Apoptosis, or Programmed Cell Death: Significance and Underlying Factors

Michael Koldehoff, M.D.

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 unclarities 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.

Resumen

El apoptosis, o la muerte celular programada, se caracteriza por cambios morfológicos y bioquímicos en la celula y juega un papel decisivo en el mantenimiento del homeostasis celular en un proceso fisiológico normal. Se quedan muchas incertidumbres en cuanto a los mecanismos moleculares que resultan en aquellos cambios y, en consecuencia, a la muerte celular.

El análisis genético del nemátodo *Caenorhabditis elegans* lleva a la descripción de varios genes y mecanismos moleculares que son homólogos estructuralmente y funcionalmente a los proto-oncogenes humanos. Entretanto, se ha identificado una variedad de reguladores positivos y negativos del apoptosis; solamente unos de los más importantes pueden describirse en este artículo.

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 warding off noxae. This regulated physiological destruction of cells is also known as programmed cell death or apoptosis (αποπτοσιο: the falling of leaves in autumn).

The regulated process of apoptosis is fundamentally different from cell necrosis or pathological cell 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.[1] 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 cytoplasm 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.[2,3] 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	APOPTOSIS
Morphological Characteristics	
• Destruction of entire groups of cells; loss of membrane integrity	• Destruction of single cells; membrane bulges; no loss of membrane integrity
	• Nucleus shrinks; the chromatin it contains is greatly compressed
• Flocculation of chromatin	• Cell compression: cells shrink
• The cell swells; formation of vesicles; complete lysis	• Budding: formation of pseudopods, some of which break off (apoptotic bodies)
• Disintegration (swelling) of organelles, especially lysosomes	• Organelles, including lysosomes, remain intact
Biochemical Characteristics	
• Passive process, independent of temperature	• Active process, temperature-dependent
• Statistically random DNA break-down	• Endonuclease onslaught defines nucleosome fragments of ca. 180-200 base pairs each; after separation typical DNA ladder structures consisting of multimers of fragments (oligonucleuosomes) appear in DNA-agarose gel
• Post-lysis DNA fragmentation (occurs late in the process of cell death)	• Pre-lysis DNA fragmentation (occurs early in the process of cell death)
• Destabilization of the cell membrane	• Strengthening of the cell membrane and cytoskeleton
Physiological Characteristics	
• Death of groups of cells	• Death of single cells (usually)
• Induced by non-physiological disturbances	• Phagocytosis by macrophages and neutrophils
• Visible inflammatory reaction	• 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 instigating 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 destruction 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 postulated 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.[6] The patho-physiological significance of DNA splitting for apoptosis is increasingly unclear in view of the evidence of suicidal cell destruction in enucleate 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 system a great variety of genes can be identifie that are directly or indirectly involved in inducing apoptosis. The Horvitz tear used the nematode *Caenorhabditis elegans* as a model and made a significant contribution to our understanding of the genetic basis of apoptosis.[7] A total of 1 genes are involved in the development related destruction of 131 of the nematode's 1,090 cells, with special significance attributed to ced-3 and ced-4 positive regulators and ced-9 as a cell death antagonist.[8]

In 1984, in connection with evidence that a translocation t(14,18) occurs the breakage site in centroblastic/centro cytic lymphoma, the location of the cell leukemia lymphoma II gene was discovered on chromosome 18q2 although its function was still unclear. 1988 Vaux *et al* proved that bcl increased the lifespan of immature pre cells, thus decisively demonstrating b 2's relationship to apoptosis. Bcl-2 is human gene homologous to ced; inhibits a number of different types programmed cell death and seems to especially effective against cell dea associated with the development of f radicals.[10]
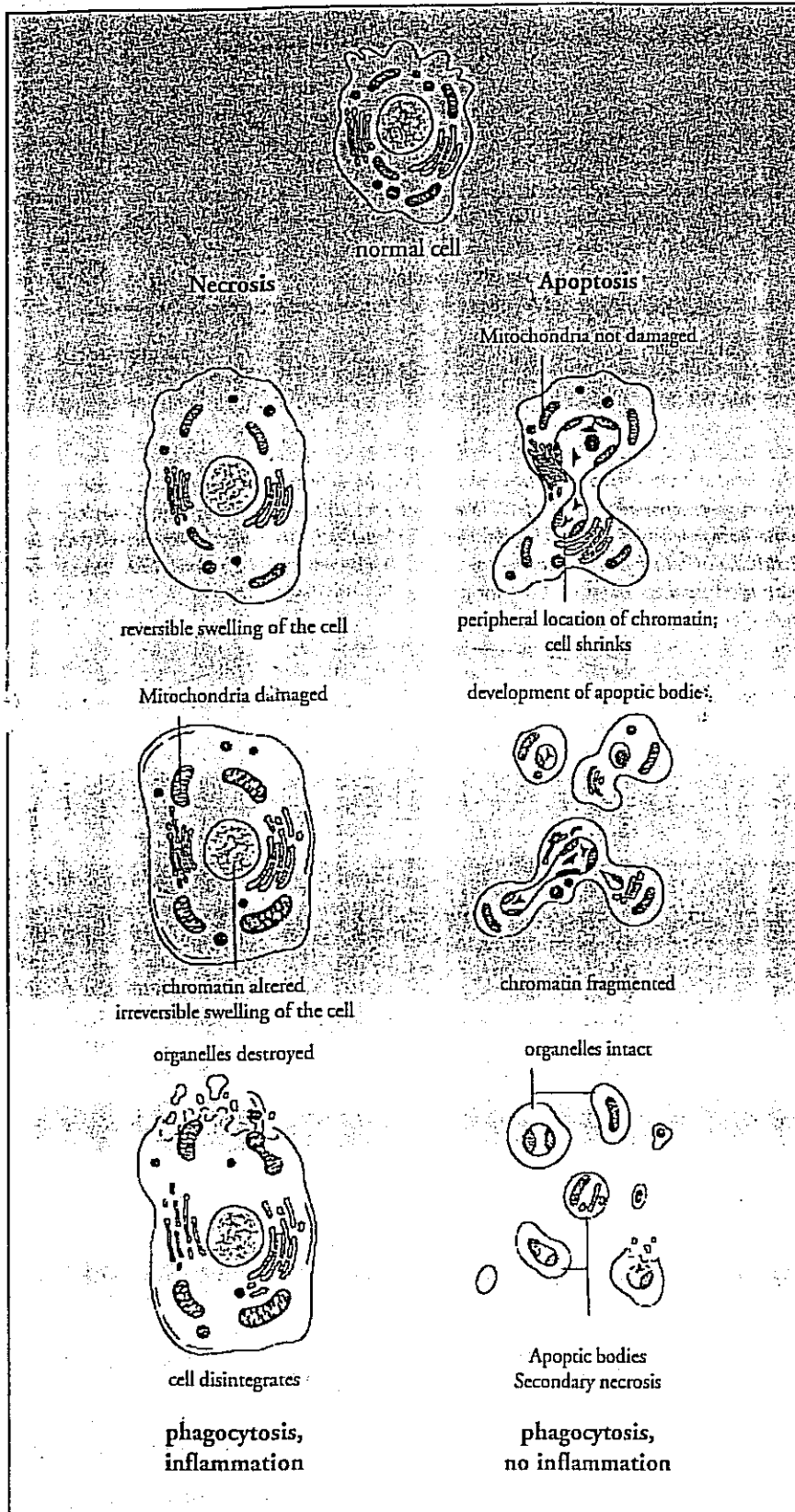
Fig. 1: Morphological changes in necrosis and apoptosis.

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-$X_{L/S}$, 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[11] (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.[12] 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.[13] In the wake of DNA fragmentation, p53 can either activate individual cell-cycle control points, causing the arrest of G_1, 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[14] (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.[15]

The enzyme PARP has recently attracted attention again because of its connection to one of the essential executioners of programmed cell death, the interleukin-1bß 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.[16] Proof of its significance for cell death resulted from the emergence of the homology of ICE to the protein coded by ced-3 in the nematode *Caenorhabditis elegans*. This protein is known to be a positive regulator of programmed cell death.[a]

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).
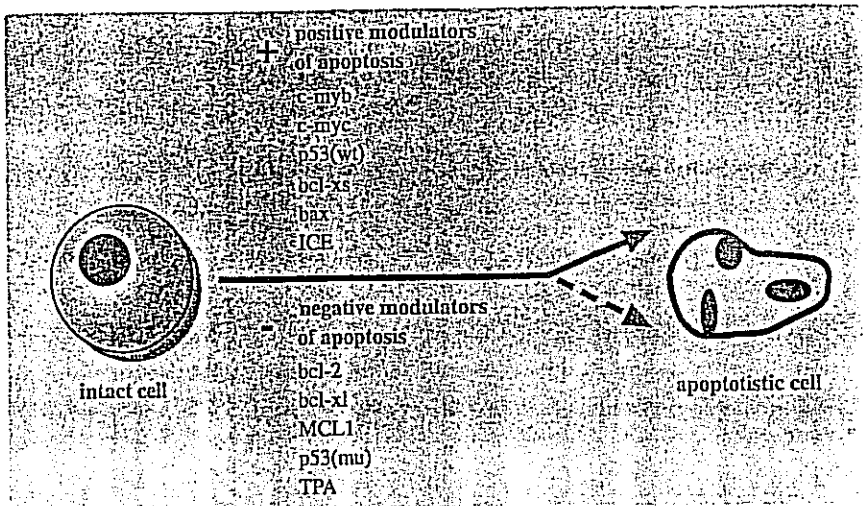

Fig. 2: Overview of the differentiation and growth factors, proto-oncogenes, and tumor-suppressor genes that play a role in the possible modulation of apoptosis. C-myb, c-myc: proto-oncogenes; p53(wt): p53 wild type; p53(mu): p53 mutant; bcl-xs, bcl-xl, bax, bcl-2, MCL1: B cell lymphoma oncogenes; ICE: IL1β converting enzyme; TPA: tumor promoting phorbol ester.
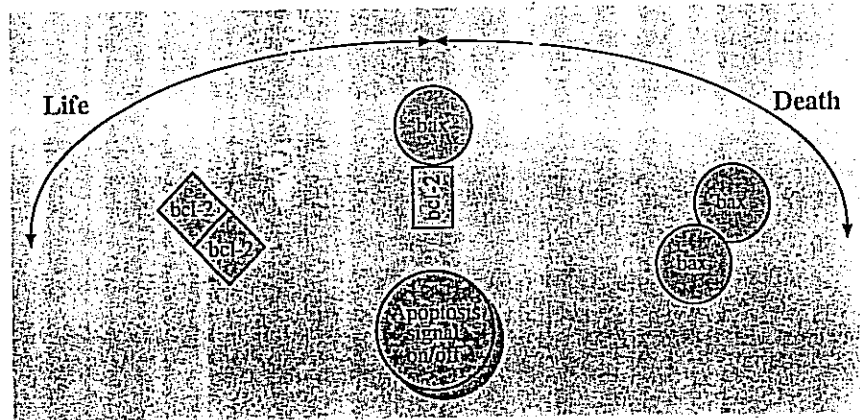

Fig. 3: Susceptibility to apoptosis (programmed cell death). The expressivity of bcl-2 and bax heterodimers and homodimers constitutes the determining factor in programmed cell death.
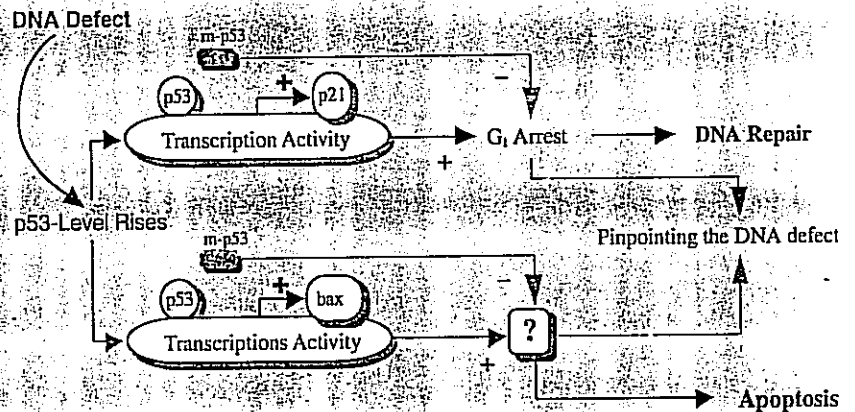

Fig. 4: Molecular working mechanism of p53 after DNA damage. P53: Tumor suppressor, wild type; m-p53: tumor suppressor, mutant; p21: cycle-dependent kinase inhibitor a.k.a. WAF1, CIP1, or SDI1; bax: member of the bcl-2 family that promotes apoptosis.
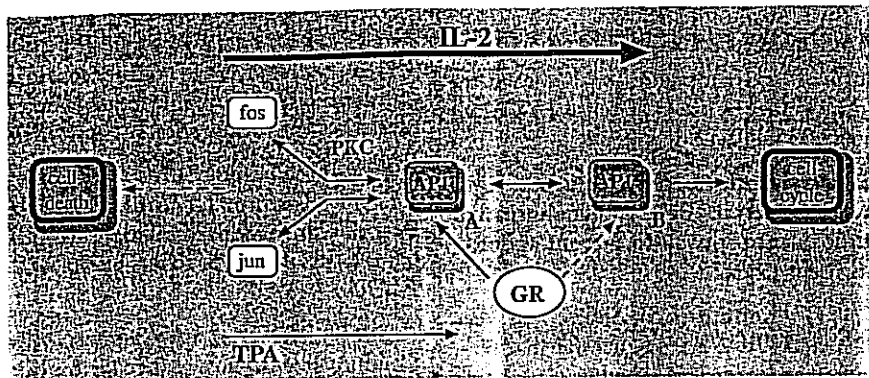
Fig. 5: The role of AP1 in mediating between cell death and the cell cycle. IL-2: interleukin 2; TPA: tumor promoting phorbol ester; AP1: activator protein 1; PKC protein kinase C; PTK: protein tyrosine kinase; GR: glucocorticoid receptor; fos, jun: transcription factors.

As with all transcription factors that have leucin 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 expressivity is known to increase after activation of protein kinases (e.g., phorbol ester) 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.[17] TPA-induced apoptosis, along with programmed PKC activation, is linked to increased expressivity 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 expressivity of myc-protein, which is involved in regulating the cell cycle in normal cells.[18] 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 expressivity of other genes via a transcription control mechanism, thus regulating normal cell proliferation, transformation, and differentiation. The overexpressivity 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 overexpressivity and the simultaneous opposite stimulus blocks the way back to the G_o phase and induces apoptosis.[19] Thus, c-myc induced apoptosis limits mitogenic factors. Although bcl-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.[20] This means that Bcl-2 expressivity 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.[21,22]

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.[23] 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 chemotherapy for malignant diseases of the lymphatic system. Also, binding the T-cell receptors of a cytotoxic T-cell to an MHC1-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 cytogenes 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 oncological 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

(1) Kerr JFR, Wylie AH, Currie AR. Apoptosis, a basic biological phenomenon with wider implications in tissue kinetics. *Brit J Cancer.* 1972;26:239-45.

(2) Savill J, Fadok V, Henson P, et al. Phagocyte recognition of cells undergoing apoptosis. *Immunol Today.* 1993;14:131-36.

(3) Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature.* 1980;284:555-56.

(4) Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol.* 1995;146:1-2, 16-19.

(5) Geschwend JE. Apoptose-Grundlagen und Bedeutung des programmierten Zelltodes fur das Prostatakarzinom. *Urologe A.* 1996;35:390-99.

(6) Oberhammer F, Wilson JW, Dive C, et al. Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or kb fragments prior to or in the absence of internucleosomal fragmentation. *EMBO J.* 1993;12:3679-84.

(7) Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode C. elegans. *Cell.* 1986; 44:817-29.

(8) Ellis RE, Jacobson M, Horvitz HR. Genes required for the engulfment of cell corpses during programmed cell death in C. elegans. *Genetics.* 1991;129:79-94.

(9) Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature.* 1988;335:440-42.

(10) Hockenberry DM, Oltvai ZM, Yin XM, et al. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell.* 1993;75:241-51.

(11) Korsmeyer SJ, Shutter JR, Veis DJ, et al. Bcl-2/Bax: a rheostat that regulates an antioxidant pathway and cell death. *Semin Cancer Biol.* 1993;4:327-32.

(12) Hockenberry DM. The bcl-2 oncogene and apoptosis. *Semin Immunol.* 1992;4:413-20.

(13) Levine AJ, Perry ME, Chang A, et al. The 1993 Walter Hubert Lecture: the role of p53 tumour-suppressor gene in tumorigenesis. *Brit J Cancer.* 1994;69:409-16.

(14) Koldehoff M. Zellzyklusregulation und die Funktion beim neoplastichen Wachstum. *Biol Med.* 1997; 26:62-66.

(15) Binder C, Wormann B. Apoptose-Regulator der Gewebehomoostase. *Onkologe.* 1996;2:82-86.

(16) Whyte M. ICE/CED-3 proteases in apoptosis. *Trends Cell Biol.* 1996;6:245-48.

(17) Rodriguez-Tarduchy G, Lopez-Rivas A. Phorbol esters inhibit apoptosis in IL-2 dependent T Lymphocytes. *Biochem Biophys Res Commun.* 1989;164:1069-75.

(18) Korsmeyer SJ. Chromosomal translocation in lymphoid malignancies reveal proto-oncogenes. *Ann Rev Immunol.* 1993;10:785-807.

(19) Klefstorm J, Vastrik I, Saksela E, et al. C-myc induced cellular susceptibility to the cytotoxic action of TNF-α. *EMBO J.* 1994;13:5442-50.

(20) Harrington EA, Bennett MR, Fanidi A, et al. C-myc induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J.* 1994;13:3286-95.

(21) Fanidi A, Harrington EA, Evan GI. Cooperative interaction between c-myc and bcl-2 proto-oncogenes. *Nature.* 1992;359:554-56.

(22) Yang E, Korsmeyer SJ. Molecluar Thanatopsis: A discourse on the BCL-2 family and cell death. *Blood.* 1996;88:386-401.

(23) Schulze-Osthoff K. The Fas/APO-1 receptor and its deadly lig and. *Trends Cell Biol.* 1995;4:421-26.

Address of the author:

Michael Koldehoff, M.D.
St. Johannes Hospital
Duisburg Hamborn
Medical Clinic II
Hematology, Oncology, and Immunology
An der Abtei 7-11
D-47166
Duisburg
Germany