The role of autophagy in cancer treatment

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Abstract: Autophagy is the natural, conserved degradation of cell that removes unnecessary or dysfunctional components through a lysosome-dependent regulated mechanism. Therefore, the cell can prevent the accumulation of toxins or unnecessary components that leads to damage of the cell and recycle these components to sustain metabolic homeostasis. In mammalian cells, there are mainly three types of autophagy. They are microautophagy, macroautophagy and chaperone-mediated autophagy (CMA). Macroautophagy is the most widely used type of autophagy, thus most of the autophagy we talked about is macroautophagy. Multidrug resistance (MDR) is an unavoidable issue after long-term chemotherapy, resulting in refractory cancer and tumour recurrence. Fortunately, autophagy as a self-degradative system has the potential to be utilized as a possible treatment for cancer. In the following essay, the mechanism and function of autophagy in cancer cell will be discussed. In cancer specifically, autophagy plays a dichotomous role, as it can inhibit tumour initiation, but it supports tumour progression. Recently autophagy has been approved to treat several diseases including cancer, in order to provide safe and efficient therapeutics that controls autophagy, a thorough understanding of the mechanism of autophagy with a special focus on druggable targets (GEMMS) will be fully discussed in the paper.

1. Introduction

1.1. Mechanism of autophagy

Autophagy is a mechanism that is characterized by the autophagosomes, or autophagic vesicles, that is capable of engulfing and degrading aggregated proteins and damaged organelles. Then, the autophagic vesicles will fuse with lysosome. Lysosome is a membrane-bound organelle in animal cell. Inside the membrane of lysosome, there is the presence of hydrologic enzymes which can degrade various bio molecules. The fusion of autophagic vesicles with lysosome will ensure the degradation of autophagic vesicles content (eg. aggregate protein, damaged organelles and etc.) Under normal cellular micro-environment, the aim of autophagy is to carry out homeostatic function such as the turnover of protein and organelle. Under environment with lack of nutrients, such as starvation, or environment with additional need of clearing aggregate protein, autophagy is unregulated to tackle with the problem (figure 1).

1.2. Process of autophagy

There are four essential steps involved in the autophagy: initiation, nucleation, maturation, and degradation. (Figure 1) The stress signal from mTORC1 initiates the autophagy. Then, the phosphoinositide signals induce class III phosphoinositide 3-kinase Vps34 and Beclin1. Ubiquitin-like protein Atg8 will combine with the phosphatidylethanolamine on the membrane, forming an early stage autophagic vesicle. A cleavage will be formed by cysteine protease, and ubiquitin-like protein Atg8 will be integrated into the bilayer, recruiting autophagy receptors. These receptors will induct adaptor protein to close the cleavage and form a complete autophagic vesicle. The autophagic vesicle is then delivered to lysosome and fuse with lysosome, degrading the biomolecule content of

autophagic vesicle. The acidic pH environment in lysosome is neutralized by chloroquine or bafilomycin

1.3. Autophagy's dichotomous role in cancer microenvironment

Acknowledging autophagy has been identified as a potential therapeutic target in cancer in the past few decades. The modern researchers have found that whether by inhibiting autophagy or inducing autophagy, both have the possibility to enhance tumour's survival rate, which completely subvert people's firmed opinion on the positive effect controlling autophagy. Several studies have shown that mice with loss of one allele of the autophagy gene Beclin 1 developed cancer [1-3]. Initially, people made hypothesis that by inhibiting autophagy can treat diseases such as breast, ovarian, and prostate cancers, which are known to harbor monoallelic loss of Beclin 123, 24, 26. However, it was shown that the human homolog of the mouse Beclin1/ Atg6 gene, BECN1 gene resides adjacent to BRCA1, but other tumour suppressor genes are also on the same chromosome. Tumorigenesis in human tumours may be more likely to found in other neighbouring genes than in BECN1.

On the other hand, many preclinical studies have successfully found the autophagy is induces by the cytoprotective anticancer agent. However, the experiment result cannot be directly translated into use of human bodies. Because yet there are only a few cytotoxic [4] agents can induce autophagy without causing other important process in the cell, such as mTOR signalling or the unfolded protein response [5].

2. Method

2.1. Using GEEMs to test autophagy's function within tumour micro-environment

In the past studies, research made a comparison between normal and autophagy-defective mice, in order to figure out the actual role of autophagy. The accumulation of ubiquitinated keratins, the autophagy cargo adaptor p62, and abnormal mitochondria is observed in the autophagy defective mice [6,7]. The chronic inflammatory state [8,9] can also cause DNA damage response, cell damage and reactive oxygen species (ROS) production, which is initially started by those damaged, malfunctioned cellular organelles. They are recognised as cancer promoter and identified as it can initiate degenerative and inflammatory diseases [10,11].

3. Result

3.1. Role of autophagy in adult mice

By knowing tumour cells are more sensitive and autophagy-dependent, thus, to figure out autophagy's role in cancer is to simply switch off autophagy, then to close examine the impact on the host. GEEMs has been widely used globally. From a close observation of the experiment, by Atg 7 or Atg 5 deficiency may introduce internal cell starvation, thus they require more nutrients than normal mice. By knocking out tissue specific Atg illustrates that autophagy is closely assonated with brain, liver, adipose and muscle [12].

But in sharp contrast, under Atg deficiency, deleting Atg 7 or Atg 5 in adult mice is surprisingly tolerated and less harmful than in neonatal samples. Most adult mice without Atg 7 or Atg 5 can live healthy for more than a month. Thus, the result demonstrates autophagy is probably more important in early stages because of autophagy's unique role on maintaining metabolism and growth. However, adult mice that have fully differentiated cell tissues and complete function is less likely to be impact as severe over time. The few potential risk of lacking autophagy is to get neurodegeneration.

3.2. The effectiveness of autophagy in RAD-Driven cancers

According to the experiments have demonstrated it is achievable to inhibit autophagy to treat RAS-Driven cancers. In genetically engineered mouse models (GEMMs), when autophagy genes were

deleted, the results shown that autophagy suppresses the development of benign tumours, but at the same time accelerates the growth of advanced cancers. [13-17]

In many research fields have concluded that autophagy might promote resistance during cancer treatment. In a recent GEMMs model, they muted Atg 7 gene to observe the result. This model is a very close model of autophagy inhibition to normal human situation. Despite losing gene Atg 7 didn't cause any severe damage to most adult mice themselves, but after a period, those mice were observed to start developing neurodegeneration.

3.3. Overview of autophagy in mice models

Mice with allelic loss of the essential autophagy gene beclin1 display defective autophagy, altered protein homeostasis (accumulation of ubiquitinated proteins and p62), and gross morphologic tissue damage that is particularly striking in liver where there is also an accelerated incidence of hepatocellular carcinoma (Figure 2) These findings suggest that autophagy stimulators may prevent both degenerative diseases and cancers arising from chronic tissue damage and inflammation, such as hepatocellular carcinomas.

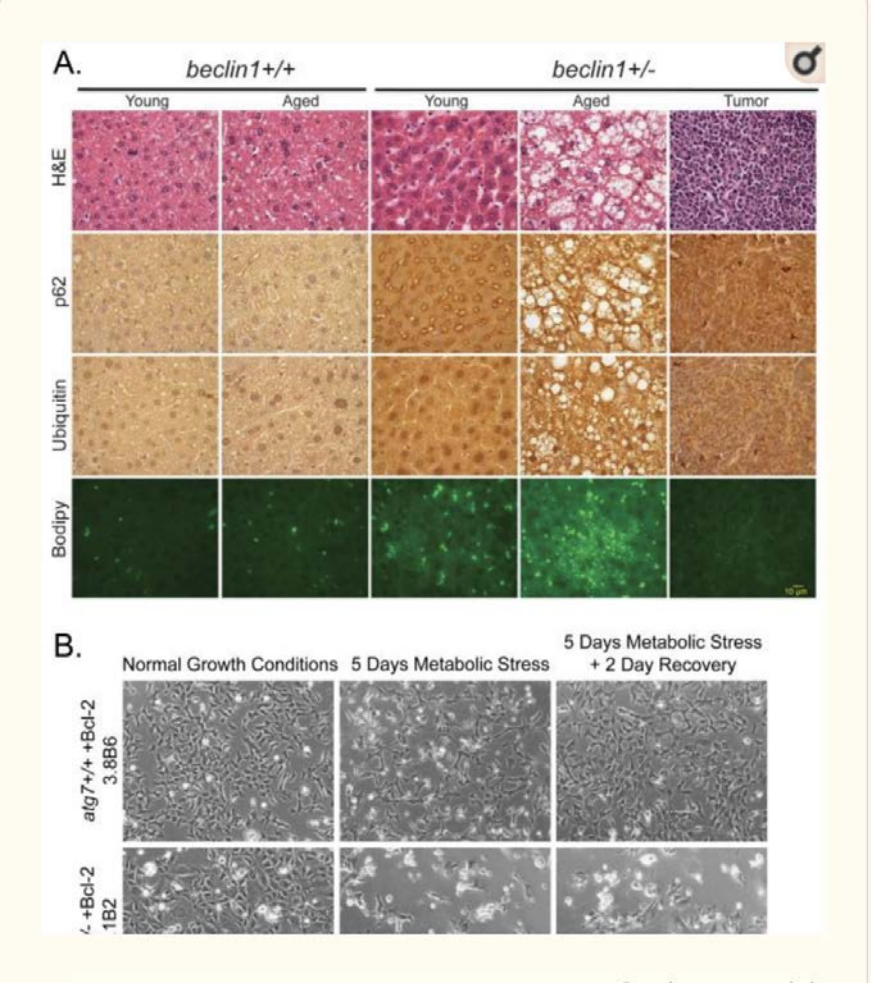

Figure 1. Role of autophagy in suppressing liver damage and cell death. A, elevated p62, ubiqulitin, and accumulation of lipids in aged beclin 1+/- mouse live. Sections of liver from young (16-month-old) and aged (>24 month) beclin +/- and +/- mice and a representative spontaneous liver tumour from a beclin 1 +/- mouse were stained with hematoxylin and eosin, and by immunohistochemistry for p62 and ubiquitin, and with BODIPY to indicate lipid droplet accumulation by fluorescence. B, autophagy promotes cell viability in metabolic stress. Representative images of immortal baby mouse kidney epithelial cells derived from atg7 +/+ and -/- mice that were untreated, treated with metabolic stress, and allowed to recover. Image reproduced with permission from Dr. C. Karp and H.-Y. Chen from the White laboratory.

3.4. Current therapeutic treatment

3.4.1. Hydroxychloroquine (HCQ) as an autophagy inhibitor

HCQ is a weak base that can trap inside compartments like lysosomes which are acidic, by inhibiting HCQ can increase the pH of those compartments. Some preclinical studies have illustrated the fact that HCQ can promote tumour cell death and enhance tumour killing while doing cytotoxic chemotherapy and target specific agents. The rationale for choosing HCQ is because several studies have demonstrated by escalating with chloroquine from the standard 150mg/day, patients are likely to suffer severe drug toxicity. But in sharp contrast, increasing the amount of HCQ in the treatment does not show a significant negative effect on patients.

3.4.2. The crosstalk between intracellular systems

To maintain the human body homeostasis, two intracellular degradation systems play a critical role. The autophagy-lysosome system and the ubiquitin-proteasome system (UPS) has its unique function of recycling waste inside the body. In many studies have concluded that UPS is served as targeting short-lived proteins and soluble misfolded proteins. Reversely, others long-lived proteins, aggregated and misfolded proteins, and intracellular components are look after by autophagy [18-20]. From previous studies, the result has shown both two degradation systems are working dependently to maintain the homeostatic, thus it is reasonable to assume by combining those two intracellular systems can generate a more efficient and effective treatment on diseases, since autophagy is actively activated by the inhibition of UPS.

3.4.3. ER stress inhibition

The study on ER stress also allocates its important roles, as a control centre to connect both two systems to work correspondingly. By knowing the ER's major function is to process the body waste, including the by-product from varies proteins. Thus, those misfolded proteins are packed and get exported from the ER lumen, then those proteasomes can degrade those proteins through some certain pathways. On top of autophagy's function of clearing out misfolded proteins [21]. Consequently, the guess of by using drug-induced ER stress to induce autophagy is generated [22-26]. The activation of autophagy can also prevent cell death since the accumulation of misfolded proteins can lead to cell malfunction and disrupt the normal body function. It is extremely useful in relevant cancer therapies which involves proteasome inhibitors. In addition, by genetically ablating autophagy can carry tumour cells to proteasome inhibitors to clear out waste proteins. In stack of using the combination of bortezomib and chloroquine can also significantly promote tumour cell death in both vitro and in vivo [27]. Thus, to support the current therapies to be more effective.

3.4.4. Limiting chronic inflammation

The inflammatory of cells are also targeted by autophagy to process to be removed from the body. The persistence of continuously unfixed damages can lead to inflammation and introduce chronic cell death. In many cancers micro-environments, it can be a potential cancer promotor. And if cells are lack of nutrients, they tend to undergo necrosis, which will cause more inflammation. On the other hand, autophagy can provide the essential internal resources to maintain metabolism and prevent extra cell damages, thus, to minimise uncertainly of cells getting further damaged and body inflammation that can start the process of tumour initiation and development.

3.4.5. The risk of malignant transformation can be reduced by autophagy induction with metformin or rapamycin

The success of reducing cancer risk in type 2 diabetic patients has been proved in many epidemiological studies and supports the idea of metformin can suppress tumorigenesis of cancer. The rationale of using metformin to treat cancer is because it activates AMPK pathway and brings metabolic responses like mTOR signalling and reduced insulin resistance. From the research, the mTOR and metabolic negative responses are the main reasons to resist cancer treatment and cause

the tumour initiation. Once these factors are suppressed, the cancer treatment then can go smoothly and even initially to prevent the cancer to occur. Given the information all above, using the combination of stimulating autophagy with metformin can be helpful to treat those patients who suffer metabolic syndrome and prevent cancer initiation.

3.4.6. p53 as an autophagy inducer

In the nucleus, one of the pro-autophagic factor is stress-activated p53, which is also a transcription-dependent agent [28]. In many past studies have shown that rapamycin (mTOR) will slow down the autophagy rate. However, those p53 targeted genes once stimulated, they are likely to treat mTOR, to maintain the homeostatic status. Reversely, AMP-responsonsive protein kinase (AMPK) is the factor which can stimulate the process of autophagy to start through transcription regulation [29,30]. Once AMPK inactivate Rheb, which is a mTORC1-interacting protein [31,32] can stop mTOR mechanism. In addition, another tumour suppressor protein, death-associated protein kinase 1 (DAPK-1) can also be regulated by the activated p53 genes by certain mechanism.

3.4.7. p53 as an autophagy suppressor

Not all p53 related genes are all proautophagic factors, such as TP53- induced glycolysis and apoptosis regulator (TIGAR), this specific gene can help the internal environment to regulate abnormal metabolic pathways and release any oxidative stress. To gain a deeper understanding of TIGAR's function, it can decline reactive oxygen species (ROS) levels directly, by actively suppressing fructose-2,6-bisphosphate levels. p53 gives especially unstressed cells the role of transcription that when p53 leaves, the level of basal autophagy on the mTOR is increased. Nevertheless, silencing p53 in mouse embryo fibroblast shows p53 plays an important role to upregulate microtubule-associated protein ARF then stimulate and start the process of autophagy. Overall, multiple studies have made a solid conclusion of p53's deficientness as an inhibitor of autophagy.

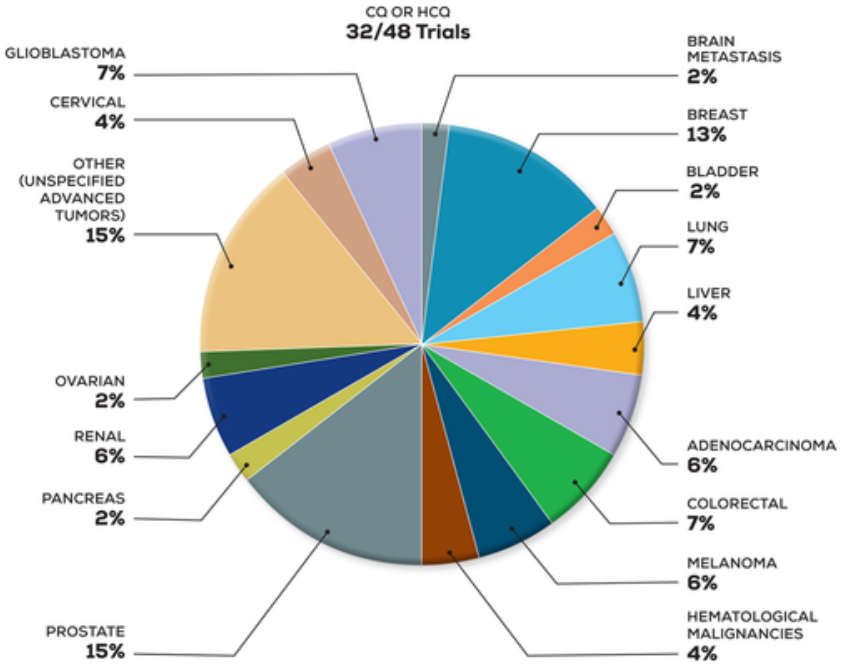

Figure 2. Current clinical trials targeting autophagy in cancer. The pie chart shows the breakdown of which cancers have clinical trails targeting autophagy registered at www.clinicaltrails.gov.

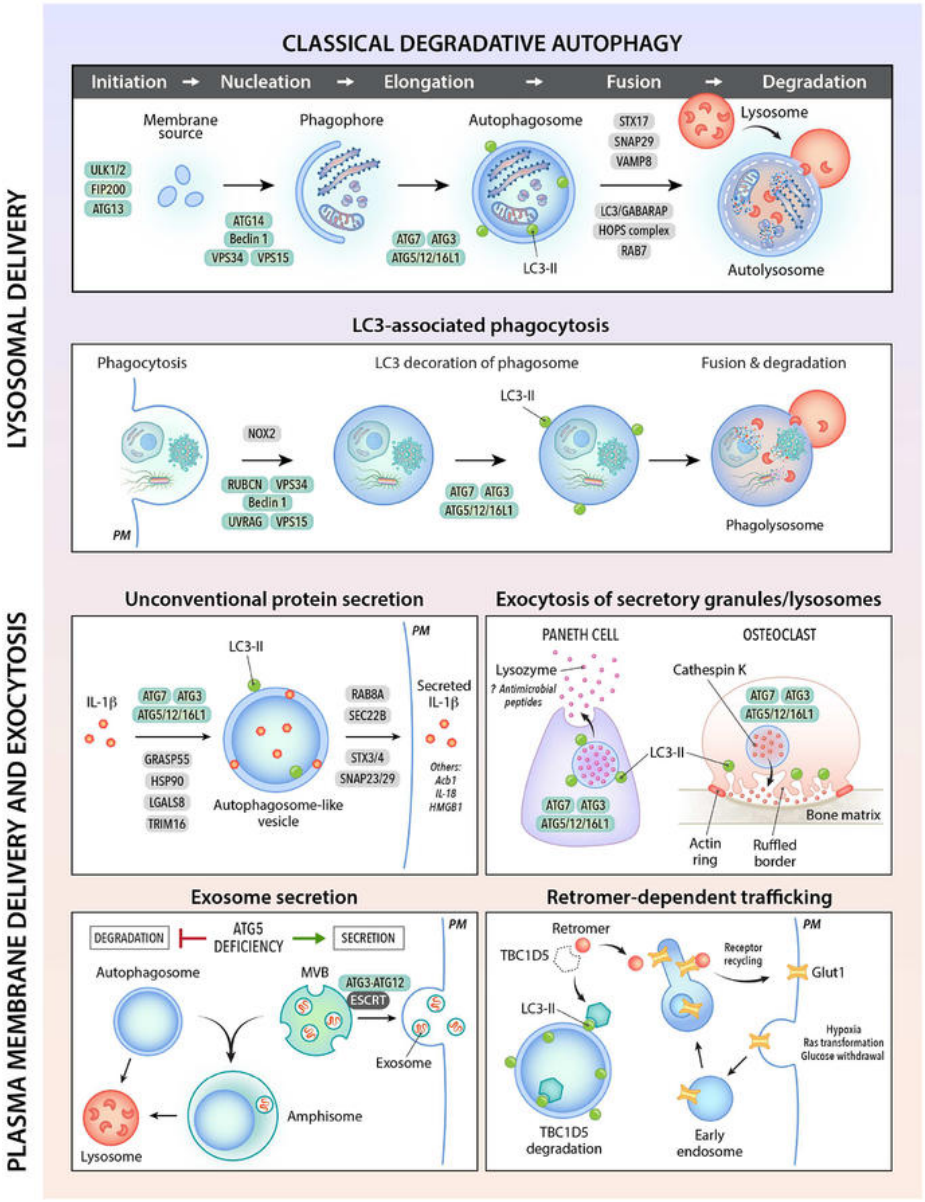

Figure 3. The autophagic pathway: autophagy is a degradative process characterized by the formation of double membrane vacuole known as autophagosomes. The process begins by the formation of the isolation membrane (phagophore), a step that depends on the activity of the complex Beclin1, among other Atg proteins, and also on the lipidation of LC3. The phagophore sequesters cytoplasmic portions and aged organelles such as mitochondria. Then, the autophagosome fuses with endocytic compartments and finally with the lysosomes to degrade and recycle the incorporated material.

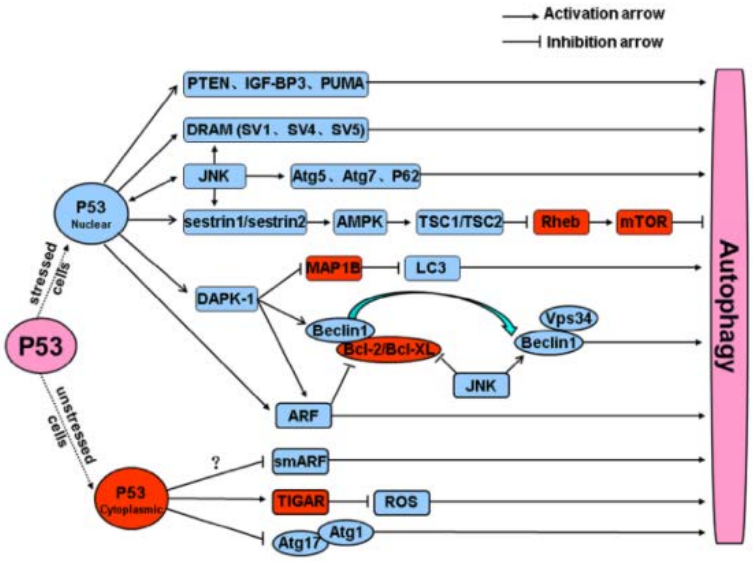

Figure 4. Based on cellular localization, p53 can be a positive or negative regulator of autophagy. In the nucleus, p53 may activate the AMPK pathway and inhibit the mTOR pathway, subsequently triggering autophagy. P53 may also transactivate multiple genes with proautophagic roles, including DRAM, PTENM IGF-BP3, DAPK-1, ARF, JNK and proapoptotic Bcl-2 proteins. . Cytoplasmic p53 suppresses autophagy via poorly understood mechanisms. P53 is not only associated with TIGAR but may also be involved in extranuclear pro-autophagic factors or interactions, respectively. Dotted lines indicate the redistribution of nuclear versus cytoplasmic p53.

3.5. Relationship among autophagy, immunity, and cancer

There has been increasing evidence from various studies shown that immunotherapy has striking benefit in refractory cancer. Patients who get cancer will have systemic autophagic syndrome. The level of autophagy is significantly increased in cancer cell, pathologically, and the level of autophagy is suppressed in immune cells. If the response of autophagic syndrome in the host cell and cancer cell is balanced, then immunotherapy, such as adoptive transfer of T cell, administration of antibodies or human recombinant cytokines will have to potential to have an effect. The therapy of dendritic cell (DC) vaccines also has the probability of treating. DC vaccine isolates the antigen presenting cells of the patient, incubate to target tumour cell and associate the specific antigen. After the incubation, the reintroduction of dendritic cell will provide a stronger immune response towards the cancer cell, facilitated by T cells activated by dendritic cells. Autophagy will direct the delivery of MHC molecules. The systemic induction of autophagy in early stage of adaptive immunity will significantly decrease the probability of immune tolerance, the induction of autophagy will improve the efficiency and efficacy of immunotherapies. Also, the inhibition of autophagy will increase the cytotoxicity of T cell and natural killer cells. Autophagy inhibition can be carried out by the introduction of hydroxychloroquine. This can also increase the effectiveness of chemotherapy and radiation therapy.

4. Conclusion

Chronic inflammation due accumulation of ubiquitinated protein, autophagy cargo adaptor and abnormal mitochondrion will cause DNA damage, cell damage and would further promote the development of cancer. Although autophagy is able to suppress tumour development, it also promotes the survival of tumour cell. Hydroxychloroquine, which is capable of promote tumour cell death, is a chemical that could be used in treatment of cancer. This brought a prospect of cancer treatment that target autophagy. Although nuclear p53 can enhance the autophagy activity. And cytoplasmic p53 oppositely can shut down autophagy through extranuclear and other mechanisms. Recent studies have pointed out the uncertainty while inhibiting cytoplasmic p53 might cause consequent biological reaction, such as K120 acetylation and K386sumoylation of p53. Thus, the safety of using p53 to

target cancer needs to be further proofed and tested. Although from past studies have shown that autophagy has an important role in regulating metabolism and organelle quality control, the role of autophagy in cancer is still undefined. While more and more specific and selective autophagy inhibitors are becoming available, the patients' different reaction to inhibitors are still unknown. And only few studies have been interested and started on looking at after the patient is induced the autophagy, will it be a sustainable solution to resist cancer. There are so many mysteries are always for scientises to explore.

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