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**Review Article**

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**The Role of Autophagy in the Progression and Treatment of Tumors**

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**Abstract**

Autophagy is a conserved homeostatic mechanism enabling cells to cope with various stresses. The pathways leading up to the activation of autophagy are interconnected with those of tumorigenesis. However, the relationship between the two events is not a straightforward one and very often context-dependent. Generally, autophagy appears to act against the tumor during the initiation stage and most often drives cancer progression subsequently. Published clinical trials for the treatment of various tumors, where autophagy was pharmacologically inhibited, were obtained and tabulated. Targeting autophagy for the treatment of tumors can be rewarding in the appropriate context, such as cancer type, grade, and microenvironment.

**Keywords:** Autophagy, Cancer, Tumorigenesis, Antitumor therapy, Autophagy modulators

**دور الالتهام الذاتي في تطور الاورام السرطانية وعلاجها****الخلاصة**

الالتهام الذاتي هو آلية توازن محافظ عليها لكي تمكن الخلايا من التعامل مع الضغوط المختلفة التي قد تتعرض لها. ترتبط المسارات المؤدية الى الالتهام الذاتي مع تلك الخاصة بتكوين الاورام السرطانية. ومع ذلك، فان العلاقة بينهما ليست علاقة مباشرة وانما تعتمد في الكثير من الاحيان على السياق المناسب. بشكل عام، يبدو ان الالتهام الذاتي يعمل ضد الورم السرطاني في البدايه وغالبا ما يؤدي الى تطور السرطان لاحقا. في هذه المراجعة، تم التطرق الى التجارب السريرية المنشوره لعلاج الاورام السرطانية المختلفه من خلال تثبيط الالتهام الذاتي فيها دوائيا، وجدولتها. يمكن ان يكون استهداف الالتهام الذاتي في علاج الاورام السرطانية مفيدا في السياق المناسب مثل نوع السرطان ودرجته والبيئه التي يتواجد فيها.

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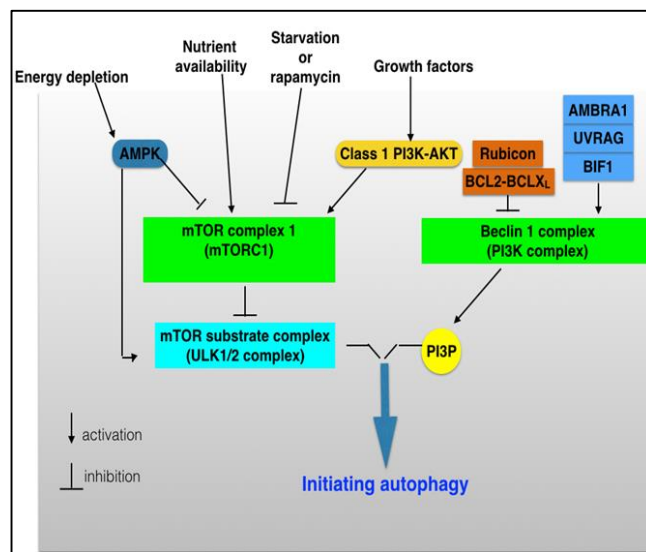
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## INTRODUCTION

Autophagy is a highly-conserved adaptive process by which cells can counteract the various physical, chemical and biological stresses that they are exposed to during their lifetime [1-4]. These stresses include temperature, ultraviolet light, oxygen tension, pH, ion and metabolite concentrations, hormones, cytokines, neurotransmitters and the presence of pathogens [4]. Autophagy is a stress-resistance mechanism used by cells to keep homeostasis [5,6]. In this process, proteins, organelles and parts of the cytoplasm are delivered to the lysosome (in mammals) and vacuoles (in plants and yeasts) for degradation and recycling. Within tolerated stress limits, autophagy is active at basal levels to perform housekeeping functions and to preserve the energetic state of the cells [1]. However, in response to elevated stress levels such as nutrient deprivation, hypoxia, accumulation of misfolded or aggregated proteins, genotoxic factors, under-synthesis of proteins or the presence of pathogens, autophagy is upregulated to preserve cellular integrity [7,8]. Autophagy is regarded as an important protective mechanism because it allows cells to survive a variety of stresses while also protecting the organism from disease [1,2,8]. Despite the existence of various forms of autophagy, macroautophagy is the most common and best-characterized type [9,10]. This form of autophagy is often referred to as simply "autophagy" and will be our main focus in this review. In autophagy, the materials to be recycled (termed cargo due to their diversity) are delivered to and get digested by the lysosomes. This ultimately generates useful compounds such as amino acids, nucleosides, nucleotides, fatty acids, sugars and adenosine triphosphate (ATP) [11,12]. As such, autophagy serves two useful purposes in providing much-needed nutrients to the cells and eliminating damaged, and potentially harmful, substances. Aberrant autophagy disturbs cellular homeostasis and contributes to the development of neurodegenerative diseases and various forms of cancer [8,11,13,14]. Recent studies also showed the involvement of autophagy in ageing and lifespan extension in several animal model organisms and illustrated that autophagy declines with age [15-17]. The link between autophagy and cancer is like a double-edged sword. The initiation of a tumor involves the action of external and/or internal stresses acting on the cell. These stresses often lead to damaging the DNA of the cell, which could result in dysfunctional proteins, and the production of reactive oxygen species (ROS) [18-20]. If the DNA damage is left unresolved, it will pass to the next generation of cells during division, elevating the risk of tumorigenesis. Cancer cells use autophagy not only to degrade and remove damaged proteins and organelles, but also to provide themselves with the energy they require to proliferate indefinitely [5,12]. This side of autophagy is characterized as a pro-survival function as it promotes the existence of cancer cells. However, some evidence also points to the anti-survival role of autophagy on cancer cells and this will be discussed in detail below [20,21]. The most plausible explanation for this apparent paradox is that the breakdown and recycling of cargo through autophagy predominantly supports survival while high cellular consumption of nutrients and energy, exceeding cellular capacity, promotes cell death [19,22].

## Induction of autophagy

Autophagy is triggered by several intracellular and extracellular stimuli, including starvation, stress, hypoxia, ultraviolet radiation, and infection [2]. The most typical trigger of autophagy is essential nutrient starvation. Signals indicating scarcity of amino acids, insulin, and insulin growth factor are thought to converge on a master regulator protein called mammalian target of rapamycin (mTOR) (Figure 1).



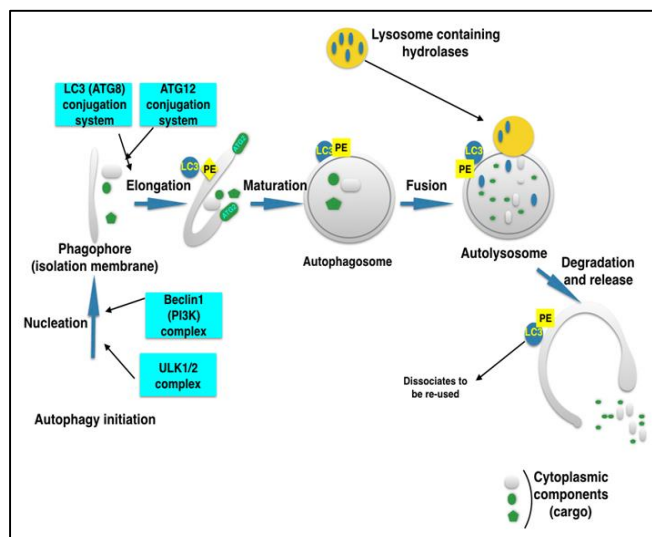
**Figure 1:** Induction and initiation of autophagy. AMPK: adenosine monophosphate-activated protein kinase; mTOR: mammalian target of rapamycin; mTORC1: mammalian target of rapamycin complex 1; ULK1/2: unc-51like kinase1/2; PI3K: phosphoinositide-3-kinase; AKT: also known as PKB (protein kinase B); AMBRA1: autophagy and Beclin1 regulator; UVRAG: UV radiation-resistance associated gene protein; Bif1: also known as Endophilin B1; Beclin1: also known as BECN1 (mammalian ortholog of Atg6) [23].

Indeed, treatment with inhibitors of mTOR, such as sirolimus (rapamycin), induces autophagy [23]. However, not all autophagy signals are transduced through mTOR. Moreover, factors other than amino acid and insulin signaling have been reported to be involved in autophagy, including reactive oxygen species, calcium, and adenosine monophosphate-activated protein kinase (AMPK) [22].

## Regulation of autophagy

Autophagy occurs at low basal levels in virtually all cells, ensuring the availability of essential nutrients and the continuous removal of superfluous and damaged (and therefore potentially dangerous) entities [24,25]. The process is rapidly upregulated when cells need to generate intracellular nutrients and energy, as in the case of starvation. It is also upregulated when there is a need for the removal of damaging components, e.g. protein aggregates or infectious microorganisms, and when cells are preparing to undergo structural remodeling, such as during development [26,27]. Regulation is key to switching on autophagy for the limited time that is required. A breakthrough in our understanding of signaling pathways occurred following the identification of the mTOR protein, which modulates cell growth, cell cycle, and protein synthesis. Because of its ability to integrate disparate metabolic, nutrient, and hormonal signals, the

mTOR signaling pathway is critical [9,10]. The sequestering and eventual recycling of cellular components takes place in several phases. These phases are usually categorized as initiation, nucleation, elongation, maturation and fusion. Following stress detection, a phagophore (isolation membrane) formation is induced and its likely source appears to be the endoplasmic reticulum (ER), the mitochondria or the cytoplasmic membrane. The phagophore then elongates and encapsulates the cargo within a double membrane structure, termed autophagosome. The autophagosome is eventually fused with the lysosome for the cargo to be degraded by its hydrolase enzymes. Finally, the broken down cargo is released back to the cytoplasm to be re-used [14,28]. Several proteins participate in the regulation of these progressive phases. A few of the genes encoding these proteins are still known by their original names when they were first discovered in yeast as simply autophagy genes (*ATG*) followed by a number [27]. An overview of the main regulatory signaling pathways is given below, pointing out the main players only. The process of autophagy starts with the detection of stress by the master regulator protein mTOR, which is a part of a larger protein complex called mammalian target of rapamycin complex 1 (mTORC1). With an abundance of nutrients, the complex is maintained in the active form to phosphorylate other target proteins that are important for growth. However, when the supply of nutrients is limited, mTORC1 is inactivated, thus reducing the overall protein synthesis and consequently triggering the initiation of autophagy to provide an alternative source of basic components [25,29]. Figure 2 depicts the roles of other major protein players, and excellent reviews on the regulation of autophagy can be found elsewhere [2,10,30].



**Figure 2:** The different phases of autophagy and its regulation. LC3(ATG8): microtubule-associated protein1 light chain 3 (autophagy gene 8 protein); ATG12: autophagy gene 12 protein; Beclin1: also known as BECN1 (mammalian ortholog of Atg6; PI3K: phosphoinositide-3-kinase; ULK1/2: unc-51 like kinase1/2; PE: phosphatidylethanolamine [30].

Once the autophagosome is formed, it fuses with the lysosome, making what is known as the autolysosome, exposing the cytoplasmic cargo to the degradative action of the acidic lysosomal hydrolase enzymes that generate the

much needed recyclable materials. These materials will pour out into the cytoplasm upon the opening of the fused structure (Figure 2).

## AUTOPHAGY AND CANCER

As outlined above, the main physiologic role of autophagy is the protection of cells against external and internal insults (cytoprotective role). However, in cancerous cells, autophagy may have opposing functions, being either cytoprotective or cytotoxic depending on the context. The tumor suppressor function acts as a mechanism, helping to mitigate the effects of metabolic stress and genome instability that would otherwise increase the risk of cancer initiation [19,31,32]. However, following the formation of the primary tumor, autophagy can either be cytoprotective or cytotoxic depending on the context of the cell and its microenvironment. Furthermore, and in certain patients, autophagy may contribute to chemotherapeutic resistance [33]. This dual role of autophagy appears to be paradoxical. The following sections of the review seek to address this issue with supporting studies and point to the crucial importance of understanding the role of autophagy in cancer for successful targeted therapy.

### The tumor-suppressor function of autophagy

The tumor-suppressor function of autophagy has been documented through several observations. For example, the expression of the *Beclin1* gene (*BECN1* or *ATG6*), an important player in the early stages of autophagy, was found to be deleted or epigenetically silenced in up to 75% of ovarian and breast cancers and up to 40% of prostate cancers [5,34-36]. This suggests that Beclin 1 protein, the gene product of *Beclin1*, may serve as a tumor suppressor [7]. Moreover, the presence of heterozygous *Beclin1* (i.e. *Beclin1*<sup>+/+</sup>) increases the frequency of spontaneous cancers. Loss of Beclin 1 protein activity blocks activation of autophagy and precludes its protective role, resulting in impairing the removal of potentially carcinogenic agents and a profound increase in DNA damage and chromosomal abnormalities, leading to an increased risk of cancer development [7,37]. An interesting observation pointed to the fact that *Beclin1* is not specifically mutated but rather lost due to the deletion of a region encompassing it on chromosome 17. That region of chromosome 17 also contains the well-known tumor suppressor gene *BRCA1* [37]. Because of the close proximity of *BRCA1*, it has been hypothesized that *Beclin1* deletion is more of a bystander event [38]. However, the worst survival probability was associated with lower *Beclin1* (but not with lower *BRCA1* mRNA) expression in all breast cancer types, indicating that *Beclin1* is a driver rather than a passenger gene [39]. Further observations linked other autophagy genes to cancer, e.g. *Atg4C* knockdown in mice was shown to increase susceptibility to developing fibrosarcoma induced by chemical agents. The *Atg4* protein is highly sensitive to ROS (reactive oxygen species) and redox modifications of its cysteine residues prevent delipidation of LC3 (a microtubule-associated protein, light chain 3), thereby promoting sustained autophagy [12,21,40]. *ATG2B*, *ATG5*, *ATG9B*, and *ATG12* mutations have also

been linked to gastric and colorectal cancers in humans. The autophagy gene *ATG8* is also frequently deleted in liver, breast, prostate and ovarian cancers and *Atg7* conditional knockout mice develop hepatomegaly and become prone to malignant transformation [41]. Similarly, mutations in another autophagy gene, namely *UVRAG* (ultraviolet radiation-resistance associated gene), resulted in lower autophagy activity and an increased occurrence of colorectal and gastric carcinomas. Substrates of the master autophagy regulator protein mTOR which promote cell cycle progression were also found to be implicated in carcinogenesis [2]. Autophagy also prevents tumor formation by controlling the level of ROS by the removal of damaged mitochondria. It is important to keep in mind that in all the mouse models, the inhibition of autophagy involved the embryonic deletion of the gene in the target cell. Therefore, the observations resulting from that deletion arose from cells that always suffered from the absence of that gene (and consequently impaired autophagy) and do not reflect autophagy loss at a later developmental stage, e.g. in established tumors [42]. Overall, there is strong evidence that some autophagy proteins display tumor suppression characteristics and the associated reduction in autophagy, due to the loss of function of these proteins, might lead to the initiation of oncogenesis because of the accumulation of dysfunctional organelles and proteins [18,20]. Paradoxically, cell death can also occur as a result of increasing cellular consumption due to unrestrained autophagy [19].

#### ***The tumor-promoting function of autophagy***

Autophagy can also promote the survival of established tumors. As cells proliferate and the tumor grows, stressful conditions are created, manifested mainly by hypoxia and nutrient deprivation, and autophagy can assist in overcoming these stresses [20]. Deletion of *Beclin1* expression, in cells forming the central zone of the tumor, enhances cell death, pointing to the paradoxical role of this autophagy protein in supporting tumor growth [20,36,43]. Under its physiologic function, autophagy also contributes to the survival of tumor cells by fulfilling their demands for nutrients and energy. In animal studies, metabolic stress of autophagy-deficient cells was shown to impair cell survival in animal studies [9]. Therefore, autophagy, from this point of view, contributes to survival and growth by enhancing stress tolerance and meeting the metabolic and energetic demands of fast-growing tumor cells. In this setting, inhibiting autophagy genes can lead to tumor shrinkage. Several studies [44-47] have found that mutations activating the canonical RAS (rat sarcoma) oncogene increase autophagy, promote tumor growth, and are associated with a poor prognosis of colon, lung, and pancreatic cancers. The interplay between cancer and autophagy will be discussed below in the context of the relevant hallmarks of tumorigenesis.

#### **AUTOPHAGY AND PROGRAMMED CELL DEATH (APOPTOSIS)**

Autophagy and apoptosis are two mechanisms by which cells, including tumor cells, respond to different stresses to maintain homeostasis. Although these two processes act

antagonistically, their crosstalk under specified biological conditions can lead to cooperation and cell death [48-51]. Key regulators are shared by autophagy and apoptosis and the impact of these regulators on the early stages of carcinogenesis is considered below. Beclin1 is an important protein in the regulation of the early stages of autophagy. However, in addition, Beclin1 is also implicated in apoptosis, thus representing a link between the two mechanisms. In vitro overexpression of Beclin1 in gastric cancer or glioblastoma cell lines induces apoptosis upon exposure to cytotoxic agents [52]. It is possible that during the early stages of cancer, Beclin1 induces both autophagy and apoptosis. Members of the ATG5-ATG12-ATG16 complex of proteins are also involved in the interplay between autophagy and apoptosis. Yoo *et al* demonstrated that exogenous expression of ATG12 in RAS-transfected intestinal epithelial cells resulted in decreased proliferation and increased cell death [53]. Furthermore, an in vitro study in which HeLa cells (laboratory cells exhibiting tumorigenesis) were treated with IFN- $\gamma$  (interferon-gamma, a cytokine) shows that death is dependent on the expression of ATG5 and its interaction with the appropriate death receptor on the cell's surface [54]. These studies point to the participation of the ATG5-ATG12-ATG16 in the induction of autophagy and activation of apoptosis during the early stages of tumor development. The well-known tumor suppressor protein TP53 (often known as p53) is another key modulator linking autophagy with apoptosis. It is the master intracellular sensor of stress caused by genotoxic agents or oncogenes. Cellular insults cause localization of TP53 to the nucleus where it upregulates transcription of a distinct set of genes involved in cell cycle control, DNA-damage repair, apoptosis and autophagy [55]. The cell cycle arrest and apoptosis mediated by TP53, during the early stages of tumor development in autophagy-defective cells, limits tumor growth. It appears that, during the early stages of tumor development, autophagy and apoptosis cooperate to prevent damage caused by carcinogenic stimuli and to remove damaged cells. The link between autophagy and apoptosis does not remain at the same steady level during the progression of a tumor. Instead, it is modified by external and internal factors influencing both processes.

#### **AUTOPHAGY AND METABOLIC PROGRAMMING IN CANCER**

For cancer cells to survive and adapt, they need to establish changes to their metabolic pathways to support the fast proliferation, migration, and other features essential for malignancy. Tumor cells achieve this by switching their metabolic utilization of glucose from the usual oxidative phosphorylation to glycolysis [56,57]. This will ensure a steady supply of energy in the form of ATP (albeit less efficiently) and the various macromolecular building blocks. Otto Warburg was the first author to identify changes in the metabolism of cancer cells [57]. He demonstrated that cancer cells consume large amounts of glucose and correspondingly excrete high amounts of lactate even when in the presence of oxygen. This was later termed the Warburg effect or aerobic glycolysis. This pathway of producing energy from glucose was thought to be less advantageous considering the reduced

amount of ATP produced from a single molecule of glucose in comparison with oxidative phosphorylation. Nevertheless, the Warburg effect occurs independently of oxygen and can be utilized in the hypoxic zones of a tumor. Moreover, the Warburg effect generates the much-needed building blocks for other metabolic pathways [57,58]. The transformation of cancer cells to aerobic glycolysis requires the activation of oncogenes such as RAS, MYC (from myelocytomatosis) and AKT (known as PKB, protein kinase B) and the inhibition of tumor suppressor genes such as p53 [59,60]. Autophagy supports this metabolic transformation through the provision of a wide variety of biomolecules. For example, monosaccharides, being the breakdown products of carbohydrates, can be used to fuel glycolysis, while the amino acids and fatty acids, being the autophagic breakdown products of proteins and fats respectively, can provide the substrates needed for the TCA (tricarboxylic acid) cycle, also known as the Krebs cycle, for example [56]. Hypoxic zones in tumors, where oxygen levels fall below 2%, trigger mechanisms to maintain homeostasis. The HIF-1 (hypoxia-inducible factor 1) protein is the primary sensor in the maintenance of homeostasis relating to the lack of oxygen in tissues. HIF-1 induces autophagy through pathways that eventually converge on the stimulation of Beclin1 or the inhibition of mTOR. Furthermore, HIF-1 also induces mitochondrial autophagy (mitophagy) through the release of Beclin1. Autophagy is also required in cancer cells to provide amino acids and fatty acid substrates derived from protein and fat degradation, respectively [61,62].

### AUTOPHAGY AND METASTASIS

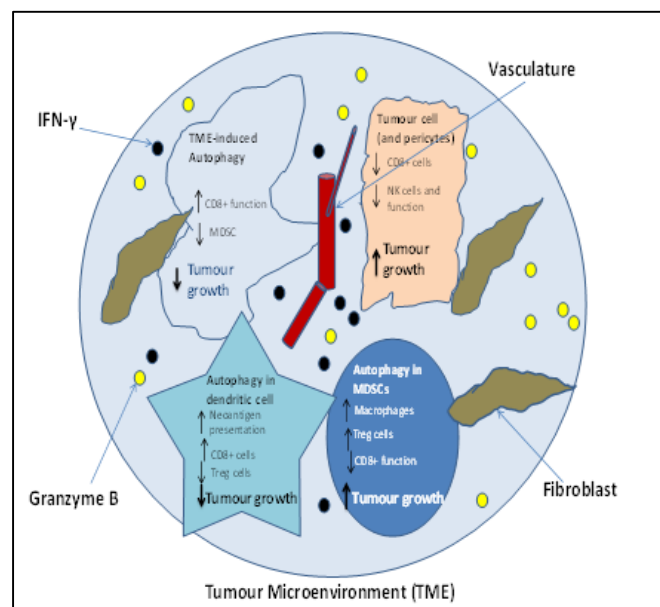
Metastasis, a stage following the establishment of a primary tumor, is the cause of death of most cancer patients [63]. The incessant proliferation of cancer cells creates a microenvironment lacking oxygen and nutrients, which drives cancer cells to migrate in search of better niches. Evidence also exists of limited migration of cancer cells during the early stages of tumor development, but metastasis is usually associated with the advanced stages of carcinogenesis [7]. Detachment of cells from the primary tumor, intravasation, survival in blood or lymph, extravasation, settlement, and colonization of the secondary site are all steps in the metastasis cascade [64]. Autophagy plays an essential role in this cascade, albeit with contradictory overall influence. A few reports show that autophagy favors metastasis [65,66], while others point out that autophagy inhibits cancer cell metastasis [67,68]. However, this may be expected considering the complexity of events involved in the metastasis cascade. With a process this complex, it is better to elucidate the function of autophagy in a particular stage in the hope of targeting that step for therapeutic gain. The process of metastasis begins with the detachment of cells from their primary mass and the activation of the epithelial-mesenchymal transition (EMT) program {the details of this program are beyond the scope of this review}. This event triggers a certain type of cell death called anoikis, which occurs when cells are detached from the extracellular matrix (ECM) [7]. The interactions between cells and the ECM require complex bonds called focal adhesion (FA), which are

essential for homeostasis [69]. Metabolic stress within the tumor microenvironment activates autophagy, which degrades essential components of FA, leading to the activation of cell motility. Tumor cells utilize activated autophagy to overcome anoikis and become resistant to dying by this route in favor of cell motility and metastasis. Fung *et al* discovered that tumor cells that shed ECM induce autophagy, which helps these cells avoid anoikis [70]. Also, detachment of hepatocellular carcinoma cells from the ECM resulted in the inactivation of the mTORC1 complex, thus leading to enhanced autophagy and avoiding anoikis [71]. Another molecule that activates autophagy and induces resistance to anoikis is miR-30a. Inhibition of this micro-RNA (miRNA) results in a decrease in Beclin1 and ATG5 and an increase in cell death [72]. Following survival against various challenges during their journey to their target metastatic organ, tumor cells must now adapt to their new microenvironment. This new microenvironment could be distinctly different from that which they left behind. The new organ may have different ECM composition, oxygen and nutrient levels. If these arriving cells do not adapt, they may enter a state of dormancy and remain clinically undetectable for a long time [73]. However, their eventual exit from dormancy can cause the often encountered tumor relapse. Autophagy helps these dormant cells to remain viable as they stay in the G0-G1 phases of the cell cycle. Green *et al* demonstrated that inhibition of autophagy in dormant cells of mouse breast cancer models decreased their viability and ability to form metastasis [74]. When the newly arrived cells do adapt to the new environment, they usually exhibit a highly flexible metabolism. For example, metastatic cells colonizing the lungs might upregulate the expression of antioxidant responses to cope with the oxidative nature of this organ. If these defenses fail, then the accumulation of ROS in these metastatic cells triggers autophagy. Peng *et al* showed that the lungs, to which primary liver tumor cells have metastasized, exhibited a higher level of autophagy compared to their initial site and that genetic inhibition of autophagy in the highly metastatic liver cancer cells blocked their colonization potential without changing EMT activation, invasion and migration [75].

### AUTOPHAGY AND IMMUNE RESPONSE AGAINST CANCER

The immune system plays a vital role in the prevention of tumor initiation, progression, metastasis and the response to anticancer therapies [76-78]. Through the surveillance of this system, tumor cells can be recognized and eliminated [79]. When considering the influence of autophagy on the immune response to cancer, the local tumor environment has to be taken into consideration [80]. This is important as the tumor microenvironment (TME) can impact cancer progression through its various constituents. The TME consists not only of the proliferating tumor cells themselves but of several other cell types, including immune cells, in addition to the secreted proteins. Understanding how the various components of TME influence tumor progression and the impact of autophagy is at its infancy. A simple summary is presented below concerning the involvement of autophagy in the immune response to the

presence of a tumor in the body with an illustrative diagram in Figure 3.



**Figure 3:** A schematic diagram of the tumor microenvironment (TME) illustrating the influence of autophagy on its various components and the outcome on cancer progression. IFN- $\gamma$ : interferon gamma; MDSCs: myeloid-derived suppressor cells; Tregs: T-regulatory cells; NK: natural killer [80].

The adaptive immune response to tumors is triggered by the neoantigen (resulting from the breakdown of dysfunctional proteins made by cancer cells) presented by dendritic cells (DCs) to naïve T cells via MHC (major histocompatibility complex) and the subsequent induction of effector T cells. Effective neoantigen presentation and the consequent response of effector T cells are essential factors for a successful immune response to a growing tumor. Reduction of MHC expression and the presentation of dysfunctional epitopes are just two mechanisms of evading detection by immune surveillance that are frequently encountered in different types of cancers. Activation of autophagy in DCs may improve their function in presenting neoantigens to T cells [81,82]. The conjugation of antigen to activators, to stimulate DCs, was shown to trigger an antitumor response in mice through an autophagy-dependent mechanism [83]. Autophagy can also create new epitopes which could increase immune recognition [84]. Therefore, when viewed from this angle, autophagy appears to be associated with efficient antigen presentation by DCs and acts to inhibit tumor progression. However, in different contexts, autophagy may play a negative role in neoantigen presentation, thus facilitating immune evasion. For example, PDAC (pancreatic ductal adenocarcinoma) cells display a low level of MHC1 and the knockdown of the *NBR1* (neighbor of BRCA1) gene increases MHC1 expression, confirming the implication of autophagy as *NBR1* functions as an autophagy cargo receptor gene. The use of autophagy inhibitors restored the expression of MHC1 and led to a reduction in tumor growth [85]. Metabolites and cytokines of TME can themselves modulate the autophagy of nearby cells and influence the immune response. For example, interferon-gamma (IFN- $\gamma$ ) can induce autophagy in gastric cancer cells and inhibit tumor growth

[86]. Furthermore, metabolic disorders such as glycolysis hypoxia in TME components can be stressful enough to induce autophagy in local cells. Autophagy can be induced in T cells exposed to elevated potassium levels to augment the persistence of these T-cells in TME and the consequent tumor regression. Moreover, the increased glycolysis activity of TME appears to enrich it with myeloid-derived suppressor cells (MDSCs), which function as suppressors of T effector cells as seen in triple-negative breast cancer [87]. Autophagy can also inhibit the immune response, enhancing cancer's ability to evade immune surveillance and thus facilitate tumor growth. MDSCs are components of the immunosuppressive compartment of TME and their autophagy has been associated with T-cell function impairment and regulatory T-cells (Tregs) recruitment [88-90]. An increase in autophagy flux was observed in MDSCs collected from melanoma patients and taken as an indication that these myeloid cells play an immunosuppressive role in TME favoring tumor growth.

### AUTOPHAGY IN THE TREATMENT OF TUMORS

Clinical efforts to inhibit autophagy in cancer therapy have focused mainly on the use of chloroquine (CQ) and hydroxychloroquine (HCQ). The long-term clinical history of these two molecules in treating a variety of diseases, as well as their affordability, make them the autophagy inhibitors of choice. However, in more recent years, more potent alternatives have emerged, including Lys05 and DQ661, and the results of their clinical use are still to be published [91]. Owing to the context-driven role of autophagy in cancer, its systemic inhibition can give rise to variable effects on different organs or tissues. Therefore, the therapeutic focus of manipulating autophagy in the treatment of tumors has been mainly to augment, and/or lower the resistance to, other anticancer medicines [6,24,92,93]. During the advanced stages of cancer, chemoresistance becomes the main obstacle to successful treatment. Chemotherapy-induced stress can induce autophagy and have a cytoprotective effect, allowing tumor cells to resist chemotherapy [31,94]. Cisplatin, a well-known chemotherapeutic compound, can induce autophagy by upregulating the expression of the *Beclin1* gene [95]. In one study, the induction of autophagy by TRP14 (thioredoxin-like protein) lowers sensitivity to Cisplatin in ovarian cancer cell lines, and when TRP14 expression was inhibited, the sensitivity to Cisplatin was markedly reversed [94]. Other studies have demonstrated similar effects, showing that autophagy acts to protect tumor cells against chemotherapeutic agents [96,97]. Occasional contradictory results were also reported during the inhibition of autophagy with certain targeted therapies. Li *et al* showed that the death of cancer cells may be achieved by the activation of autophagy, using the mTOR inhibitor Rapamycin, in cancer cells treated with Cetuximab [98]. Results from clinical trials published until 2020 where the autophagic flux was evaluated are given in Table 1 [91]. The presence of autophagy is usually assessed from measurements of vesicle accumulation in peripheral blood mononuclear cells (PBMCs) and serial tumor biopsies. A first glance at the information displayed in Table 1 reveals that only HCQ was used as an autophagy

inhibitor in these published clinical trials. However, CQ was also used in other published trials but without practical assessment of autophagy and therefore these trials were left out from Table 1. Concerning Table 1, HCQ showed limited clinical responses as a single agent in metastatic pancreatic ductal adenocarcinoma (PDAC). Monotherapy with HCQ showed inconsistent inhibition of autophagy and resulted in negligible therapeutic efficacy [99]. Although the study of the combination of HCQ and Erlotinib was mainly concerned with the collection of toxicity data, it did suggest a clinical benefit for this therapy in a subset of cancer patients [100]. Dose-limited toxicity hindered the achievement of consistent autophagy and the significant improvement in overall survival when HCQ was given in combination with Temozolomide and radiotherapy [101]. Two out of six patients with refractory BRAF (a proto-oncogene) wild-type melanoma had a near-complete response upon the administration of HCQ and Temozolomide [102]. Vogl et al concluded that the combination of HCQ and Bortezomib is a feasible and potentially useful strategy for improving outcomes in myeloma [103]. Similarly, HCQ and the mTOR inhibitor Temsirolimus yielded significant antitumor activity [104].

The preliminary efficacy results of the combination of HCQ and the histone deacetylase inhibitor Vorinostat were also encouraging to necessitate the planning of further studies [105]. The study conducted by Chi et al was interesting as it used an autophagy inhibitor (HCQ) together with an autophagy inducer (Sirolimus) and found that the response obtained, without tumor volume reduction, may indicate that non-proliferation glycolysis occurred mainly in cancer-associated fibroblasts [106]. The combination therapy of HCQ and Gemcitabine, a chemotherapeutic anti-metabolite agent, yielded clinically significant results in that 61% of patients had a decrease in CA19-9, a surrogate biomarker for cancer, following treatment, with 50% of patients demonstrating a reduction of  $\geq 50\%$  [107]. This reduction in CA19-9 was greater than expected with a single cycle of Gemcitabine treatment. As mentioned earlier, the main aim of the trials mentioned in Table 1 was not to elucidate treatment response but to investigate the toxicity and establish mechanisms. Nevertheless, the response of the tumor to the combination therapy was recorded as a secondary endpoint and the results were found clinically encouraging and warranted pursuing further.

**Table 1:** Cancer clinical trials employing the autophagy inhibitor HCQ pre-2020 where the autophagy influx was followed.

Autophagy inhibitor	In conjunction with	Tumor type	Clinical trial phase	Clinical outcomes	Reference
HCQ	Erlotinib	NSCLC	I	5% overall response rate	100
HCQ	None	Metastatic PDAC	II	Median PFS =46 days Overall survival= 69 days	99
HCQ	Temozolomide and radiation	Glioblastoma	I and II	Median survival =15.6 months with survival rates at 12, 18 and 24 months of 70%, 36% and 25% respectively.	101
HCQ	Temozolomide	Melanoma	I	PR=14% and SD=27%	102
HCQ	Bortezomib	Myeloma	I	14% very good PR, 14% minor responses and 45% period of SD	103
HCQ	Temsirolimus	Solid tumor + melanoma	I	67% in the dose escalation regimen and 74% in melanoma achieved SD	104
HCQ	Vorinostat	Solid tumor	I	One patient with renal carcinoma had durable PR and two patients with CRC had prolonged SD	105
HCQ	Sirolimus (Rapamycin)	Sarcoma	Case series	PR=60%, SD=30% and PD=10%	106
HCQ	Gemcitabine	PDAC	I and II	61% had a decrease in CA19-9 and 50% demonstrated $\geq 50\%$ reduction in CA19-9	107

Abbreviations: HCQ: hydroxychloroquine; NSCLC: non-small cell lung carcinoma; PDAC: pancreatic ductal adenocarcinoma; PFS: progression-free survival; PR: partial response; SD: stable disease; CRC: colorectal cancer; CA19-9: level of protein called CA19-9 in blood.

## Concluding remarks

Understanding the role of autophagy in tumorigenesis is still a developing field. Manipulating autophagy for induction of cell death and its inhibition through exploring context-dependent mechanisms can have therapeutic benefits. Needless to say, normal cells should not be affected while manipulating autophagy as a basal level of this process is required for cell function.

## Conflicting interests

Nothing declared.

## Data sharing statement

N/A

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