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An overview on the role of autophagy in cancer therapy

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Abstract

Autophagy is a highly regulated catabolic process through which cells recycle their own constituents by delivering them into lysosomes. Several studies have demonstrated that autophagy plays a wide variety of physiological and pathophysiological roles in cells. In cancer, autophagy has been described to have paradoxical roles, acting both as tumor suppressor and as tumor promoter. In particular, it may exert different functions in response to cancer therapy, causing cancer resistance or increasing sensitivity to chemotherapeutic drugs and radiation. Therefore, autophagy could provide new means for the enhancement of antitumor drugs and radiation effectiveness.

Introduction

Autophagy (self-eating) is a highly conserved catabolic process with critical functions in the maintenance of cellular homeostasis under normal growth conditions and in the preservation of cell viability under stress [1]. Autophagy is an intracellular process in which cellular components, such as proteins and organelles, are delivered to the lysosome leading to the degradation and recycling of cytosolic compounds, thus providing cells with essential amino acids, nucleotides, and fatty acids, that enable production of elements required for energy and macromolecule biosynthesis [2,3]. There are three main types of autophagy, differing mainly in the mechanism by which the cytosolic material is presented to the lysosome [1]: i) macroautophagy, ii) microautophagy and iii) chaperone-mediated autophagy (CMA). In macroautophagy, double-membrane vesicles, called autophagosomes, sequester cytosolic material. Those vesicles merge with lysosomes (forming the autophagolysosome), their cargo is degraded, by lysosomal hydrolases, and the recycled macromolecular precursors are transported back into the cytoplasm, where they can be used as metabolic intermediates. In microautophagy, no intermediary vesicles are present, and the cytoplasmic material is directly engulfed by the lysosome [2]. In CMA, specific proteins, associated to heat shock protein (HSP) hsc70 and its co-chaperones, are translocated to the lysosome. Those proteins contain a specific amino acid motif (KFERQ, or biochemically related), which is recognized by the HSP, and once unfolded, they are translocated directly into the lysosome, via the lysosome-associated membrane protein 2A (LAMP2A) [4,5]

Several studies have already demonstrated that autophagy plays more roles than the initially expected, including: cellular adaptation to starvation, intracellular protein and organelle clearance, development, anti-aging, elimination of microorganisms, cell death and antigen presentation [6].

Deregulation of autophagy has been associated to several diseases, including neurodegenerative diseases, diabetes and cancer [7].

In this short review, we will mainly address the role of autophagy (and its different functional forms) in cancer, and its implication in cancer therapy. The majority of the studies published on autophagy, particularly those related to cancer therapy refer to "macroautophagy". In fact, the broad term 'autophagy' usually means "macroautophagy", unless otherwise specified, and therefore, in this review, we will also use this terminology [1]. Nevertheless, it is important to mention that recent studies have shown that CMA may be also important for tumor growth, progression and therapy and that pharmacological approaches that inhibit macroautophagy may also affect CMA [8,9].

Autophagy in cancer

Cancer was one of the first diseases to be associated to autophagy [10-14]. Nevertheless, the exact molecular mechanisms and the role of autophagy in cancer cells is not yet clearly defined, being even paradoxical. While at early stages, autophagy usually acts as a tumor suppressor allowing cells to discard damaged cellular contents, decreasing ROS and DNA damage, in more advanced stages of tumor development, it may help cancer cells to survive under low-oxygen and low-nutrient conditions, acting as a tumor promoter [3,15]. Actually, the dependence of tumor cells on autophagy is highly variable. While some tumor models (like pancreatic cancer) display increased autophagy levels in basal situations (including in plenty nutrient conditions), with autophagy having a role in the maintenance of tumor growth [16], results from other studies, comparing the levels of autophagy in tumor cells with their corresponding non-tumor cells, show disparate data between different tumor models (for a thorough

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review please see [17]).

Importantly, autophagy plays also a role in cancer response to therapy since cancer therapies mostly inflict stress and damage to cells to induce cell death [18]. The outcomes of therapy-induced autophagy in cancer cells may represent also a "double-edge sword" and depends on the particular type of cancer, on the stage of disease progression or even on the type and duration of autophagy [18-21]. Indeed, several studies showed that increased autophagy leads to resistance to both chemo- and radiotherapy, while several others show that many anticancer drugs induce autophagy-related cell death in cancer cells [22,23].

The fact that many of the currently used clinically approved anticancer strategies have been described as inducing autophagy, makes the understanding of the functional role of autophagy within a specific cancer context much more relevant, as it could provide new means for the enhancement of antitumor drugs and radiation effectiveness.

Functional forms of autophagy and their implications for cancer therapy

Although, traditionally, autophagy has been seen as a pro-survival (cytoprotective) mechanism, different studies have shown that it may result in other outcomes. Currently, at least four distinct functional forms of autophagy have been described [24,25]: i) Cytoprotective, when cells die or arrest if autophagy is inhibited; ii) Cytotoxic, when autophagy induction results in cell death and its blockage results in cell survival; iii) Cytostatic, when autophagy induction results in cell growth arrest and iv) Nonprotective, if autophagy does not affect cell growth once blocked. These forms are distinguished on only based on their functional characteristics, having similar morphologic, biochemical or molecular profiles [24].

Autophagy modulation as a therapeutic strategy to improve anticancer strategies

As already referred, the different functional forms of autophagy affect the cellular response to anticancer therapies. The knowledge whether autophagy is cytoprotetive or is cytotoxic/cytostatic, will help defining strategies for its modulation (through its decrease or increase, respectively) to interfere with the cellular sensitivity to therapy.

Targeting cytoprotective autophagy has been at the basis for multiple clinical trials. Indeed, if increased autophagy confers tumor resistance to death-inducing agents, its inhibition will allow an enhanced response to treatment [26]. There are several autophagy inhibitors already identified and that have been classified as: early-stage inhibitors, if blocking autophagosome formation [such as 3-Methyladenine (3-MA), wortmannin, and LY294002] orlate-stage inhibitors, acting at the level of the autophagosome-lysosome fusion and degradation steps [such as chloroquine (CQ), hydroxychloroquine (HCQ), bafilomycin A1, and monensin]. Studies using, not only these pharmacological autophagy inhibitors, but also genetic silencing or knockdown of autophagy-associated genes, resulted in increased tumor cell sensitivity to the autophagy-inducing stimulus, usually via the promotion of apoptosis [24,26].

Several clinical trials have been evaluating the use of autophagy inhibitors (particularly HCQ) in combination to chemo- and radiotherapy to improve its efficacy [27,28]. A study carried out in melanoma patients using HCQ in combination with the mTOR inhibitor (temsirolimus) showed an improvement of the median progression-free survival to 3.5 months and increased the rate of stable disease in patients [27,29]. Also, its combination with a proteasome inhibitor (bortezomib) in relapsed/refractory myeloma patients resulted in a higher rate of partial response and stable disease [30]). More recently, the use of HCQ in combination with gemcitabine in pancreatic ductal adenocarcinoma patients caused significant decreases in the disease biomarker, CA 19–9, with the mean overall survival being extended to nearly 3 years [28,31]. Although clinical trials with these compounds indicate that autophagy inhibition in patients is possible, there is still room for improvement, since CQ/HCQ have also shown significant variability of autophagy inhibition levels among patients. Moreover, these type of compounds, although being already FDA approved, have to be administered in higher concentrations to inhibit autophagy and are retained for long periods of time in patients (some studies showing patients retaining HCQ) in their system up to 5 years [28,32].

On the other hand, autophagy induction may help improve the effect of anticancer therapies when autophagy is cytotoxic, by inducing cell death by itself or by the activation of other cell death mechanism, namely apoptosis [33,34]. Several drugs/natural extracts, some of which already used in the clinic, have been described to induce autophagy-mediated cell death in different cancer cells [23]. For example, the combination of Vitamin D with radiation promoted cytotoxic autophagy in breast tumor cells [35,36]. Resveratrol and curcumin caused cell death in several human tumor cell lines through apoptosis and autophagy [37,38]. Naphthazarin, a naphthoquinone compound acting as microtubule depolymerizing agent was shown to induce cell death in lung cancer cells through apoptosis and autophagy [39]. In addition, the small molecule STF-62247 induced autophagic cell death in Von Hippel Lindau (VHL)-deficient renal cell carcinoma cells [40] and TXA1, a thioxantonic small molecule, decreased the viability of melanoma and breast cancer cells through the induction of autophagy [41].

Autophagy in immunotherapy

The role of immune response has been gaining particular interest for cancer therapy. Recently, autophagy has also been described as playing an important role in the regulation of immune recognition and response [42]. It has been demonstrated that autophagy increases tumor cells immunogenicity, being involved in tumor antigen processing and in the subsequent activation of the effector T cells. Thus, strategies aiming at autophagy induction could serve as adjuvant to stimulate the antitumor immune response. For example, the use of tumor autophagosome-derived vaccines have been found to induce cytotoxic immune cells and, consequently, antitumor activity in mice bearing lung carcinoma and melanoma cell lines [43].

Recent studies show that, since increased levels of autophagy in cancer cell suppresses the antitumor immune response, autophagy inhibition improves antitumor immune response in immunotherapeutic strategies, such as adoptive transfer of T cells, vaccines, administration of antibodies or recombinant cytokines [44] Based on published findings, autophagy inhibition may increase the cytotoxicity of effector T and NK cells once they have been activated to lyse the tumor cells. The combination of high doses of IL-2 with chloroquine increased long term survival, decreased vascular leakage associated toxicity, and enhanced immune cell proliferation and infiltration in the liver and spleen [45]. Autophagy plays also a fundamental role in increasing the immunogenicity of the tumor cell, participates in the antigen processing and in the subsequent activation of the effector T cells, and its induction could be exploited as adjuvant strategy to stimulate the

antitumor immune response [43,46]

The understanding under which circumstances inducers or inhibitors of autophagy affect the therapeutic efficacy of anticancer treatments will be important to improve the rational use of such modulators, since the data available do not yet allow us to realize this [46].

Summary

Autophagy plays an important role as a stress response mechanism to chemotherapeutic drugs and radiation in cancer cells. There are at least four functional forms of autophagy that may occur in response to chemotherapy or radiation: cytoprotective, nonprotective, cytotoxic and cytotastic. Currently, is not possible to predict which form will be induced by a particular therapy, since these forms of autophagy have no clear-cut morphologic, biochemical, or molecular distinctions. In some circumstances, autophagy protects tumor cells from cancer therapy while, in others it is associated with cancer cell killing. Modulation of autophagy may represent an important therapeutic opportunity to enhance the efficacy of anticancer therapies. The future challenge for autophagy research in cancer therapy is to find ways to identify which functional form of autophagy is activated, in specific tumor models, and which tumors may be most effectively treated by autophagy modulation. A better understanding of the role of autophagy in different tumor models will provide new therapeutic tools for more effective cancer therapeutic strategies.

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