Apoptosis and *in vivo* models to study the molecules related to this phenomenon

Apoptose e modelos *in vivo* para estudo das moléculas relacionadas a este fenômeno

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ABSTRACT

Apoptosis or programmed cell death is a physiological process, essential for eliminating cells in excess or that are no longer necessary to the organism, acting on tissue homeostasis, although the phenomenon is also involved in pathological conditions. Apoptosis promotes activation of biochemical pathways inside cells called caspase pathway, of the proteins responsible for the cleavage of several cell substrates, leading to cell death. Antiapoptotic members of the Bcl-2 family (B cell CLL/lymphoma 2), that belong to the intrinsic route of the activation of caspases, such as Bcl-xL (extra-large B-cell lymphoma) and Bcl-w (Bcl-2-like 2), act predominantly to prevent that pro-apoptotic members, such as Bax (Bcl-2-associated X protein) and Bak (Bcl-2 relative bak) lead to cell death. Antiapoptotic molecules are considered potentially oncogenic. Murine models are known to be valuable systems for the experimental analysis of oncogenes *in vivo*, and for the identification of pharmacological targets for cancer and to assess antitumor therapies. Given the importance of tumorigenesis studies on the immune responses to cancer and the possibility of investigating the participation of antiapoptotic molecules in tumor progression *in vivo*, the development of new models may be platforms for studies on tumorigenesis, immune antitumor responses, investigation of the ectopic expression of antiapoptotic molecules and immunotherapies for tumors.

Keywords: Apoptosis/immunology; Apoptosis/physiology; Genes, bcl-2/metabolism; Caspases; Cell death; Neoplasms

RESUMO

A apoptose, ou morte celular programada, é um processo fisiológico essencial para a eliminação de células em excesso ou que não são mais necessárias ao organismo, atuando na homeostase dos tecidos; entretanto, esse fenômeno também está envolvido em condições patológicas. A apoptose promove a ativação de vias bioquímicas dentro das células, denominada via das caspases, proteínas responsáveis pela clivagem de diversos substratos celulares, levando as células à morte. Membros antiapoptóticos da família Bcl-2 (B cell CLL/lymphoma 2), pertencentes à via intrínseca de ativação das caspases, como Bcl-xL (B-cell lymphoma-extra large) e Bcl-w (Bcl-2-like 2) atuam predominantemente prevenindo que os membros pró-apoptóticos, como Bax (Bcl-2-associated X protein) e Bak (Bcl-2 relative bak) ocasionem a morte celular. Moléculas antiapoptóticas são consideradas potencialmente oncogênicas. Sabe-se que os modelos murinos são sistemas valiosos para a análise experimental de oncogenes *in vivo*, bem como para a identificação de alvos farmacológicos do câncer e para avaliar terapias antitumorais. Em vista da importância dos estudos de tumorigênese e respostas imunes contra o câncer e da possibilidade de investigar a participação de moléculas antiapoptóticas na progressão tumoral *in vivo*, o desenvolvimento de novos modelos poderá servir como plataforma para estudos de tumorigênese, respostas imunes antitumorais, investigação de expressão ectópica de moléculas antiapoptóticas e imunoterapias contra tumores.

Descritores: Apoptose/imunologia; Apoptose/fisiologia; Genes bcl-2/metabolismo; Caspases; Morte celular; Neoplasias

INTRODUCTION

Apoptosis or programmed cell death is a physiological process, essential for eliminating cells in excess or that are no longer necessary to the organism, which acts on tissue homeostasis. On the other hand, the phenomenon is also involved in pathological conditions. Programmed cell death is characterized by morphological and molecular aspects. The morphological aspects include reduction of cell volume, condensation of chromatin, DNA cleavage and fragmentation of cell into apoptotic bodies, which are recognized and removed fast by phagocytes, not leading to immunological response. Among the molecular aspects one can mention the release of mitochondrial cytochrome c, and activation of caspases that cleave several cell substrates, thus leading cells to death[1].

Due to the difficulty and controversy about the designation of apoptosis, it is commonly defined as a

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result from the activation of biochemical pathways inside cells, that is, activation of proteins called caspases[1]. However, some studies indicated that apoptosis is more complicated. In tumors and in the nervous system, new forms of cell death were identified regardless of caspases, presenting mixed characteristics of apoptosis, necrosis and other forms of cell degeneration[2].

CASPASES

Caspases are proteases with cysteine in their active site which are capable of specifically recognizing and cleaving residues of aspartate in other proteins[3]. Caspases activate through two main pathways: an extrinsic pathway, mediated by receptors located in the cell membrane and called death receptors, and an intrinsic pathway, mediated by internal stimuli of intracellular stress, such as DNA lesion or deviation in the cell cycle or in metabolic pathways[4]. These different paths eventually lead to the activation of proteases known as caspases that play an essential role in cell death process. Once activated, most caspases are capable of catalyzing the activation of multiple other members of this family, resulting in magnification of the proteolytic cascade. Some members, such as caspase-8, are in a proximal position in the proteolytic cascade and work as regulators and initiators, while others such as caspase-3 are more distal and act as cell fragmentation effectors[5]. In the extrinsic path, the bond to cell surface receptors, like FAS (type-II trans membrane protein) or TNF (tumor necrosis factor), promote the activation of caspase 8, which activates caspase 3 directly or cleaves pro-apoptotic members of the Bcl-2 family (B cell CLL/lymphoma 2) that promote the release of mitochondrial cytochrome c. In the mitochondria, cytochrome c release is regulated by members of the Bcl-2 family that have pro- and antiapoptotic proteins. As a response to stress, pro-apoptotic members of this family are translocated from the cytoplasm to the mitochondria, where they promote the release of cytochrome c. In the cytoplasm, cytochrome c binds to Apaf-1 (apoptotic protease activating factor 1) and to pro-caspase 9, forming the apoptosome complex, activating caspase 9 that, in turn, activates caspase 3; in this way, the cleavage of specific substrates begins and results in the morphological and biochemical aspects of cell death[6].

PRO- AND ANTIAPOPTOTIC PROTEINS

The antiapoptotic members of the Bcl-2 family, such as Bcl-xL (extra-large B-cell lymphoma) and Bcl-w (Bcl-2-like 2) act predominantly to prevent that pro-apoptotic Bax (Bcl-2-associated X protein) and Bak (Bcl-2 relative bak) members link and disrupt the integrity of intracellular membranes, particularly the external mitochondria membranes, leading to release of cytochrome c and cell death[7]. These molecules are considered potentially oncogenic. The Bcl-2 protein, for example, originates from the translocation between chromosomes 14 and 18, and it was found in the human follicular lymphoma[8]. Despite single mutations in the antiapoptotic genes in this family are rare events in tumors, they may be indirectly associated with their increased expression, i.e., the activation of the NF-kB path (nuclear factor-kB) in tumors, leading to the activation of Bcl-xL and A1 (annexin 1)[9].

The Bcl-w protein has a structure very similar to that of Bcl-2 and Bcl-xL, and its main characteristic is the presence of domains named BH (Bcl2 homology)[9,10]. This molecule is found in a form weakly linked to the mitochondrial membrane and sequesters pro-apoptotic members, preventing the release of cytochrome c. In response to apoptogenic stimuli, protein BIM (activator subtype of BH3-only proteins) links to Bcl-w, inserting it in the mitochondrial membrane and hence preventing sequestration of pro-apoptotic members[11]. It was reported that Bcl-w protects cells from death in face of a wide range of cytotoxic injuries, including privation of cytokines, irradiation with gamma and UV (ultraviolet) rays and chemotherapy drugs[12]. The molecule is found in several tissues of structures such as the colon, brain, testicles, and myeloid, lymphoid and epithelial cells[13]. Despite the structural similarity to other antiapoptotic members with functions related to tumorigenesis, little has been described on the involvement of the molecule in the phenomenon of tumorigenesis and tumor progression.

Murine models are known to be valuable systems for experimental analysis of *in vivo* oncogenes, identification of the pharmacological targets of cancer and to evaluate antitumor treatments. In this sense, some studies showed the function of some molecules in the behavior of tumors *in vivo* when overexpressed in tumor cells. For example, the SSC-S2 molecule, also known as protein 8 induced by TNF-alpha (TNFAIP8) characterized as the antiapoptotic molecule and oncogene, had its *in vivo* function elucidated by the work of Zhang et al.[14]. In the study, the overexpression of this molecule in breast cancer cells was associated with the increased frequency of tumor colonization in the lungs, showing a new function of this molecule in tumor progression and potential target in antitumor therapy.

The members of the Bcl-2 family have been associated with the pathogenesis of several blood neoplasms, including non-Hodgkin follicular lymphoma. The overexpression of Bcl-2 in transgenic mice was described to induce the development of lymphoid hyperplasia and splenomegaly[15]. Another study showed that the overexpression of Bcl-2 in melanoma cells increased

the activity of proteinases related to metastasis and tumor growth *in vivo*, showing the crucial function of this molecule in the invasive phenotype and in tumor progression[16].

As mentioned previously, despite presenting interesting characteristics when analyzed *in vitro*, the participation and function of the Bcl-w molecule in tumor progression *in vivo* has been little investigated up to now. According to some studies, the participation of Bcl-w was shown indirectly in *in vivo* tumor progression in the work of Oltersdorf et al.[17]. The authors used ABT-737 (chemotherapeutic drug that inhibits the antiapoptotic Bcl-w, Bcl-2 and Bcl-xL molecules) in tumor animal models to induce the regression of tumors and increase survival of animals, thus showing a possible participation of Bcl-w in the establishment of tumors *in vivo*. Moreover, the overexpression of Bcl-w in cell lineages of gastric adenocarcinoma was described to promote increased invasiveness because it increases expression of MMP2 (2 matrix metallopeptidase) through the PI3K (phosphoinositide 3-kinase), AKT (protein kinase B) and Sp1 (transcripton factor specificity protein 1) pathways[18]. Bcl-w knockout mice are sterile because they have altered spermatogenesis[19], although no other abnormality was observed in other tissues, showing that this molecule may have a redundant function[20]. Whether this molecule may change tumor progression *in vivo* or not is a fact that remains obscure, and the elucidation of the participation in tumor progression may enable the development of new treatments for tumors.

Given the importance of studies on tumorigenesis and immune responses to cancer, and the possibility of investigating the participation of antiapoptotic molecules in tumor progression *in vivo*, the development of new murine models of T lymphomas can be platforms for studies on tumorigenesis, antitumor immune responses, investigation of ectopic expression of antiapoptotic molecules and immunotherapies for tumors.

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