessing the impact of autophagy inhibitors might be beneficial in determining whether tumor cells rely on autophagy activation for survival during treatment.[73–76] Despite the limited understanding of the function of autophagy in cancer, the significant surge in research papers on autophagy and cancer reflects a heightened interest in this area of study. Hence, in order to enhance cancer treatments, it is essential to ascertain the molecules that exert positive or negative control over autophagy in cancer cells, and get a deeper comprehension of the relationship between autophagy and cancer cell death.

Conclusion

Autophagy serves as a crucial stress response mechanism to chemotherapeutic medicines and radiation in cancer cells. Radiation or chemotherapy may trigger at least four distinct types of autophagy: cytoprotective, nonprotective, cytotoxic, and cytotastic. At present, it is not feasible to anticipate the specific kind of autophagy that will be triggered by a given treatment, since these many types of autophagy lack distinguishing morphological, biochemical, or molecular characteristics. Autophagy may either shield tumor cells from the effects of cancer treatment or contribute to the elimination of cancer cells, depending on the specific conditions. Manipulating autophagy might be a significant therapeutic strategy to improve the effectiveness of anticancer treatments. The next challenge in autophagy research for cancer

treatment is in determining the exact functional form of autophagy that is triggered in different tumor models, as well as identifying which cancers may be most efficiently treated by autophagy regulation. Gaining a more comprehensive understanding of the function of autophagy in various tumor types can provide novel therapeutic evaluations for more efficient cancer treatment tactics.

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