UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA DE BOTUCATU

Jofer Andree Zamame Ramirez

Autofagia em células tumorais: um mecanismo de carcinogênese e resistência aos quimioterápicos

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Jofer Andree Zamame Ramirez

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***Dedico este trabalho a minha família*.**

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“Aprender a leer es lo más importante que me ha pasado en la vida”.

Mario Vargas Llosa

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Revisão da literatura apresentada na forma de manuscrito, redigido de acordo com as normas para submissão à revista Cellular Oncology (Springer). Porcentagem de similaridade com textos da literatura=6.7% de acordo com a plataforma Academic Plagiarism.

Autophagy in tumor cells: a mechanism of carcinogenesis and resistance to chemotherapeutic agents

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Abstract

Autophagy is a dynamic physiological macromolecular process, whereby intracellular substrates are exposed to lysosomes for degradation and recycling of damaged organelles, alleviating cellular stress conditions. Several studies have shown that autophagy plays a critical role in tumoral cell survival, performing a protective role by correcting carcinogenic damages. However, this physiological process can be subverted in some cells, leading to the promotion of carcinogenesis or allowing cell escape by increasing its resistance to chemotherapeutics. This review covers the basic mechanisms and genes involved in autophagy as well as the controversial findings on their role tumor cells; we also reviewed the processes by which drug resistance may be determined, for a better understanding of how autophagy works and how it can be handled as an antitumor therapeutic intervention.

1. Introduction: Autophagy as a physiological process

The term autophagy was first used by deDuve in 1963(1), and was defined by Klionsky in 2003 as "self-eating" at subcellular level(2). Since then, many scientists have investigated the mechanisms that trigger this process. Current definition of autophagy is a conserved cellular degradation process, in which portions of cytosol and organelles are sequestered into a double-membrane vesicle, called autophagosome and fused with lysosome for breakdown and eventual recycling of resulting macromolecules(3). This process is required for the maintenance of cellular homeostasis and generation of amino acids to sustain viability during periods of nutrient privation, or under other kinds of metabolic stress like intracellular bacterial or viral infections, mutations or exposition to drugs(4-6).

The autophagy process has three different forms: macroautophagy, microautophagy, and chaperone-mediated autophagy(3). Macroautophagy, which is often simply referred as autophagy, is the most characterized form and has been extensively investigated (4, 6, 7), being defined as the sequestration of a bulk of cytoplasm and organelles in a double-membrane vesicle called phagophore. This phagophore is originated from endoplasmatic reticulum and formed by expansion of autophagosome, and fuses with lysosome, forming autophagolysosome. Inside autophagolysosome, damaged organelles are degraded by lysosomal hydrolases, and finally, the resultant *ATP* and peptides are used to keep the cell viability (3, 8). In contrast, microautophagy is characterized by direct uptake of cytoplasmic substrates by invagination of lysosomal membrane, while chaperone-mediated autophagy occurs by shuttling soluble proteins into the lysosome, via lysosomal chaperone proteins such as *hsc70*(3, 8).

Survival and homeostatic functions of autophagy have been evolutionarily conserved from yeast to mammalian. When cells have rich nutrient condition, autophagy is produced

at low levels (basal autophagy), providing tissues with a cleaning mechanism, to control intracellular quality through cytoplasmic rotation and removal of damaged or unnecessary organelles (9-12). However, when a cell is under different kinds of stress, such as nutrient deprivation, hypoxia, intracellular infections (13) and exposition to drugs(14), autophagy is rapidly induced in order to maintain the amino acid pool in the cytoplasm, and cell survives possibly through protein neo-synthesis, energy production and gluconeogenesis(12), that maintain cell *ATP* production.

Autophagy involves a concerted action of highly conserved gene products and is controlled by two pathways involved in the regulation of cell growth and metabolism, the *mTOR* (mammalian target of rapamycin) and the *AMPK* (*AMP-activated protein kinase*) /*UVRAG* (*UV irradiation resistance associated gene)* signaling pathways. During normal situations (nutrient availability), *mTOR complex I* (*mTORCI*) inhibits autophagy since it phosphorylates *Atg13*, causing disaggregation of *ULK1 complex* (*ULK1, Atg13 and FIP200*), required to form autophagosome. However, when *mTOR* is inhibited by dephosphorylation (caused by cell starvation), *ULK1* complex works and autophagy is induced (8). Conversely, *AMPK*, is activated during energy deprivation or hypoxia by increased conversion of *ADP to ATP*, and can inactivate *mTORC1*, and trigger autophagy (10, 11). In parallel, *AMPK* and *UVRAG* pathways activate the pro-autophagy kinase *Vps34* (*Class III PI3 Kinase*) by phosphorylating and recruiting *beclin-1* (proautophagy and tumor suppressor gene), which produces *PI(3)P* that is essential for to initiate autophagosomes. Autophagy triggering is illustrated in Figure 1.

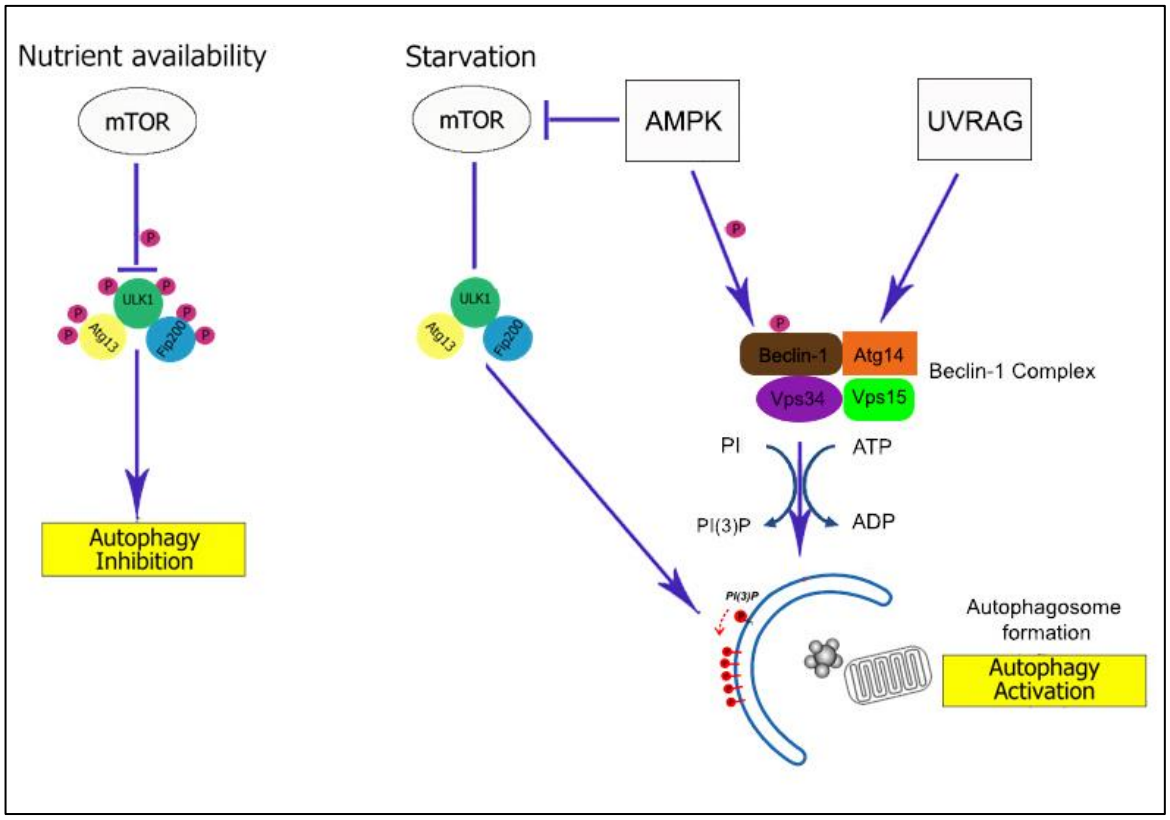

Figure 1: Autophagy inhibition and activation routes. *Nutrient availability allows mTOR to disaggregate ULK1 complex, releasing ULK1, Atg 13 and FIP200, and avoiding the formation of autophagosome. Nutrient starvation provokes mTOR dephosphorilation and reduces its activity. It keeps ULK1 complexed and able to induce autophagosome formation, triggering autophagy. In such a condition AMPK pathway is also triggered, increasing conversion of ADP to ATP, and inactivating mTORC1. AMPK also phosphorylates beclin-1 to induce PI(3)P and start autophagy.*

Autophagy starts with phagophore formation and there are two models to explain the biogenesis of phagophores. In the first, the maturation model, it derives from a preexisting endoplasmic reticulum (ER) membrane. This theory, created by Klionsky and Dunn, is supported by the observation of similar membrane thickness of these organelles. They used specific antibodies for ER and phagophores to demonstrate the similarity between these two membranes by immunoelectron microscopy(2).

The second, called assembly model, is commonly observed in yeast cells and states that phagophore is assembled *de novo* from lipids in the cytoplasm to form a pre-autophagosomal structure (*PAS*)(15, 16), that eventually form phagophore and autophagosome membrane. Use of a *green-fluorescent protein* (*GFP*) labeled anti-*Aut7* (related with *LC3*) allowed to show that *PAS* plays a crucial role just before or during the formation of autophagosome in yeasts. Anyway, independent of the proposed models, autophagy can be summarized in four steps (12, 16, 17) as shown in figure 2.

- **Nucleation:** It is the initial formation of autophagosomes and requires the *beclin-1 complex* (*beclin-1, VPS34, Atg14L, VPS15*). This complex is formed and regulated by *UV irradiation resistance associated gene* (*UVRAG*), produces *PI(3)P* by phosphorylating *phosphatidylinositol* (*PI*) of the hydroxyl group at position 3 of the inositol ring. Accumulation of *PI(3)P* generates a platform to recruit effector proteins. *PI3P-binding proteins* (*Atg2-Atg18*) are required to form the isolation membrane for sequestering autophagic substrates.
- **Elongation:** Refers to the closure of isolation membrane to form autophagosomes. This process requires conjugation of *Atg* proteins ("*Autophagy proteins*") that causes sequestration of cytoplasmic constituents. Meanwhile, *PI(3)P* recruits *Atg12-Atg5-Atg16 complex* (*Atg5 complex*), that conjugate *LC3* (*LC3-I*/*Atg8*) to *phosphatidylethanolamine* (*PE*) to form *LC3-II*. This protein increases stability of autophagosome elongation by setting microtubule-associated proteins, like bricks building a wall.
- **Maturation (Fusion):** Molecular mechanism underlying autophagosome maturation is largely unknown, but according to some theories this process is mediated by *TECPR1* (*Tectonin Beta-Propeller Repeat Containing 1*) from lysosome, binds to *Atg12-Atg5* complex and *PI(3)P* to promote autophagosome-

lysosome fusion and stability. Alternatively, autophagosomes can fuse with endosomal vesicles, such as endosomes and multivesicular bodies, to form amphisomes, which ultimately fuse with lysosomes.

- **Degradation:** After fusion with lysosomes, sequestered materials are hydrolyzed and inner membrane of autophagosomes and cargoes are degraded by lysosomal enzymes. Breakdown products are released back into the cytosol for further recycling.

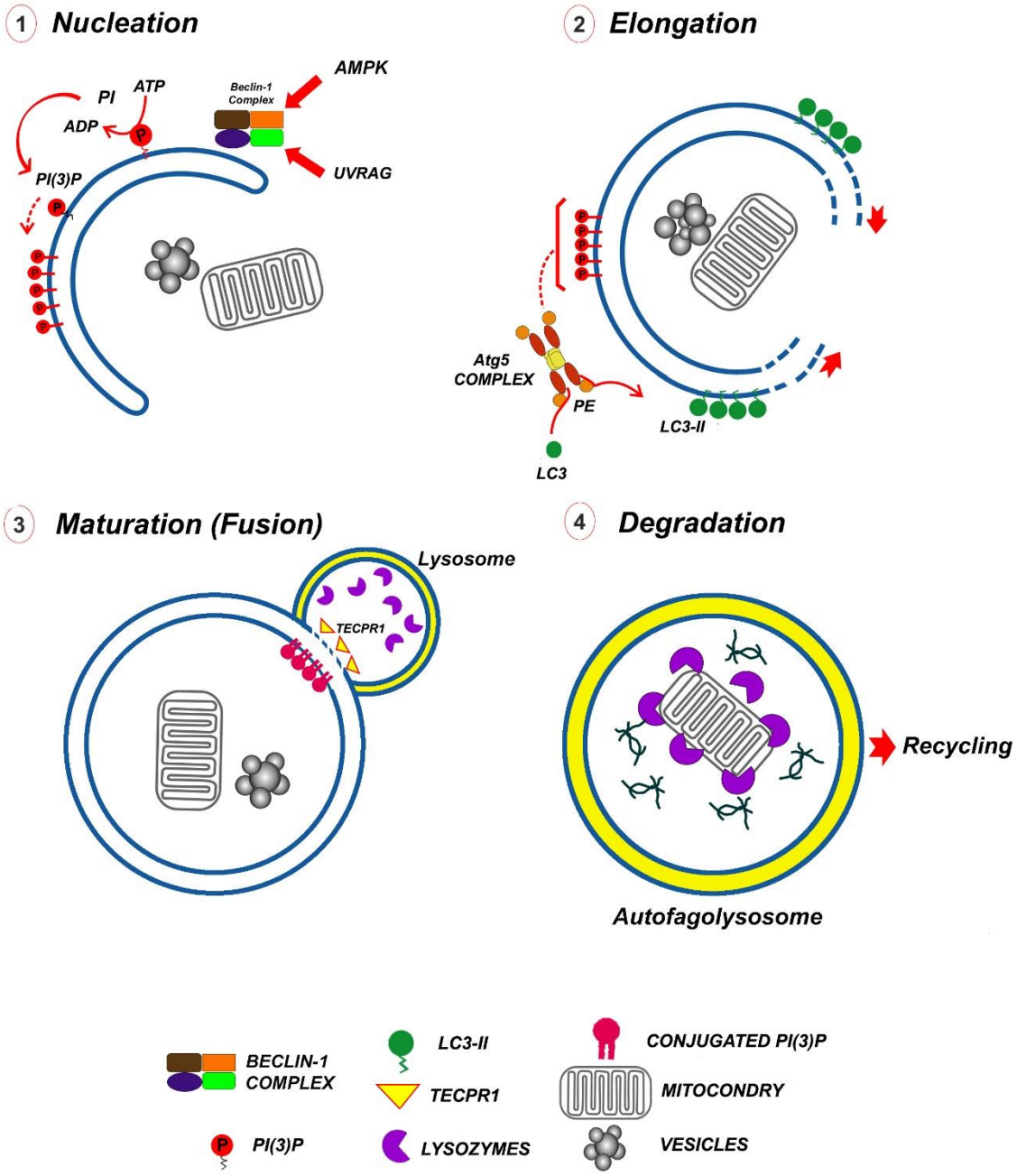

Figure 2: Autophagy steps. *After autophagy induction, UVRAG stimulates recruitment of beclin-1 complex, which includes beclin-1, VPS34, Atg14L, and VPS15, In order to form an autophagosome, elongate it and close the isolation membrane, two protein conjugation systems are required, the Atg12–Atg5–Atg16 complex (Atg5 complex) and the Atg8/LC3–phosphatidylethanolamine (PE) Finally, closed autophagosomes fuse with lysosome, which enzymes degrade autophagosome contents.*

2. The controversial role of autophagy in cancer

Autophagy and autophagic defects have been implicated in different diseases, including neurodegeneration, myopathy, Crohn's disease and cancer. However, the role of autophagy in carcinogenesis is controversial, since there are both evidence that it suppresses tumor transformation, and that it can promote or facilitate survival and adaptation of tumor cells. Thus, autophagy appears to play a protective role in the early steps of tumor development, regulating oncogenic genes and molecules, while established cancer appears to be benefited by autophagy. Therefore, in this review, we will first present the evidence of a protective role of autophagy in the early phases of carcinogenesis and then, those found as pro-carcinogenic events in the later steps of this process.

2.1. Autophagy as a tumor suppressor

One of the most important connections between autophagy and tumor suppression is the regulation of *reactive oxygen species* (*ROS*). The increase of *ROS* production induces nitration and deamination reactions on *DNA* bases, accelerating mutagenesis, increasing the activation of oncogenes, and thus stimulating carcinogenesis(18, 19). Mitochondria are considered the main source of intracellular *ROS* and its production increases as these organelles age or become damaged. In this context, autophagy helps to avoid damage through selective degradation of defective mitochondria (mitophagy). Consequently, in early steps of cancer, inhibition of autophagy facilitates genomic instability by activating oncogenes and inducing genotoxic effects as observed in autophagy-defective cells(20). Thus, selective removal of potentially damaged mitochondria reduces excessive *ROS* production and thereby limits tumor-promoting effects. Accordingly, inhibition of

autophagy leads to accumulation of defective mitochondria, with consequent cell transformation(21).

The gene *p53* is called “guardian of the cellular genome”, because his product (protein p53) inhibit cell cycle progression. Normal activity of p53 induces generation of the protein p21 that complex to cell division stimulating protein cdk2 (22). Fail of p53, do not block cell cycle, allowing the development of transformed cells. P53 can be activated by variety of stresses and subsequently works as a transcription factor to orchestrate several biological outputs, such as transient cell cycle arrest(22), apoptosis (22), cell senescence (22), metabolism (23), and regulation of autophagy(24). Being a suppressor gene for oncogenesis, defects or suppression of *p53* break the genomic stability and facilitate cancer development. However, during autophagy, part of the *p53* molecules is degraded, resulting in its underexpression and tumor growth facilitation(25).

Increasing evidence indicate that *p53* and *AMPK/mTOR* pathway are the most important mediators of the senescence response (26, 27). Thus, *p53* can function as a suppressor of cell senescence by blocking the *mTOR* pathway (26). Under serum starvation, an increased autophagic flow was observed in *HCT-116 p53*+/+ but not in *HCT-116 p53*-/- cells, suggesting that *p53* promotes autophagy. Then, p53-dependent autophagy protects cancer cells from starvation-induced death, while inhibition of autophagy (by the treatment with autophagy inhibitors) results in a high rate of cell death. Also, *PALB2* (Partner and localizer of *BRCA2*) knockout mice develop breast adenocarcinoma when *p53* is mutated and partial inhibition of autophagy by monoallelic loss of beclin-1 increases apoptosis and delays tumor growth in a *p53* dependent way (28, 29).

Autophagy also permits degradation of protein aggregates. Defects in the autophagic process is associated with accumulation of protein aggregates and autophagy substrate *p62/SQSTM1*. Protein *p62* is a selective autophagy substrate that accumulates when autophagy is reduced. This protein contains three regions called *PB1* (*Phox and Bem1p*) domain that permits protein oligomerization, *UBA* (*ubiquitin-associated domain)* required for binding to polyubiquitinated proteins, and *LIR* (*LC3-interacting region*) necessary for association with *LC3*. Interestingly, *p62* levels are commonly elevated in breast and prostatic tumors, suggest an association between reduced autophagy and carcinogenesis (30, 31).

Another possible tumor suppressor role of autophagy was shown in studies of *beclin-1* (*BECN1* gene). Overexpression of *BECN1* promotes autophagy in cancer cells and inhibits tumorigenesis in a murine model(33). This gene plays an important role in autophagy, regulating and binding to *BCL2* (gene of B-cell lymphoma 2), *UVRAG* (UV Radiation Resistance Associated), *Atg14* (Autophagy-related gene 14) and *VMP1* (Vacuole Membrane Protein 1), which are important genes for autophagosome formation. *BECN1* resides on chromosome 17q21, commonly deleted in breast, ovarian, and prostate cancers(34). *BECN1* deletion can be monoallelic, as happens in breast cancer cell lines (35). Furthermore, *BECN1* +/- mice suffer from a high incidence of tumors, including mammary gland neoplasia, lymphoma, lung adenocarcinoma, and hepatocellular carcinoma (36, 37), suggesting that *BECN1* may be a haploinsufficient tumor suppressor.

Analysis by *RT-qPCR* showed expression of beclin-1 is upregulated in mamospheres of breast cancer cell lines starved. Knocking out beclin-1 gene in adult mice causes avoidance of autophagy and increases the incidence of lymphomas and carcinomas, indicating that autophagy can work to eliminate tumorigenic defects. This same scenario also occurs in ovarian and prostate cancer(38).

Mutations of *UVRAG* are also found in human colon cancer cells. This protein recruits beclin-1 increasing autophagy events, while mutations interfere with its tumor-suppressing functions and enhance transformation of colorectal cancer cells, as happens with many human colon cancer cell lines (HCT15, HCT116, KM12, LIM2405, LS180, RKO and SW48)(17, 39, 40).

2.2. Autophagy as a tumor promoter

Besides the above-mentioned evidence that autophagy can protect cells from malignant transformation, several studies point out this phenomenon as a way to escape from antitumor response.

Tumor cells usually have high proliferation rates, which translate into higher bioenergetic and biosynthetic requirements than non-transformed cells. These requirements can be satisfied by increasing autophagy as a mechanism to obtain both *ATP* and metabolic intermediates(32). Importantly, for tumor cells, in which oncogene *Ras* is active, high levels of basal autophagy and dependence on this mechanism for survival are observed (34, 41). Then, autophagy is thought to promote tumor cell survival by increasing tolerance to stress and providing a pathway to get nutrients to supply their energetic requirements (32). Small *GTPases* of *Ras* family are involved in signaling pathways important for proliferation, cell survival, and metabolism. *Ras*-activating mutations are present in 33% of all human cancers(42), being linked to the development of some of the most lethal cancers, including those of the lung, colon, and pancreas (34). Activation of *Ras* oncogene is initiated by cell surface receptors, that induces *RasGEFs* (*guanine-nucleotide exchange factors*) to exchange *GDP* by *GTP* on *Ras*. Once activated, *Ras* stimulates diverse downstream effectors leading to the initiation of an array of cell signaling networks, including *AMPK/UVRAG* autophagy pathway(43). Since *Ras* works as a positive regulator

of the *mTOR* pathway, it would be expected this gene would negatively regulate autophagy. However, *Ras* is also involved in the regulation of a vast number of other signaling pathways, and its implication in autophagy regulation is multifaceted(42, 43). *In vitro* studies showed that several cell lines with *Ras*-activating mutations exhibit high levels of basal autophagy and marked autophagy-dependent survival under conditions of nutrient deprivation. Accordingly, silencing these genes involved in autophagy promotes the accumulation of dysfunctional mitochondria, low oxygen consumption, and decreased cell growth(20). In summary, current evidence points towards autophagy as a mechanism that ensures adequate mitochondrial metabolism in *Ras+* cancers by supplying mitochondrial intermediates via degradation of macromolecules under both basal and starvation conditions(41).

The role of autophagy has also been studied in other different contexts independent of Ras. For instance, in a model of breast cancer, the inhibition of autophagy by *FIP200* deletion suppresses mammary tumor initiation and progression. Deletion of *FIP200* results in multiple autophagy defects including accumulation of ubiquitinated protein aggregates and *p62/SQSTM1*, deficient *LC3* conversion, and increased number of mitochondria with abnormal morphology in tumor cells(44). Authors observed the *FIP200* ablation increase the number of mitochondria with abnormal morphology in mammary tumor cells and significantly reduces their proliferation (45). Thus, it would be interesting to determine whether *FIP200* contribute to dependence on autophagy for cell proliferation.

Other genes involved in autophagy are *LC3* genes or microtubule-associated protein 1 light chain 3 (*MAP1LC3A* and *MAP1LC3B*). Authors observed that 65 out of 67 samples obtained from patients with breast carcinoma have significantly higher expression of *MAP1LC3A/B* protein as compared with normal tissues(45). It indicates that expression of *LC3* is closely associated with development of breast cancer.

Immunohistochemical analysis of *ULK1* expression in two independent cohorts of nasopharyngeal carcinoma (*NPC*) indicates a correlation of high expression of this protein with resistance to therapy, whereas patients with good therapeutic response express lower levels(46). It suggests that high *ULK1* expression is closely associated with an aggressive clinical feature of *NPC* patients. Since *ULK1*, one of three main genes required to form phagophore, is regulated by *mTOR* complex, increased levels of this molecule mean higher levels of autophagy and the consequent facilitation of tumor cell grow.

Therefore autophagy can work both for tumor suppression (*Beclin-1, UVRAG, ULK1* and *p53*) and for oncogenesis (*FIP200, LC3, p62* and *Ras*) depending on the level of alteration they present, and the imbalance towards one of these hands, as illustrated in figure 3.

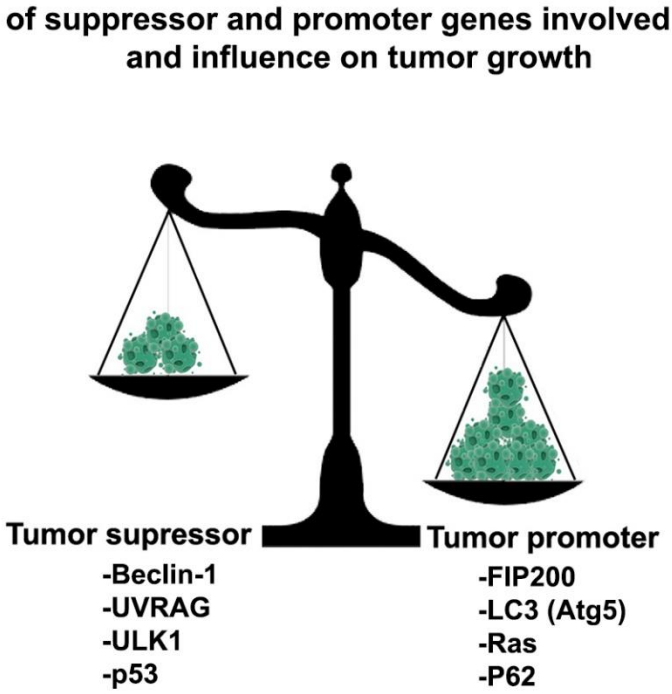

Figure 3: Autophagic imbalance. *Proposed genes by which autophagy may suppress or promote tumorigenesis. Genes Beclin-1, UVRAG, ULK1 and p53, expressed in early stages of cancer, trigger autophagy as a process to fix all damages caused by stress and thus prevent tumor growth. But, in later stages, changes occurring in tumor suppressor genes provide the balance for the oncogenesis side. Genes FIP200, LC3 (Atg5), Ras and p62, when are overexpressed or mutated, behave like oncogenes, promoting oncogenesis. As illustrate in the image, genes that stimulate tumor growth are more prevalent and more expressed in final stages of tumorigenesis.*

3. Autophagy and resistance to chemotherapy

Growing knowledge on the mechanisms involved in tumor formation and metastatic progression has not been directly translated into more effective treatment and cure of cancer. Reasons for failure of presently available anticancer treatment are mainly related to

cellular heterogeneity of tumors and rise of inherent or therapy-induced resistance of tumor cells to the therapeutic agents (47). The inability of conventional treatments to completely eradicate all invasive tumor cells is the major cause of treatment failure, allowing tumor recurrence or relapse. It has been proposed that small subsets of cancer cells, called cancer stem cells (CSCs) are responsible for cancer genesis, tumor growth, recurrence, and development of drug resistance(29). CSCs have been identified as immortal tumor initiating cells that can self-renew and have pluripotent capacity(48), and was found in several kinds of solid tumors(49). In recent years, CSCs have gained intense interest as key tumor-initiating cells that may also play an integral role in recurrence following chemotherapy. Then, a number of mechanisms of chemoresistance have been identified in CSCs (36, 50, 51), including autophagy.

Gong et al.(52) observed that muted *beclin-1* is crucial for maintenance of CSC and tumor development in athymic mice, this study highlighting the role of autophagic pathway for CSC maintenance and consequently for tumor survival and growth.

Serum-deprived mesenchymal stem cells (SD-MSCs) support MCF-7 tumor growth. Tumor injected with SD-MSCs exhibited higher cellularity, decreased apoptosis, and differentiation of tumoral cells. Muted *Beclin-1* staining indicated autophagic areas surrounded by actively proliferating tumoral cells. In addition, *in vitro* studies demonstrated that SD-MSCs survive using autophagy and secrete paracrine factors that support tumor cells following nutrient/serum deprivation(51).

Several studies show that inhibition of autophagy turns cancer cells sensitive to a broad spectrum of therapies (53-55). For example, *LC3* expression positively correlates with expression of aldehyde dehydrogenase 1 (*ALDH1*) in pancreatic cancer tissues. *ALDH1* is a CSC marker and a high co-expression of *LC3/ALDH1* can be associated with

both poor overall survival and progression-free disease (53). In pancreatic cancer cell lines, higher *LC3-II* expression was observed in the sphere-forming cells than in the bulk cells. Blockade of autophagy by silencing *ATG5*, *ATG7*, and *BECN1* or administration of chloroquine (*CQ*) markedly reduces the CSC population, *ALDH1* activity, sphere formation, and resistance to gemcitabine *in vitro* and *in vivo*(54).

The combination of temsirolimus, an *mTOR* inhibitor (clinically used in renal cancer and melanoma) with hydroxychloroquine (*HCQ*), drug that increases the pH of the lysosome, disrupting its functions and blocking its union with the autophagosome, augments cell death in preclinical models. A phase I dose-escalation study evaluated the effects of the maximum tolerated dose (MTD=200 mg/day), safety, preliminary activity, pharmacokinetics, and pharmacodynamics of the combination of these two drugs. Authors studied 27 patients with advanced solid malignancies and observed by positron emission tomography (*PET*) that such a combination produced metabolic stress in tumors of those patients that experienced clinical benefit. Pharmacodynamic evidence of autophagy inhibition was observed in tumor biopsies of patients treated with daily *HCQ*. This study indicates that combination of *HCQ* with temsirolimus is safe and tolerable, modulates autophagy in patients, and has significant antitumor activity(55). Moreover, active clinical trials are ongoing to test the clinical efficacy of autophagy inhibitors (mainly *CQ/HCQ*) as cancer therapeutics(56-60). At least 14 clinical trials using *CQ/HCQ* as adjuvant in cancer therapy are currently ongoing as summarized in Table 1.

In a single blind phase I trial (NCT01480154) it was observed that combination of *HCQ* at maximum tolerated dose with AKT-inhibitor downregulated autophagy and extend survival among patients with advanced solid tumors(56).

A phase II trial (NTC01026844) was proposed to use Erlotinib, a epidermal growth factor receptor inhibitor, commonly used for treatement of lung cancer, in conjunction with *HCQ* at different concentrations for the treatment of lung cancer. The study began in 2009 and was completed in 2017, but of little response against drugs, authors resolves finished and discarding what was found(57). Another phase II study (NTC01842594) used Sirolimus (Rapamicyn), an mTOR inhibitor (1mg) along with *HCQ* (200mg) against soft tissue sarcoma of 10 patients, with moderate toxicity (nausea, diarrhea and skin rash) being found in 30% of patients(58). Moderate toxicity was also observed in 52% of patients with untreated B-CLL lymphoma, in this phase II study (NTC00771056) (59).

Other studies enrolled in table 1 are still in the "ongoing" phase, recruiting patients or in phase of pre-publication analysis. It is possible that some of them did not achieved the hypothesized clinical results, making it difficult to be published. For instance a phase I/II trial (NCT01206530) was launched in 2010 to test the efficacy of combined therapy of *HCQ* with fluorouracil, leucovorin, calcium, oxaliplatin and bevacizumab to treat colorectal cancer (CRC)(60);but until now results are not publically available.

Nevertheless, none of these trials has reached Phase III, implying that inhibiting autophagy remains an incompletely know approach, requiring efforts understand the complexity of events that such approaches can trigger.

Table 1: Currently approved clinical trials using adjuvant CQ/HCQ as cancer therapies

Trial ID	**Start Year**	**Phase of Trial**	**Cancer Type**	**Dose of Adjuvant**	**Therapeutic agents**
NCT01480154	2011	I	Advanced solid tumors, melanoma, prostate or	HCQ MTD	Akt inhibitor MK2206

			kidney cancer		
NTC01026844	2009	II	Non-Small Cell Lung cancer	HCQ (400-600-800-1000mg/day)	Erlotinib
NTC01842594	2012	II	Soft tissue sarcoma	HCQ (200mg/day)	Sirolimus
NTC00771056	2008	II	B-Cell Chronic Lymphocytic Leukemia	HCQ (400mg/day)	---
NCT02316340	2015	II	Refractory metastatic colorectal cancer	HCQ (600 mg/day) clinical efficacy with progression free survival	Vorinostat
NCT01978184	2013	II	Pancreatic cancer	HCQ (1200 mg/day) antitumor Efficacy	Gemcitabine, abraxane
NCT01649947	2011	II	Recurrent non-small cell lung cancer	CQ (200 mg BID two times in day) response to therapy	Bevacizumab, carboplatin, paclitaxel
NCT01494155	2011	II	Pancreatic cancer	HCQ (400 mg BID) progression-free survival	Proton beam radiation therapy and capecitabine
NCT01828476	2013	II	Metastatic castrate refractory prostate cancer	Response with/without HCQ treatment	Navitoclax and abiraterone acetate
NCT01006369	2009	II	Metastatic colorectal cancer	HCQ (200 mg/day) partial response and overall survival	XELOX-bevacizumab
NCT02013778	2013	I, II	Liver cancer and hepatocellular carcinoma	HCQ MTD	Transarterial chemoembolization (TACE)
NCT01550367	2012	I, II	Metastatic renal cell carcinoma	HCQ (600–1200 mg/day) safety and toxicity	IL-2 (aldesleukin)

NCT01206530	2010	I, II	Colorectal cancer	HCQ (600–800 mg/day)	Fluorouracil leucovorin calcium oxaliplatin, bevacizumab
NCT01510119	2011	I, II	Kidney cancer	HCQ MTD rate of progression in 6 months	Rad001(everolimus)

Source: National Cancer Institute (USA)

4. Conclusion

Although the controversy about the action of autophagy as tumor promoter or tumor suppressor, there is evidence that it inhibition helps to kill tumor cells mostly dependent on the kind of tumor and the therapeutic agents to be associated with. Therefore, we need a better understanding of the functional relevance of autophagy in the tumor microenvironment and enhance the ongoing dialogue between the laboratory and clinical researches in order to provide a new focus on therapeutic strategy to prevent resistance and increase the effects of cancer therapies.

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Anexos

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Prezado Dr. Ramon

Com referência a Pesquisa "**Autofagia em células tumorais: um mecanismo de carcinogênese e resistência aos quimioterápicos**", a ser conduzido por **Jofer Andreé Zamame Ramirez**, orientado por Vossa Senhoria não necessitará de obtenção de parecer ético, uma vez que trata-se de estudo envolvendo **"Revisão Crítica da Literatura"**.

Atenciosamente,

Profª Drª Silvana Andréa Molina Lima
Coordenadora do CEP.