Apostosis, or Programmed Cell Death: Significance and Underlying Factors

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Abstract

Apoptosis, or programmed cell death, is characterized by morphological and biochemical changes in the cell and plays a decisive role in maintaining cellular homeostasis in a normal physiological process. Many uncertainties persist with regard to the molecular mechanisms that lead to these changes and hence to cell death.

Genetic analysis of the nematode Caenorhabditis elegans has led to the description of various genes and molecular mechanisms that are both structurally and functionally homologous to human proto-oncogenes. Meanwhile, a variety of positive and negative regulators of apoptosis have been identified; only a few of the most important can be described and discussed here.

Introduction

In all higher life forms, the integrity of the overall organism is intimately related to the development of mechanisms that regulate cell proliferation, differentiation, senescence, and (ultimately) cell death. From time to time, the elimination of some of the organism's own cells becomes inevitable, both in the course of morphogenesis and differentiation and in wounding off noxes. This regulated physiological destruction of cells is also known as programmed cell death or apoptosis (antineoplastos: the falling of leaves in autumn).

The regulated process of apoptosis is fundamentally different from cell necrosis or pathological cellular death, which sets in when the integrity of the cell is destroyed under circumstances of extreme mechanical force, heat, or chemical influences, for example.

Apoptosis

The work of Kerr et al constitutes a milestone in the process of distinguishing between pathological cell death (necrosis) and regulated physiological cell destruction, apoptosis. Morphologically, apoptosis is delineated by several characteristics: expansion of the cell's surface, rapid loss of cell volume, and condensation of nuclear chromatin followed by disintegration of the nucleus into multiple basophilic bodies that are enclosed within the cell membrane.

On the molecular level, a magnesium/calcium-dependent endonuclease breaks down the DNA into fragments of 180-200 base pairs (bp) each. Without releasing cytoplasmic components, the cell breaks down into membrane-enclosed apoptotic bodies. Their modified surface structure makes these bodies recognizable to specific receptors on neighboring macrophages or epithelial cells, which phagocytize them completely. Typically, no inflammatory reaction occurs. In contrast to necrosis, no granulocytic inflammatory reaction occurs in programmed cell death because apoptotic cells are phagocytized and removed within a very short time. Another important difference is that in apoptosis, membrane integrity is preserved until the final stage and the membrane's function as a permeability barrier remains intact (Table 1, Figure 1).

Necrosis

A typical morphological characteristic of necrosis is swelling of the cell caused by damage to the plasma membrane. Because of this damage, the cell is no longer capable of maintaining osmotic balance. As the process continues, further breaching of the membrane soon results, and the cell bursts open. No characteristic alterations are observed in the nucleus. During this phase when the cell swells and bursts, chromatin remains intact to a great extent, but it is soon broken down by nucleases that have been released. The contents of the cell move out into their surroundings and the ensuing phagocytosis of cell remnants is accompanied by signs of inflammation (Table 1, Figure 1).
**NECROSIS**

**Morphological Characteristics**
- Destruction of entire group of cells; loss of membrane integrity
- Necrosis: cell death with no loss of membrane integrity
- Nuclei intact; no morphological changes

**Biochemical Characteristics**
- Passive process, independent of temperature
- Statistically random DNA breakdown

**Physiological Characteristics**
- Death of groups of cells
- Induced by non-physiological disturbances
- Visible inflammatory reaction

**APOPTOSIS**

**Morphological Characteristics**
- Destruction of single cells; membrane bulges; loss of membrane integrity
- Apoptotic bodies in a process of phagocytosis
- Nuclei fragmented into sub-nuclear units
- Cell compression: cells shrink
- Budding formation of pseudopods, some of which break off (apoptotic bodies)
- Organelles, including lysosomes, remain intact

**Biochemical Characteristics**
- Active process, temperature-dependent
- Endonuclease onslaught defines nucleosome fragments of ca. 180-200 base pairs each; after separation typical DNA ladder structures consisting of multimers of fragments (aligenculesomes) appear in DNA-agarose gel
- Pre-lysis DNA fragmentation (occurs early in the process of cell death)
- Strengthening of the cell membrane and cytoskeleton

**Physiological Characteristics**
- Death of single cells (usually)
- Phagocytosis by macrophages and neutrophils
- No inflammatory reaction

Tab. 1: Differences between necrosis and apoptosis (modified according to 4, 5)

**Modulators of Apoptosis**

Ever since apoptosis, or programmed cell death, was first described, a multiplicity of inactivating factors have been identified. Two general groupings can be distinguished: One group includes positive trigger mechanisms that stimulate apoptosis by means of positive signals, while the second group contains negative trigger mechanisms that hinder the programmed implementation of apoptosis (Figure 2).

As of now, the biochemical mechanisms corresponding to the morphological phases of cell death have been only partially explained. However, a decisive biochemical criterion of apoptosis is the appearance of breaks in double-strand DNA at regular intervals (ca. 200 base pairs). An electrophoretogram shows the fragments to be so-called DNA ladder structures. The possible cause of this is the activation of an endonuclease which, after removal of the H1 histone, cuts the DNA in the exposed left-hand internucleosomal area, thus bringing about the typical change in nuclear chromatin structure. Only recently has there been any indication that breakages in DNA strands can also occur in other locations in the context of apoptosis. The patho-physiological significance of DNA splitting for apoptosis is increasingly unclear in view of the evidence of suicidal cell destruction in eukaryotic cells.

**Regulation of Apoptosis**

The concept of programmed cell death implies the existence of genes that code for a cascade of proteins which, in turn, are capable of inducing the destruction of a cell. In eukaryotic cell systems, a great variety of genes can be identified that are directly or indirectly involved in inducing apoptosis. The roundworm Caenorhabditis elegans as a model and made a significant contribution to our understanding of the genetic basis of apoptosis. A total of 11 genes are involved in the development-related destruction of 131 of the nematode's 1,050 cells, with special significance attributed to ced-3 and ced-4, positive regulators and ced-9 as a cell death antagonist.

In 1984, in connection with evidence that a translocation t(14,18) occurs in the breakpoint site in centroblastic centrocytic lymphoma, the location of the cell leukemia lymphoma II gene was discovered on chromosome 14q2. Although its function was still unknown, in 1988 Vaux et al. proved that bcl-2 increased the lifespan of immature pre-B cells, thus decisively demonstrating bcl-2's relationship to apoptosis. Bcl-2 is a human gene homologous to ced-3; it inhibits a number of different types of programmed cell death and seems to be especially effective against cell death associated with the development of free radicals.
This typical representative of the so-called survivor gene is the protagonist of a whole family of homogenous proteins that either inhibit or promote cell death. In addition to bcl-2, these include bcl-XL, bax, bad, bak, mcl-1 and A1. Homodimer/ heterodimer linkage fine tunes this system. Thus the ultimate deciding factor in whether a cell lives or dies seems to be the degree of heterodimerization between bcl-2 and bax, its main antagonist.\(^1\) (Figure 3).

In contrast to other oncoproteins, bcl-2 itself does not stimulate cell proliferation but only serves to protect the cell against induction of apoptosis. However, since bcl-2 protein is capable of blocking a great number of apoptosis inducers, its area of effectiveness seems to lie near the far end of the apoptosis cascade.\(^2\) The expressivity of bax protein, in turn, is probably regulated via a p53-dependent transcription control. Thus the expressivity of bax and the mutually antagonistic relationship between it and bcl-2 presumably belong to the apoptosis metabolism controlled by p53. In any case, p53 works in both directions. It codes for a multifunctional transcription factor that guards the genetic stability of the cell.\(^3\) In the wake of DNA fragmentation, p53 can either activate individual cell-cycle control points, causing the arrest of G1, or it can lead to apoptosis induction. In the latter case, its interactions with bcl-2/bax may be significant, since p53 influences the transcription of both genes.\(^4\) (Figure 4).

For its part, p53 expressivity seems to be linked to activation of the enzyme PARP (poly-ADP ribosylpolymerase). This makes it clear that the regulation of apoptosis is a highly complex process. The enzyme PARP, activated by DNA damage, is a catalyst for the formation of ADP ribose polymers at the breakage site, splitting NAD into its ADP ribose component and nicotinamide. This limits uncontrolled access on the part of DNA-repairing enzymes and prevents premature faulty transcription. The negative aspect is the consumption of NAD and of the ATP needed to resynthesize it. The resulting energy deficiency is seen by many authors as the cause of the ultimate death of the cell.\(^5\)

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Fig 1: Morphological changes in necrosis and apoptosis.
The enzyme PARP has recently attracted attention again because of its connection to one of the essential executors of programmed cell death, the interleukin-1β converting enzyme (ICE). Although the proteolytic degradation of PARP in the context of apoptosis was described long ago, ICE with its homologs ICH-1/Nedd-2, ICH-2/TX, and CPP32/YAMA has only recently been identified as the molecule responsible for this.1 Proof of its significance for cell death resulted from the emergence of the homology of ICE to the protein coded by cell-3 in the nematode Caenorhabditis elegans. This protein is known to be a positive regulator of programmed cell death.3

The genes responsible for regulating apoptosis are balanced by genes that are significant for cell proliferation. A shift in this balance leads to malignant transformations. This insight is reflected in the fact that the incidence of mutations of the p53 gene are very high in human neoplasias. In addition, almost all p53 mutations in human tumors that are monitored by DNA sequencing affect DNA linkage sites, thus preventing p53 protein from accumulating on specific chromosomal DNA sequences. During the normal development of a cell population, control of programmed cell death is almost independent of p53. In damaged cells, in contrast, blockage of the cell cycle and induction of apoptosis are usually bound to p53 protein's function as a transcription factor.

Other genes that are known to regulate proliferation, such as c-fos, c-jun, cdc-2 and especially c-myc, are also involved in the destruction of cells. The alkaline DNA-linkage sites and leucin zippers of fos and jun are motifs typical of transcription factors. Both proteins occur in preparations that can be isolated from promoters by means of affinity chromatography via the AP1-DNA consensus sequence. The AP1 sequence transmits signals of protein kinase C, whose activators include the phorbol ester TPA. Thus the AP1 linkage sequence is also described as a phorbol ester response element and abbreviated as TRE (TPA response element).

Fig. 2: Overview of the differentiation and growth factors, proto-oncogenes, and tumor-suppressor genes that play a role in the possible modulation of apoptosis. C-myc, c-myc: proto-oncogenes; p53(wt): p53 wild type; p53(mux): p53 mutant; bel-2, bel-6, and TPA: tumor promoting phorbol ester.

Fig. 3: Susceptibility to apoptosis (programmed cell death). The expressivity of bel-2 and bax heterodimers and homodimers constitutes the determining factor in programmed cell death.

Fig. 4: Molecular working mechanism of p53 after DNA damage. p53: Tumor suppressor; wild type; p53-: tumor suppressor, mutants; p21: cycle-dependent kinase inhibitor a.k.a. WAF, G1P, or SDH; bax: member of the bel-2 family that promotes apoptosis.
As with all transcription factors that have leucine zippers and alkaline sites, dimerization is a prerequisite of the binding of DNA. The c-fos and c-jun genes occupy a central position in growth regulation. Their expression is known to increase after activation of protein kinases (e.g., phospholipase C) or growth factors (e.g., IL-2) that bind to tyrosine kinase receptors. These findings confirm the great significance of fos and jun as effectors of factors influencing growth, differentiation, and transformation.

Most recently, the protein kinase C group in particular has been credited with both restricting and inducing apoptosis. TPA-induced apoptosis, along with programmed PKC activation, is linked to increased expression of transcription factors including c-fos, c-jun, and others (Figure 5).

Analysis of chromosomal anomalies, especially in cases of leukemia, has revealed a great deal about the regulation of cell growth and how it is disturbed in tumor cells. In the cells of Burkitt's lymphoma, a recombination occurs between the proto-oncogene c-myc on chromosome 8 and an immunoglobulin locus on chromosome 14 (heavy chain), 2 (light κ-chain), or 22 (light λ-chain). This rearrangement deregulates the expression of myc-protein, which is involved in regulating the cell cycle in normal cells. The myc-family consists of at least seven closely related genes that code for nuclear phosphoproteins with molecular weights of 60-68 kD. Myc-proteins are DNA-binding proteins that regulate the expression of other genes via a transcription control mechanism, thus regulating normal cell proliferation, transformation, and differentiation. The overexpression of c-myc in many malignant tumors confirms the central role of this proto-oncogene in oncogenesis.

In untransformed cells, circumstances unfavorable to growth lead to a decrease in c-myc and proliferation comes to a stop. In contrast, in tumor cells the combination of c-myc overexpression and the simultaneous opposite stimulus blocks the way back to the G0 phase and induces apoptosis. This c-myc induced apoptosis limits mitogenic factors. Although bel-2 alone, in contrast to other oncoproteins, does not stimulate cell proliferation or cause transformation, it does cooperate with c-myc and the members of the ras-family, which are capable of transforming cells. This means that Bel-2 expression must have a double function in tumorigenesis: On the one hand, it must extend the lifespan of mutated cells; on the other, it must also divert the activity of c-myc in the direction of proliferation by blocking its apoptotic function, which could cause a transformation.

The Fas/APO-1 gene occupies a special place in the regulation of apoptosis. It codes for a membrane protein of the TNF-receptor family that activates the cell death cascade after binding specific antibodies. Therefore, it too can be counted among the mediators of cell death. The natural Fas ligand Fas-L is to some extent homologous to TNF (tumor necrosis factor); like the latter, it is present in membranes in soluble form. The receptor known as Fas/APO-1 was identified by means of monoclonal antibodies (CD95) that induce apoptosis after binding with lymphocytes.

Fas/APO-1 is produced not only by lymphocytes but also by epithelial cells such as enterocytes and hepatocytes. Thus, induction of apoptosis via the Fas-L ligand associated with it is not restricted to the immune system. Under certain circumstances, TNF receptors are also mediators of apoptosis. An additional positive apoptosis signal is the negative selection of thymocytes mediated by T-cell receptors. The apoptotic effect of glucocorticoids has long been put to use in the context of chemotherapie for malignant diseases of the lymphatic system. Also, binding the T-cell receptors of a cytotoxic T-cell to an MHC-I-presented viral peptide of an infected-cell is a corresponding positive apoptosis signal.

Conclusions

The discovery that growth (especially tumor growth) may be due not only to cell proliferation but also to longer cell life spans opens up completely new perspectives for tumor research with regard to both etiology and clinical applications. Apoptosis and its inhibition help us better understand the function of known oncogenes, tumor suppressor genes, and cytokines in the different stages of tumorigenesis. Current interest focuses on the regulatory mechanisms of programmed cell death and proof of its clinical significance for different tumor processes.

We now know that onco logic therapy of quickly proliferating tissues is effective not only because of its actual cytotoxic effect but also because it induces apoptosis. This knowledge should spur further research on apoptosis. In future, molecular markers may help us make better choices in designing successful and rational cancer choices for our patients.
References


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