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Apoptosis and Autophagy Pathways, A New Therapy Approach in Head and Neck Cancer

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ABSTRACT

Tumor development is linked to apoptosis and autophagy mechanisms that generally use kinase signaling pathways and several transcriptional regulators to decide tumor cell fate. Apoptosis and autophagy are cellular processes that play an important role in maintaining cellular homeostasis, but some of their checkpoints's pathways can be real targets for tumor therapy. If the end of the molecular process of apoptosis is characterized by the removal of the damaged cells (cell death), the molecular process of autophagy is the lysosomal degradation, followed by lysosomal recycling of proteins and organelles, therefore being considered a survival mechanism. Recently it has been shown that autophagy could be associated with a chemotherapy resistance, but how the autophagy contributes to chemo resistance in head and neck cancer (**HNSCC**) still not be elucidated. Some of the therapeutic agents used in HNSCC treatment such as cetuximab or sorafenib applied alone or in combination with radiation have shown promising results. Both drugs are able to induce the modulation of apoptosis as well as the activation of

autophagy. Autophagy has an important place within the complex network created to generate an adequate response to different stimuli, therefore the inhibition of autophagy may lead to a better sensitization of tumor cells to cytotoxic drugs or the reduction of chemoresistance. In this chapter, we will discuss the possible role of autophagy in anticancer therapy and we will focus on the analysis of the possible connections that exist between apoptosis and autophagy as well as on the molecules involved in these connections in order to reveal new potential targets for tumor prevention and cancer therapy. Understanding and integrating this new information could lead to the development of new multi-target therapeutic strategies that might improve the response to treatment and increase the survival rate for patients with cancer, especially those with HNSCC.

INTRODUCTION

Head and neck cancer includes four types of cancer which develop in the oral cavity, oropharynx, hypopharynx and larynx, and by the time a pacient is diagnosed in most cases the disease is already in its advanced stages [1]. Forsooth, over the last couple of years there has been an increase in the incidence of head and neck squamous cell carcinoma (**HNSCC**) around the world which has led to the rising of this type of cancer all the way to the sixth place in the rankings of the most aggressive malignancies [2]. Conventional HNSCC therapy depends on the location of the tumor and the stage of the disease. Using these conventional therapeutic strategies, it is intended to optimize disease control by minimizing long-term toxicity and maximizing survival outcomes for patients [3]. In current clinical management algorithms for early-stage disease, the choice of treatment addresses surgery and / or radiotherapy to help preserve organs or reduce the risk of recurrence. Given the fact that, at the time of diagnosis, the majority of HNSCC patients present an advanced locoregional disease state, combined treatment strategies that may affect the quality of life are used on them [4]. Standard combinatorial therapy is based on radiotherapy associated with cisplatin therapy alone or combined with other chemotherapeutic agents, such as taxanes (paclitaxel and docetaxel) and 5-fluorouracil, while for non-receptive patients, radiotherapy can be combined with the anti-epidermal growth factor receptor (**EGFR**) monoclonal antibody (cetuximab) treatment. However, patients with HNSCC only have modest responses to second-line systemic therapies [5].

The low success rate of the treatment in HNSCC cases could be due to genomic complexity and intratumoral genetic heterogeneity that can lead to both to chemotherapy resistance and loco regional or distant recurrence or metastasis [6,7]. The development of HNSCC is a progressive process consisting of several stages, from precancerous lesions to malignant tumors, a process which involves genetic changes, which activates the oncogenes (that induce cell growth by inhibiting cell death) and inactivate the tumour suppressor genes that normally exert an anti-tumoral activity by suppressing DNA replication and promoting cell death [8]. Tumor progression comprises the expression of the malignant phenotype and the tendency of malignant cells to acquire more aggressive characteristics over time. A prominent characteristic of the malignant

phenotype is the propensity for genomic instability and uncontrolled growth. The aggressiveness of tumors directly correlates with the regulatory molecules expression that control the proliferation, survival or programmed cell death processes [9]. The molecular mechanisms that lead to the appearance of a highly malignant phenotype and to an unfavorable prognosis in HNSCC patients are poorly understood, therefore a rigorous study of HNSCC pathogenesis is required to elucidate these mechanisms in order to develop innovative treatment strategies. Many of the drugs used to treat cancer act to activate the programmed cell death processes (apoptosis, necrosis) which lead to the inhibition of excessive tumor cell proliferation. Extensive studies of the effects of anti-cancer therapy on apoptosis have led to a better understanding of the regulatory mechanisms that control the apoptotic process as well as the identification of the proteins that play a vital part in the activation or inhibition of apoptosis in the tumorigenesis process. However, it seems that the molecular mechanisms governing the unlimited multiplication of tumor cells are much more complex and involve, in turn, the activation or inhibition of several signal transduction pathways, determining the possibility of a certain interconnectivity between them. The present study, cares to highlight the possible connections that might form between the apoptotic process and the autophagic process and which, in turn, might be able to control the response to anti-cancer therapy, thus allowing the development of multi-target therapy.

APOPTOSIS VERSUS AUTOPHAGY IN CARCINOGENESIS

Apoptosis (type I programmed cell death) is a process characterized by cellular events such as chromatin condensation, nuclear shrinkage and apoptosis bodies production which are associated with biochemical and morphological changes. Depending on the nature of stimuli that act at the level of the mitochondria or which induce the activation of membrane receptors, the apoptotic signaling cascade is activated by the intrinsic or extrinsic pathways [10,11]. To trigger apoptosis during tumor development the important signals such as extracellular triggers such as growth/survival factor depletion, hypoxia, radiation and loss of cell-matrix interactions, but also the intracellular triggers including DNA damage (produced by cell-cycle checkpoint defects or exogenous toxins), telomere malfunction and inappropriate proliferative signals produced by oncogenic mutations interfere. In cancer, there is a loss of balance between cell division and cell death and cells that should have died do not receive the signals to do so. However, being a double-edged sword, apoptosis can be the cause of the problem as well as the solution, in the quest of finding new drugs targeting various aspects of apoptosis [12-14].

Apoptosis is subverted during tumorigenesis, through the systematic loss of regulatory control mechanisms, ultimately resulting in the generation of a malignant phenotype and resistance to chemotherapy and radiation therapy. The mechanisms that contribute to the evasion of apoptosis can be divided into: disrupted balance of pro-apoptotic and anti-apoptotic proteins, reduced caspases function and impaired death receptor signaling. Apoptosis induction is arguably the most potent defense against cancer. The cellular mechanisms associated with

apoptosis are highly conserved and might explain why mutations in genes that regulate apoptosis pathways (e.g., p53, Bcl-2 family members, and PTEN) are common in most human cancers, including HNSCC and they underscore the importance of apoptosis resistance in the process of carcinogenesis. Apoptosis acts as a natural barrier able to restrict malignant cells from surviving and disseminating. However, cancer cells frequently find different strategies to avoid programmed cell death pathways by generating genetic mutations or epigenetic modifications. Deciphering of the apoptotic mechanisms leading to a comprehensive view of how defects along the apoptotic pathway contribute to carcinogenesis and how apoptosis can be used as a vehicle of targeted treatment in cancer [15,16].

Autophagia (also known as Type II programmed cell death to distinguish it from apoptosis or Type I programmed cell death) is described as a process of degradation and recycling of cellular components damaged by the formation of an autophagosome in which the target components are sequestered, later on being subject to degradation by acid lysosomal hydrolases as a result of fusion of the autophagosome with the lysosome. This complex process could be divided into five steps, including initiation, elongation, maturation, fusion and degradation, all controlled by over 30 products of autophagy-related genes (**ATG**). Three types of autophagy must be considered: basal housekeeping autophagy important for protein and organelle turnover, starvation and stress-induced autophagy that promotes cell survival and autophagy that exceeds the safe threshold and leads to cell death [17-19]. In tumor biology, autophagy as a double-edged sword and has both pro-, and anti-tumor effects. Numerous studies suggest that autophagy always is a protector during tumorigenesis, even if it plays dual roles as tumor suppressor and promotor in different stages [20]. Autophagy protects normal cell homeostasis during early stages of tumorigenesis by limiting genome instability via retarding stem cells involved in the damage/repair cycle and by inhibiting the formation of inflammatory microenvironment. On the other hand, during late stage of tumorigenesis, autophagy protects tumor cells survival by supporting metabolic demand and decreasing metabolic damage. Moreover, autophagy enhances migratory behavior of tumor cells by promoting anoikis resistance and dormancy [21,22].The normal cells that have lower autophagy capacity due to activation of autophagy-inhibitory pathway are prone to tumorization and the incipient tumor cells that have more autophagy bearing capacity or higher autophagy level possibly due to the up regulation of autophagy-promotive pathway are easier to survive in the microenvironment and accumulate more mutations to promote malignant progression. Autophagic activity appears to be regulated and continuously switched on and off during the phases of tumorigenesis. This mechanism is carried out by the protein-protein interaction (**PPI**) network containing protein interactors (e.g.,enzymes and adaptors) of autophagy proteins as well as by regulatory networks of transcription factors and miRNAs. The coordinated action of these networks controls the activity of autophagy in cancer [23]. The identification of key regulators of autophagy such as Atg genes and the proteins involved in the initiation and progression of autophagy might lead to the discovery of the complex interactions

between the pathways controlling autophagy and those regulating apoptosis, which would allow highlighting the importance of these processes in cancer [24]. It is possible that the autophagy is important in the regulation of cancer development and progression and in determining the response of tumor cells to anticancer therapy, but the role of autophagy in these processes is complicated and may have opposite consequences for the tumor [25,26].

CROSSTALK BETWEEN APOPTOSIS AND AUTOPHAGY IN HNSCC CANCER

The autophagy was considered a possible mechanism of tumor suppression because the apoptosis occurs concomitantly with specific features of autophagy. In cultured cells, it has been observed that autophagic gene exhaustion rather induces apoptosis than protects cell against death induced by different stresses. Currently, the role of autophagy in inducing cell death is unclear and requires further investigation [27,28].

In HNSCC and some other types of cancer, autophagy contributes to cell death via spread crosstalk between pro-apoptotic signaling pathways. Understanding the correlation between autophagy and apoptosis is an important objective of research in the field of tumor biology. It is now known that there is an interdependence between apoptotic and autophagic pathways, and studies have shown that BCL2, which is an important regulator of apoptosis, may also be an important regulator of the autophagy pathway [16]. Some studies sustain that there ia a functional relation between apoptosis and autophagy, meaning that autophagy can act as an adaptation way to stress and therefore it surpresses apoptosis, whereas in other cellular settings, autophagy may be an alternative pathway to cell death which is called autophagic cell death (or type II cell death) [29]. It appears that similar stimuli can induce either apoptosis or autophagy. Whereas a mixed phenotype of autophagy and apoptosis can sometimes be detected in response to these common stimuli, in many other instances, autophagy and apoptosis develop in a mutually exclusive manner, perhaps as a result of variable thresholds for both processes, or as a result of a cellular 'decision' between the two responses that may be linked to a mutual inhibition of the two phenomena. Recently, several pathways that link the apoptotic and autophagic mechanisms or that polarize the cellular response between autophagy and apoptosis have been deciphered at molecular level [30]. Caspases have a key role in apoptotic cell death and play a critical role in directing autophagy-apoptosis crosstalk. Processed caspases can shut off the autophagic response by degradation of Atg proteins (i.e., Beclin-1, Atg5, and Atg7). In some special cases, the pro-autophagic proteins can be even converted into pro-apoptotic ones to mediate apoptotic cell death after having been cleaved by caspases. On the other hand, autophagy can influence the apoptotic cascades by regulating the amount and activity of caspases [31]. Crosstalk between apoptosis and autophagy includes the Beclin 1-BCL-2 interaction; caspase-mediated Beclin1 cleavage; UVRAG-BAX interaction; ATG12-ATG3 conjugation; ATG12-Mcl-1 interaction; ATG5-FADD interaction; Calcium-dependent, nonlysosomal, cysteine protease-(Calpain-) mediatedATG5 cleavage. The

complex molecular crosstalk between autophagy and apoptosis and the role of these proteins involved in the crosstalk between these pathways may have particularly important roles in cancer. Autophagic proteins such as Atg12 interact with Bcl-2 and Mcl-1 and promote staurosporine-induced apoptosis, acting upstream of the mitochondria. In addition, Atg12 forms an Atg3 complex with a role in regulating mitochondrial integrity through mitophagy-mediated degradation of the depolarised mitochondria. Another Atg protein, Beclin-1, increases cisplatin-induced apoptosis by increasing caspase-9 activity whereas p62 autophagy adapter protein activates caspase-8 during the extrinsic apoptotic pathway [32,33].

In summary, it can be said that the regulatory molecules involved in the autophagy and apoptosis processes could interact and modulate the functional status of each other. There are cases in which apoptosis can affect autophagy and cases in which autophagia may end with the induction of apoptosis. Dependent on cell type, the environmental and stimuli, autophagy has differential effects on the apoptosis such as: (i) autophagy acts as an antagonist of apoptosis, (ii) autophagy can facilitate apoptosis and (iii) autophagy and apoptosis cooperate to induce tumor cell death. The regulatory molecules of apoptosis and autophagy pathways are inter-connected and can become capable of activating any of these pathways. Furthermore each path has several genes whose expression is critical to their realization. This interdependence between apoptosis and autophagy is very complex and sometimes contradictory but critical to the overall fate of the tumor cell [34]. Depending on the cellular context and death trigger, apoptosis and autophagy often co-operate in a balanced interplay that involves autophagy, or they are employed by cells in a complementary fashion to facilitate cellular destruction.

Thus, the molecular information about the mechanisms of crosstalk between apoptosis-and autophagy-associated cell death is crucial for the successful future development of anticancer therapies [35-38].

APOPTOSIS AND AUTOPHAGY IN DRUG RESISTANCE

In tumorigenesis processes of HNSCC tumor cells can lead to apoptosis resistance, which in turn induce resistance to radiation therapy and chemotherapy [39,40]. Drug resistance whether intrinsic or acquired, can be attributed to a wide variety of mechanisms including tumor cell heterogeneity, drug efflux, metabolism, tumor microenvironment stress-induced genetic or epigenetic alterations as a cellular response to drug exposure [41]. Although many anticancer therapeutic strategies can induce autophagic cell death, a majority of pertinent studies that have been conducted indicate that autophagy is a protective mechanism associated with increased resistance to chemotherapy in human cancer cells HNSCC. The activation of autophagy either leads to cancer cell chemo resistance via EGFR signaling, PI3K/AKT/ mTOR pathways, p53, VEGF, MAPK14/p38a signaling and microRNA or potentiates autophagic cell death through AMPK/ AKT1/mTOR axis, which depends on the tumor type and treatment characteristics [42].

The therapeutic approach in HNSCC has seen a widespread development over the last few years, leading to the emergence of a new paradigm in the treatment of cancer known as molecular therapy, which uses specific drugs that target regulatory molecules involved in processes responsible for tumor progression [16,43].

An exemple of a drug used in the treatment of head and neck cancer is cetuximab-a recombinant monoclonal antibody that inhibits tyrosine kinase receptor activation. At molecular level cetuximab induces the inhibition of the PI3K / Akt / mTOR signaling pathway and decreases levels of HIF-1α and Bcl-2 as result we have cell cycle arrest, triggers apoptosis, and, in addition, leads to the activation of autophagy [44]. Studies have shown a direct dependence between triggering the autophagic process and the intensity of the apoptotic process, as demonstrated by the use of a specific inhibitor for apoptosis, which also prevented autophagy. Another drug used in advanced or metastatic head and neck cancers is sorafenib which inhibits tumor proliferation and apoptosis by its actions on EGFR / Ras / Raf / MEK / ERK signaling pathways. It has also been observed that treatment with sorafenib may induce autophagy and using either pharmacological inhibitors or essential autophagy gene knockdown (*Atg7 gene*), mechanism that can amplify the apoptosis process of sorafenib in vitro treated tumor cells [45-47]. In addition, there are studies trying to demonstrate the role of natural compounds in potentiating the effects induced by conventional therapy using the ability of these compounds to act on more molecular targets [48]. Therefore, the idea of dual targeting of apoptosis and autophagy will provide a new therapeutic strategy that will help reduce resistance and enhance anticancer effect.

In conclusion, the controversed theory about the prosurvival or anticancer effect of autophagy still largely analyzed. Data analyzed, both in vitro and in vivo seem to further support the idea that autophagy facilitates the resistance of tumor cells to chemotherapy, and the inhibition of autophagy could potentiate the therapeutic desensitization of resistant tumor cells to anticancer drugs by using genetic or pharmacological inhibitors of autophagy.

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