MOLECULAR ASPECTS OF APOPTOSIS

ANTONELA CHEŞCĂ

„Transilvania" University of Braşov

Abstract: *Apoptosis is a complex process involving a variety of molecular aspects. It has been considered that apoptosis depends mainly on caspases. An important part in this process is played by mitochondria. There are certain ways to study the mechanisms of apoptosis. In conclusion, the study of molecular aspects of the Programmed Cell Death (PCD) offers the possibility to distinguish apoptosis from necrosis.*

Keywords: *apoptosis, caspases, mitochondria, necrosis, molecular mechanisms*

Rezumat: *Apoptoza este un proces complex care implică o varietate de aspecte moleculare. În acest context, se consideră că apoptoza depinde în pricipal de caspaze. Un rol important în acest proces îl au mitocondriile. S-au consemnat câteva căi pentru studiul mecanismelor apoptozei. Concluzionând, studiul aspectelor moleculare ale morţii celulare programate (MPC), oferă posibilitatea distingerii apoptozei de necroză.*

Cuvinte cheie: *apoptoză, caspaze, mitocondrii, necroză, mecanisme moleculare*

The term "apoptosis" is of Greek origin, and is used to describe the falling of leaves from a tree. The process lays at the basis of certain phenomena regarding the idea of leaving life, when the cell has accomplished its function. Within this context, the cell is decomposed very slowly and in stages in its molecular components, following that afterwards, to be used by the other cells of the body. Apoptosis is not only participating in certain stages of the individual development of the organism, but it also removes the cells infected by viruses, as well as the cells that become the focus centre of the toxic products.

The pattern of the death of cells through apoptosis or necrosis depends on the intracellular concentration of NAD+ and ATP. Thus, it was proved that the decrease of the level of NAD+ and ATP may induce cell necrosis. (1) In a healthy organism, the programmed cell death (PCD) represents the mechanism to maintain haemostasis. Both apoptosis' hypofunction and hyperfunction result in disorders of homeostasis. It is considered that PCD represents a natural stage of activity regarding the animal cells.

Apoptosis is a multi-stage process. The first stage consists in receiving the signal, the occurrence of a piece of information, its transmission, either outside the cell or its dissemination in the intracellular compartments. The signal is fixed on the receptor and submitted to an analysis. Afterwards, the signal is transmitted by messengers of different ranks into the nucleus, where the initiation of the cell suicide programme takes place, through the activation of the lethal genes or by the repression of those anti-lethal. The accomplishment of the PCD in the nuclear systems confirms the fact that the presence of the nucleus is not a binding component in order to perform this process.

Regarding the animal and human cells, apoptosis depends in most of the cases on the proteolytic activation of caspases. These represent a family of cysteinic proteases, conservative from the evolution point of view, which split up the proteins after the remains of the aspartic acid. According to the structural homology, caspases are classified into a number of sub-groups caspases 1 (1,4,5); caspases 2; caspases 3 (3, 6-10). (4)

Although it involves a complex mechanism, apoptosis may cover all the stages through the overproduction of process-promoting proteins. From this point of view, Bax and Bak induce the PCD in the presence of caspases' inhibitors.

As a result of the action of caspases, the following sequence occurs: activation of pro-caspases with the formation of caspases, split of the antiapoptotic Bcl-2 family proteins; hydrolysis of the lamina proteins, stabilizing the nuclear membrane with the condensation of chromatin; destruction of the proteins participating in the regulation of the cytoskeletal function; inactivation and disorders of the mechanism for the regulation of the proteins taking part in the DNA recovery, RNAm splicing, DNA replication. The target of caspases is represented by the poly (ADP-ribose) polymerase (PARP). (4,8).

There are certain methods for the accomplishment of the PCD. (8) One of these is determined by the physiological inductors and takes place through the cellular receptors, which include in their function the mechanisms of the apoptotic programme. Schematically, the following sequence takes place: inductor, receptor, adaptors; initial caspases, regulators; final caspases. (Fas) receptor interacts with the (FasL) ligand (T-Kiler trans-membrane protein); it becomes

activated and includes the virus-infected PCD. Thus, upon the interaction with FasL on the surface of the T-lymphocytes or of the antibody to the Fas-receptor, the beta-lymphocytes are dying. (9)

FasL – ligand is the component part of the family of the tumour necrotic factors (TNF), representing a large family of homotrimeric ligands. Fas represents the trans-membrane proteins that interact with the ligands trimmers. The interaction between the receptor and ligand brings about the formation of the receptor clusters, by fixing the adapters intracellular loci. The latter are fixed on the receptors and enter into action with the effectors, with the initial caspases, which are inactive for the moment. The interaction described is accomplished through specific types of proteins: DD (death domain), DED (death effector domain), CARD (caspase activation and recruitment domain). All of them have a similar structure, containing six alpha-catenae.

The most studied is pro-caspase 8. The aggregate FasL - Fas - FADD (Fas - associated DD-protein) – pro-caspase 8 becomes activated and is called apoptosoma (Green D.S., 1998) or disc - death-inducing signalling complex (5,8), or apoptozic chaperonins. (1,7)

Pro-caspases have a low proteolytic activity (1-2%) and could be found in manometric form. Their concentration at cell level could lead to the formation of active caspases through self-splitting. As a result, the N-regulator domain is detached from the pro-caspase (with the molecular weight of 30-50 kDa), while the rest of the molecule is dissociated in two sub-units, the largest ones being of 20 kDa and the smallest, of approximately 10 kDa.

Afterwards, their association in tetramer takes place, with two independent catalytic centres, occurring in the cytosol of the cell.

Specific ways of activating the caspase 8 are described. Regarding the stage of the initial caspase activation, the cell life may be preserved. The regulators that block or accelerate the destructive effect of the initial caspases are also described.

Caspase 8 activates the final caspases (effector caspases) and through proteolysis, the caspase 3 is generated, after the PCD process has become irreversible.

Caspase 3 may be self-activated, stimulating the activation of other caspases and DFF, which lead to the irreversible lithiasis of the RNA in nucleosomal fragments.

At the level of the cells submitted to the action of the apoptosis inductor, the membrane potential is suddenly reduced, generating the increase of the mitochondrial internal membrane permeability. Recently, it has been confirmed that an important part in apoptosis is played by mitochondria. (3,6) In the most early stages of apoptosis, a depolarisation of the mitochondrial internal membrane occurs. (2) Both apoptosis and the mitochondrial internal membrane depolarisation are protected through the use of specific inhibitors.

A detailed analysis confirms the presence of a fundamental law, which has a large utility in the animal world. Finally, it is supposed that different causes may provoke apoptosis, but only one single mechanism allows the cell to accomplish its mortal ultimatum. The reason for which the cell chooses a certain very complex mechanism for the accomplishment of apoptosis could be found in ample studies. (10,11)

In 1994, it was said that the aerobe cell holds an echeloned defence system, involved in the formation of superoxide. This system functions together with the antioxidant mechanisms that neutralize the superoxide or the products of the subsequent transformations.

Studies showed that at the level of the cell not containing mitochondrial DNA (p), apoptosis may be generated by the tumour necrotic factor (TNF) and not by the antimycin A. It was proved that within the witness cells (p+) that contain mitochondrial DNA, both substances induce apoptosis. It has been confirmed that the nature of the primary signals that bring about apoptosis is different. TNF is an external signal of self-destruction. Antimycin A is a specific inhibitor that blocks the transportation of electrons between the heme bh and the cytochrome b.

Regarding the mitochondrial proteins, there are other apoptogenic factors, such as: cytochrome c, pro-caspases 2, 3, 9; protein AIF (apoptosis inducing factor), that presents a flavoprotein with the molecular weight of 57 kDa. Cytochrome c together with the cytoplasmic factor APAF-1 (apoptosis protease activating factor -1) participates in the activation of the caspase 9. APAF -1 plays the part of the skeleton, where the self-catalitic process of the caspase 9 occurs. ATP dependent conformational alterations lead to the fixation of the cytochrome c together with the occurrence of a new conformation. Oligomerization leads to the CAKD opening for the pro-caspase 9 with the identical field. In the end, an apoptosoma of the molecular weight of approximately 1,3 mlm Daltons is formed, containing at least 8 subunits of APAF-1. The conformational approach of the pro-caspase 9 molecules on the multi-metric background APAF-1-cytochrome c, leads to the activation of the caspase 9. This is split up and activates the pro-caspase 3.

It was proved that flavoprotein AIF brings about the condensation of the chromatin in the HeLa cells, as well as the fragmentation of DNA, while its addition to the rat liver mitochondria, for example, may determine the release of the cytochrome c and of the caspase 9. The effects described are not altered by the caspases' inhibitor, L-VADfmk, that precludes the apoptosis induced by the cytochrome c. AIF – is a mitochondrial effector of the PCD that activates independently of caspases. In certain cases, PDC takes place as a result of the combined effect of two ways, with the participation of the plasmatic membrane receptors and of the mitochondrial cytochrome c. DNA lesion may bring about the accumulation of the gene p53 in cell that stops the multiplication of cells and (or) induces apoptosis.

In more than 50% cases, the gene p53 is inactive in the cancerigen cells, generating the deregulation of the cellular haemostasis.

P53 protein is a transcription factor that regulates the activity of the genes. It is assumed that the response to the formation of p53 depends on the level of alteration of the cellular genome.

Different ways of apoptosis may interact between them. Thus, in certain cases, the receptoric way will lead to the activation of the pro-caspase 8. In such a case, the mitochondrial way of apoptosis is also involved. The caspases 8 interacts with the cytosolic protein Bid of the Bax family, splitting it up in two component parts. The C terminal end of Bid interposes in the mitochondrial membrane, inducing the release of the cytochrome c and its fixation on APAF-1.

There is also the way of transmitting the PCD signal with the participation of the endoplasmic reticulum, where pro-caspase 12 is localized.

Endoplasmic reticulum-dependent apoptosis may be found in Alzheimer's disease, for example. In this regard, it was proved that the cortical neurons in the caspase 2 deficient mice are resistant to the apoptosis induced by an amyloid protein and not to the apoptosis induced by the cytochrome c. The main difference between apoptosis and necrosis consists in the fact that up to the most important morphological stage, that is up to the formation of the apoptosis compounds and their fagocitosis, the cellular membrane preserves its integrity. The chosen cellular death way will be established after the analysis of the multiple factors, one of them being represented by the injured action of the active forms of oxygen.

An early phenomenon in apoptosis involves the oxidant disorders regarding the relation between the cytoskeleton and the membrane bilayer, for which certain proteins of the cytoskeleton are responsible. The result is given by the increase of the motility of phosphatidylserin, which normally can be found in the cytosolic layer of the membrane and after the disorders of the membrane-skeleton relations, the "flip-flop" type diffusion takes place, following to be found in the external part of the bilayer.

The prolonged activation of the glutamic receptors, which occurs in the disorders of the neurons' function, presents a factor that produces the cellular death. Such a process is considered a specific effect of glutamate. Such mechanisms occur in the aging mechanisms, such as in the Parkinson's disease, Alzheimer's disease or in acute disorders at the level of the cerebral blood flow.

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