

Cell death: Apoptosis versus necrosis (Review)

DARJA KANDUC¹⁻³, ABRAHAM MITTELMAN^{1,4}, ROSARIO SERPICO⁵, EBERTA SINIGAGLIA⁶, ANIMESH A. SINHA⁷, COSTANZO NATALE⁸, RAFFAELLA SANTACROCE⁵, M. GRAZIA DI CORCIA⁸, ALBERTA LUCCHESI^{2,5}, LUCIANA DINI^{2,9}, PAOLO PANI¹⁰, SALVATORE SANTACROCE⁵, SIMONE SIMONE³, ROMANO BUCCI^{2,11} and EMANUEL FARBER¹²

¹Department of Microbiology and Immunology, New York Medical College, Valhalla, NY, USA;

²CARSO Cancer Research Center, Regione Puglia, and ³Department of Biochemistry and Molecular Biology,

University of Bari, Bari, Italy; ⁴Department of Medicine, Division of Oncology/Hematology, New York Medical College,

Valhalla, NY, USA; Departments of ⁵Dentistry and Surgery, and ⁶Biomedical Sciences and Oncology, University of Bari,

Bari, Italy; ⁷Department of Dermatology, Joan and Sanford I. Weill Medical College, Cornell University, New York,

NY 10021, USA; ⁸Division of General Surgery, United Hospitals, University of Foggia, Foggia; ⁹Department of Biology,

University of Lecce, Lecce; ¹⁰Department of Medical Sciences and Biotechnology, University of Cagliari,

Cagliari; ¹¹Division of Internal Medicine, United Hospitals, University of Foggia, Foggia, Italy;

¹²Department of Pathology, School of Medicine, University of South Carolina, Columbia, SC, USA

Received March 8, 2002; Accepted April 9, 2002

Abstract. Cell death and the subsequent post-mortem changes, called necrosis, are integral parts of normal development and maturation cycle. Despite the importance of this process, the mechanisms underlying cell death are still poorly understood. In the recent literature, cell death is said to occur by two alternative, opposite modes: apoptosis, a programmed, managed form of cell death, and necrosis, an unordered and accidental form of cellular dying. The incorrect consequence is the overlapping of: a) the process whereby cells die, cell death; and b) the changes that the cells and tissues undergo after the cells die. Only the latter process can be referred to as necrosis and represents a 'no return' process in cell life. In this review, we discuss the excellent basic research developed in this field during last decades and problems that remain to be resolved in defining both experimentally and mechanistically the events that lead to and characterize cell death.

Contents

1. Introduction
2. The pathobiology of cell death
3. The biochemistry of cell death

4. The genetics of cell death
5. Apoptosis versus necrosis: the misconceptions
6. Cell death and cancer development
7. Conclusions

1. Introduction

Cell death is part of normal development and maturation cycle, and is the component of many response patterns of living tissues to xenobiotic agents (i.e. micro organisms and chemicals) and to endogenous modulations, such as inflammation and disturbed blood supply (1,2). Cell death is an important variable in cancer development, cancer prevention and cancer therapy (3-5). In the treatment of cancer, the major approach is the removal, by surgery, of the neoplasm and/or the induction of cell death in neoplastic cells by radiation, toxic chemicals, antibodies and/or cells of the immune system (6-9). On the other hand, this pathobiological process remains poorly understood and the physiological and biochemical factors that lead to cell death are still not clear. One main factor is the existing confusion between 'apoptosis' process, as compared and contrasted with 'necrosis', leading to the overlapping of the ante mortem changes, i.e. the process of cell death, and the post-mortem changes, i.e. the necrosis process.

2. The pathobiology of cell death

The elegant scientific exploration of sub-cellular molecular anatomy of the last decades have reinforced the cell concept as 'the smallest integrating unit in biology: a pseudo-intelligent computer that receives, screens, changes, reacts to and adapts to a host of environmental signals, all of this activity apparently

Correspondence to: Dr D. Kanduc, Department of Biochemistry and Molecular Biology, University of Bari, Via Orabona 4, I-70126 Bari, Italy

E-mail: d.kanduc@biologia.uniba.it

Key words: cell death, apoptosis, necrosis

designed, through evolution, for cell survival and host survival' (10). As such, there are essential cellular components (the plasma membrane, mitochondria, lysosomes, endoplasmic reticulum, the nucleus, and other cellular organelles) each can be considered a 'vulnerable site' whose destruction or malfunction threatens the functioning of the cellular unit (11,12).

Plasma membrane is an obvious vulnerable site: a functional/structural change that compromises the physiology of the plasma membrane can lead to cell death. Extensive literature suggests that membrane changes related to Ca^{++} influx appear among the many possible 'critical changes' that may compromise cell integrity (13,14).

Another favourite site is the mitochondria (15,16). However, studies with ischemic and with toxic chemicals failed to implicate mitochondria in acute episodes of cell death (17-19). It remains to be established what level of energy production is needed to maintain basic cell integrity.

Lysosomes have received considerable attention in the pathogenesis of cell death. Release of lysosomal hydrolytic enzymes is no doubt important in the post-mortem autolysis, but is not a cause of cell death (11).

Endoplasmic reticulum is an important site for the control of intracellular Ca^{++} including a calcium pump and has been suggested as a possible vulnerable site (14). Again, this remains to be established.

The nucleus and its DNA have been predominant areas of study as vulnerable in proliferating cells (20). Lymphocytes, and a few other hematolymphopoietic cells, are the primary cell populations that show cell death following interference with DNA synthesis (21) and, however, the role of DNA synthesis blockage seems to be indirect, its effects mediated through alterations involving protein synthesis (22-26). Accordingly, not all DNA-binding compounds induce cell death (27), whereas cycloheximide, an inhibitor of protein synthesis, or actinomycin D, an RNA synthesis inhibitor, are capable of inducing large-scale apoptosis (23).

Inhibition of protein or RNA synthesis is a critical event in cell death occurring in lymphocytes and in other cell types such as hepatocytes (by appearing the disorganization of the rough ER, which is regularly seen in perivenular parenchymal areas associated with cell death, the morphological equivalent of a disrupted protein synthesis). Nonetheless a few studies show that inhibition of protein synthesis is lethal mainly to lymphocyte populations, and does not eliminate mitoses throughout the whole body (28,29). One explanation might be that different cells and tissues in the same organism show wide variations as to the kinetics of cell death. Lymphocytes, and perhaps some other cells of the hematolymphopoietic system, show an extremely rapid cell death (from minutes to 1 h) and a very rapid post-mortem change (1-3 h). In contrast, hepatocytes exposed to nitrosamines exhibit a very slow process of cell death (many hours) and a very slow process of post-mortem changes (days). Moreover, a number of cells exhibit a wide range of cell death kinetics and morphologies with different xenobiotic chemicals (30). These observations stress the need of pinpointing and establishing the timing of cell death in specific cellular population.

Nevertheless, it appears definitively ascertained that the intranucleosomal cleavage of chromatin and the fragmentation

of nuclear proteins during apoptosis are Ca^{2+} -modulated nuclear processes (31). Many other experimental biochemical observations trace back to the calcium-activated pathway hypothesis. For example, the loss of glycogen and the disorganization of the rough endoplasmic reticulum occurring before cell death are well known phenomena thoroughly described years ago (32). Recently, it has been shown that the Ser/Thr kinase glycogen synthase kinase 3 (GSK3) is a component of the Ca^{2+} signalling pathway. Indeed, GSK3 regulates the activity of NF- Atc (33), a family of transcription factors involved in cell death pathway (34). Furthermore, transient increases in calcium resulted in a prolonged increase in GSK3 tyrosine phosphorylation concomitant with alterations in the phosphorylation state of the cytoskeletal protein, tau (35). So, the increase in intracellular calcium appears as a membrane change that might be implicated in a currently acceptable hypothesis for cell death for many cells.

3. The biochemistry of cell death

Among the many suggested biochemical alterations associated with cell death, those relating to free radicals appear of possible importance. Free radicals may interact with lipids, proteins and other cellular constituents thus leading to cell death (11,36). Xanthine oxidase in the genesis of superoxide (O_2^-); the mixed function oxygenase cytochrome P450 system in the genesis of electrophile reactants, and the nitric oxide (NO) in the genesis of the potent oxidant peroxynitrite (ONOO-) (37), are examples of reactions capable of generating an array of free radicals that can lead to cellular alterations. However, the demonstration of specific membrane changes as critical events in cell death has not been that successful. The clearest demonstration thus far is the induction of lipid peroxidation in phospholipids, involving especially the fatty acids in the 2 position of glycerophospholipids such as phosphatidylcholine (38). One interesting step following lipid peroxidation is the genesis of a variety of hydroxy aldehydes such as 4-hydroxynonenal (39,40). Over thirty different aldehydes have been identified following exposure of a rat to carbon tetrachloride (41). Although some of these may be generated spontaneously, others may be facilitated by the action of phospholipase A_2 (42,43). Thus, there is evidence that oxygen radicals created under different physiological and toxic conditions may induce cell death by selectively altering the structure and the function of the plasma membrane. Testing of this hypothesis under specific experimental and clinical conditions remains to be carried out.

4. The genetics of cell death

Although any experimental exploration at molecular level should develop on a feasible hypothesis at the physiological and biochemical level, we are witnessing the explosion of excellent molecular research on the so-called 'death genes'. Two distinct families of proteins have emerged, the TNF receptor-associated factors (TRAFs) and the death domain homologues. The cloning of members of these gene families and the identification of the protein-interaction motifs found within their gene products has initiated the molecular identity of factors (TRADD, FADD/MORT, RIP, FLICE/MACH,

and TRAFs) associated with both of the p60 and p80 forms of the TNF receptor and with other members of the TNF receptor superfamily (44,45). Death gene classification have also been introduced. Pro-death genes include p53, the ced-3/ICE proteases, and the Bax family; anti-death genes include ced-9/Bcl-2 and the adenovirus protein E1B (46-48). On the whole, one point is missed: a gene that codes for a protein that participates in either the process of cell death or the post-mortem changes that follow, should not be indicated as a gene responsible for the specific cell death, if any such genes exist. Whether there are genes or a specific gene expression pattern that defines the occurrence of the cell death under specified conditions is often implied but not yet proved.

Apoptosis has been carefully dissected at molecular level in the nematode *Caenorhabditis elegans*, an excellent model system in which the stages of physiological cell death can be observed during development. The ced- (i.e. cell death) 3 gene product has been characterized as a cysteine protease essential for programmed cell death to occur. In mammals, six different homologs of ced-3 have been identified, and it has been shown for several of them that overexpression can cause apoptosis and that this death can be inhibited by interfering with protease function. However, the molecular mechanisms for initiating caspase activation remain poorly understood for organisms across the phylogenetic scale. On the contrary, it is clear that almost all mammalian cells express several cell death proteases, even when they are not undergoing apoptosis (49,50).

5. Apoptosis versus necrosis: the misconceptions

The term 'apoptosis', defined as a controlled type of cell death that can be induced by a variety of physiologic and pharmacological agents, was first coined by Kerr *et al* (51) on the basis of the following main morphologic criteria: cellular shrinkage, condensation and margination of the nuclear chromatin, DNA fragmentation, cytoplasmic vacuolisation, cell lysis.

Extensive literature has accumulated perpetuating a number of incorrect concepts: a) apoptosis is a genetically controlled, energy-dependent method of cellular deletion without inflammation (52), whereas necrosis is associated traditionally with inflammation (53); b) apoptosis occurs in response to any mild injury whereas necrosis is said to occur in response to more severe forms of the same types of injury (54); c) apoptosis occurs via a coordinated, predictable and pre-determined pathway, while necrosis results from the additive effect of a number of independent biochemical events that are activated by severe depletion of cell energy stores; d) necrosis is difficult to prevent, whereas the apoptotic pathway can potentially be modulated to maintain cell viability. It is assumed that necrosis is 'ordinary' cell death with the characteristics of a passive process while apoptosis is a 'special' form of cell death with the characteristics of an active process (55-59).

In the final synthesis, the following realization emerges: apoptosis and necrosis are two distinct forms of cell death (60-62).

Preliminarily, we suggest that to compare apoptosis to necrosis is scientifically unjustified. It is unsound to compare

the process whereby cells die, i.e. cell death, and the changes that the cells and tissues undergo after the cells die, that is necrosis. These two processes are temporally dislocated and represent the two extremes of a continuum: the necrosis process can start only and exclusively when the cell dies and is an irreversible process, a 'no return' way in the cell life (11).

Morphologically, condensation and margination of the chromatin are not exclusive parameters of apoptosis and can occur at a even higher extent in many different mammalian organs or tissues during the changes that cells and tissues undergo after the cells die. Following cell death and lysosomal enzyme release, dead cells may undergo karyorrhexis (nuclear break-up thoroughly described already in 1966) (see ref. 63) and karyolysis (progressive loss of nuclear material) (11). Therefore it is impossible to assume chromatin condensation, including the inconstant nucleosomal laddering effect, as characterizing and exclusive parameters of apoptosis. *Ad abundantiam*, cells with the characteristic morphologic appearance of apoptosis may not show evidence of DNA fragmentation (64,65) and sometimes the DNA ladder formation in cell culture systems can also be caused by mycoplasmal nucleases (66).

Moreover, the inflammation seen more often in necrosis than in apoptosis is mostly evidence of the phagocytosis of cell debris produced by the necrotic process. Likewise, the possibility of recovery during apoptosis is simply the rescue capacity of a not yet dead cell, provided it is equipped with adequate molecular repair systems.

Kinetically, ante-mortem and post-mortem changes vary with the type of cell and the agent. Again, some lymphocytes show a short period of cell death and an inordinately rapid (within 2 to 3 h) set of post-mortem changes. By contrast, nitrosamine-treated rat hepatocytes show necrosis in hepatocytes (usually in zone 3, because of the preferential localization of bio-transformation) only after 36 to 48 h, even though the nitrosamines undergo their complete metabolic conversion to highly reactive derivatives by 4 to 8 h. Most cells in the various tissues and organs, including liver cells exposed to different xenobiotics and other nitrosamines, fall between the two extremes in this spectrum.

One main consequence of these kinetic differences is that selecting lymphocytes as typical cells for the study of apoptosis (21,67-69) introduces a bias in analysing the processes of cell death and of post-mortem changes. That is illustrated by the study of cell death in the small intestine of the rat. Cytotoxic agents and X-rays induce clear-cut morphologic changes in the cells lining the crypts. These changes have been interpreted for a long time as acute lethal effects on the proliferating mucosal epithelial cells (70). Actually, the targets of the rapid cell death are some lymphocytes in the submucosa (71,72). This cell death appears within a few hours and is followed shortly by phagocytosis of the dead lymphocytes (72). As a matter of fact, mucosal epithelial cells disappear through a slower process and the villi become atrophic.

It is frequently assumed that the death of cells can be passive. This non-biological point of view on cell death ignores the role of cell death in cell development and adaptation. It cannot be assumed that 'ordinary' cell death or 'necrosis' is a passive process while the presumed special form of cell

death, 'apoptosis' is active. Both the ante-mortem and post-mortem changes are active since both are enzyme-catalysed biochemical reactions.

There are at least 3 different meanings for the term 'programmed death' a) the selective cell death characterizing embryologic development and maturation of organisms, tissues or organs (73), i.e. the 'developmentally programmed' cell death. The programmed cell death in the ovarian cycle (74,75) is an elegant example of a logical and rational use of the word 'programmed'; b) the 'physiologically programmed' cell death seen in organs or tissues that undergo temporary hyperplasia and then return to their 'resting physiological' level (76). In this case the return of the liver to its physiological cell level after hyperplasia is regulated by homeostatic rather than programmed mechanisms; c) the sequence reactions that lead to cell death and are induced by a wide variety of xenobiotic chemicals and micro organisms. The mechanisms underlying such sequence of reactions are unknown, even though it is not valid to consider this as gene-determined unless the gene can be shown to code for the program, and not simply for one of the protein components in the sequence.

In all, it seems that the concept of 'apoptosis' in its present formulation does not add new insights into the problem of cell death from the point of view of the experimental cell pathobiology.

6. Cell death and cancer development

Properties of carcinogenic agents (chemical agents as well as radiations) are the growth-inhibition power and the ability to induce cell death. These properties are widely used in anti-cancer chemo- and radiotherapies. By contrast, the mechanisms relating cell death and cancer induction have received scarce attention. In general the cell death induced by radiations and carcinogenic agents have been hastily ascribed to toxicity, even if toxic compounds produce a different response (77). Given the great progress in basic cancer research, it should be wise to reconsider the relationship between cell death and cancer development by using emerging technologies to create new conceptual paradigms.

7. Conclusions

Current assays for cell death rely on 'post-mortem' changes, not the process(es) involved in the death of the cell. Instead of adding new insights into the problem of cell death from the point of view of the experimental cell pathologist, the concept of 'apoptosis' has led to compare the changes of the cells that die to the series of structural-biochemical changes that occur after the cells die. Morphologic criteria using whole-mount preparations that do not require embedment and sectioning procedures, might be an effective tool to distinguish cell death changes from the necrotic ones. The microscope might be a catch-all for identifying characteristic perturbations that occur in specific experimental situations. Using appropriate protocols and tools now available, it should be possible to determine the boundary between pre- and post-mortem changes. In conclusion, it appears mandatory to refer to the recommendations of the Committee of the Society of

Toxicologic Pathologists on the nomenclature of cell death in order to use terms that accurately and concisely convey the level of appropriate information (78).

References

1. Clavien PA, Rudiger HA and Selzner M: Mechanism of hepatocyte death after ischemia: apoptosis versus necrosis. *Int J Oncol* 17: 869-879, 2000.
2. Vaupel P and Hockel M: Blood supply, oxygenation status and metabolic micromilieu of breast cancers: characterization and therapeutic relevance. *Hepatology* 33: 1555-1557, 2001.
3. Zerban H, Radig S, Kopp-Schneider A and Bannasch P: Cell proliferation and cell death (apoptosis) in hepatic preneoplasia and neoplasia are closely related to phenotypic cellular diversity and instability. *Carcinogenesis* 15: 2467-2473, 1994.
4. Schulte-Hermann R, Hufnagl K, Low-Baselli A, Rossmann W, Wagner A, Ruttkey-Neky B, Bursch W, Mullauer L, Parzefall W and Grasl-Kraupp B: Apoptosis and hepatocarcinogenesis. *Digestion* 59: 64-65, 1998.
5. Schulte-Hermann R, Bursch W, Low-Baselli A, Wagner A and Grasl-Kraupp B: Apoptosis in the liver and its role in hepatocarcinogenesis. *Cell Biol Toxicol* 13: 339-348, 1997.
6. Kacinski BM and Flick M: Apoptosis and cutaneous T cell lymphoma. *Ann NY Acad Sci* 941: 194-199, 2001.
7. Zornig M, Hueber A, Baum W and Evan G: Apoptosis regulators and their role in tumorigenesis. *Biochim Biophys Acta* 1551: F1-F37, 2001.
8. Kong AN, Yu R, Hebbar V, Chen C, Owuor E, Hu R, Ee R and Mandelkar S: Signal transduction events elicited by cancer prevention compounds. *Mutat Res* 481: 231-241, 2001.
9. Dragan YP, Bidlack WR, Cohen SM, Goldsworthy TL, Hard GC, Howard PC, Riley RT and Voss KA: Implications of apoptosis for toxicity, carcinogenicity, and risk assessment: fumonisins B (1) as an example. *Toxicol Sci* 61: 6-17, 2001.
10. Farber E and Rubin H: Cellular adaptation in the origin and development of cancer. *Cancer Res* 51: 2751-2761, 1991.
11. Popper H: Hepatocellular degeneration and death. In: *The Liver Biology and Pathobiology*. Raven Press Arias IM, Jakoby WB, Popper H, Schachter D and Shafritz DA (eds). Raven Press, New York, pp1087-1103, 1988.
12. Ferri KF and Kroemer G: Organelle-specific initiation of cell death pathways. *Nat Cell Biol* 3: 255-263, 2001.
13. McConkey DJ, Hartzell P, Nicotera P and Orrenius S: Calcium-activated DNA fragmentation kills immature thymocytes. *FASEB J* 3: 1843-1849, 1989.
14. Xu K, Tavernarakis N and Driscoll M: Necrotic cell death in *C. elegans* requires the function of calreticulin and regulators of Ca(2+) release from the endoplasmic reticulum. *Neuron* 31: 957-971, 2001.
15. Saggioro D, Barp S and Chieco-Bianchi L: Block of a mitochondrial-mediated apoptotic pathway in Tax-expressing murine fibroblasts. *Exp Cell Res* 269: 245-255, 2001.
16. Wang NS, Unkila MT, Reineks EZ and Distelhorst CW: Transient expression of wild-type or mitochondrially targeted Bcl-2 induces apoptosis, whereas transient expression of endoplasmic reticulum-targeted Bcl-2 is protective against Bax-induced cell death. *J Biol Chem* 276: 44117-44128, 2001.
17. Robin ED: Of men and mitochondria: coping with hypoxic dysoxia. *Am Rev Respir Dis* 122: 517-531, 1980.
18. Mittnacht S Jr and Farber JL: Reversal of ischemic mitochondrial dysfunction. *J Biol Chem* 256: 3199-3206, 1981.
19. Elsasser A, Suzuki K, Lorenz-Meyer S, Bode C and Schaper J: The role of apoptosis in myocardial ischemia: a critical appraisal. *Basic Res Cardiol* 96: 219-226, 2001.
20. Kanduc D, Bannasch P and Farber E: A critical perspective in cancer research. *Int J Oncol* 15: 1213-1220, 1999.
21. Stahnke K, Fulda S, Friessen C, Strauss G and Debatin KM: Activation of apoptosis pathways in peripheral blood lymphocytes by *in vivo* chemotherapy. *Blood* 98: 3066-3073, 2001.
22. Ben-Ishay Z and Farber E: Protective effects of an inhibitor of protein synthesis, cycloheximide, on bone marrow damage induced by cytosine arabinoside or nitrogen mustard. *Lab Invest* 33: 278-290, 1975.
23. Martin SJ, Lennon SV, Bonham AM and Cotter TG: Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA or protein synthesis. *J Immunol* 145: 1859-1867, 1990.

24. Hinz T, Flindt S, Marx A, Janssen O and Kabelitz D: Inhibition of protein synthesis by the T cell receptor-inducible human TDAG51 gene product. *Cell Signal* 13: 345-352, 2001.
25. Gerner C, Frohwein U, Gotzmann J, Bayer E, Gelbmann D, Bursch W and Schulte-Hermann R: The Fas-induced apoptosis analyzed by high throughput proteome analysis. *J Biol Chem* 275: 39018-39026, 2000.
26. Ray SK, Matzelle DD, Wilford GG, Hogan EL and Banik NL: Cell death in spinal cord injury (SCI) requires *de novo* protein synthesis. Calpain inhibitor E-64-d provides neuroprotection in SCI lesion and penumbra. *Ann NY Acad Sci* 939: 436-449, 2001.
27. Chatterjee S, Zaman K, Ryu H, Conforto A and Ratan RR: Sequence-selective DNA binding drugs mithramycin A and chromomycin A3 are potent inhibitors of neuronal apoptosis induced by oxidative stress and DNA damage in cortical neurons. *Ann Neurol* 49: 345-54, 2001.
28. Verbin RS and Farber E: Effect of cycloheximide on the cell cycle of the crypts of the small intestine of the rat. *J Cell Biol* 35: 649-658, 1967.
29. Longnecker DS, Shinozuka H and Farber E: Molecular pathology of *in vivo* inhibition of protein synthesis. Electron microscopy of rat pancreatic acinar cells in puromycin-induced necrosis. *Am J Pathol* 52: 891-915, 1968.
30. Dini L, Coppola S, Ruzittu MT and Ghibelli L: Multiple pathways for apoptotic nuclear fragmentation. *Exp Cell Res* 223: 340-347, 1996.
31. Santella L and Carafoli E: Calcium signaling in the cell nucleus. *FASEB J* 11: 1091-1109, 1997.
32. Bannasch P: The cytoplasm of hepatocytes during carcinogenesis. Electron and light microscopical investigations of the nitrosomorpholine-intoxicated rat liver. *Recent Results Cancer Res* 19: 1-100, 1968.
33. Graef IA, Mermelstein PG, Stankunas K, Neilson JR, Deisseroth K, Tsien RW and Crabtree GR: L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. *Nature* 401: 703-708, 1999.
34. Schuh K, Kneitz B, Heyer J, Bommhardt U, Jankevics E, Berberich-Siebel F, Pfeffer K, Muller-Hermelink HK, Schimpl A and Serfling E: Retarded thymic involution and massive germinal center formation in NF-ATp-deficient mice. *Eur J Immunol* 28: 2456-2466, 1998.
35. Hartigan JA and Johnson GV: Transient increases in intracellular calcium result in prolonged site-selective increases in Tau phosphorylation through a glycogen synthase kinase 3beta-dependent pathway. *J Biol Chem* 274: 21395-21401, 1999.
36. Halliwell B: A super way to kill cancer cells? *Nat Med* 6: 1105-1106, 2000.
37. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B and van der Vliet A: Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 391: 393-397, 1998.
38. Marathe GK, Harrison KA, Murphy RC, Prescott SM, Zimmerman GA and McIntyre TM: Bioactive phospholipid oxidation products. *Free Radic Biol Med* 28: 1762-1770, 2000.
39. Bestervelt LL, Vaz AD and Coon MJ: Inactivation of ethanol-inducible cytochrome P450 and other microsomal P450 isozymes by trans-4-hydroxy-2-nonenal, a major product of membrane lipid peroxidation. *Proc Natl Acad Sci USA* 92: 3764-3768, 1995.
40. Hartley DP, Kolaja KL, Reichard J and Petersen DR: 4-Hydroxynonenal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunochemical detection and lobular localization. *Toxicol Appl Pharmacol* 161: 23-33, 1999.
41. Wacker M, Wanek P and Eder E: Detection of 1,N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats. *Chem Biol Interact* 137: 269-283, 2001.
42. Lo HH, Teichmann P, Furstenberger G, Gimenez-Conti I and Fischer SM: Suppression or elevation of cytosolic phospholipase A2 alters keratinocyte prostaglandin synthesis, growth, and apoptosis. *Cancer Res* 58: 4624-4631, 1998.
43. Ma Z, Bohrer A, Wohltmann M, Ramanadham S, Hsu FF and Turk J: Studies of phospholipid metabolism, proliferation, and secretion of stably transfected insulinoma cells that overexpress group VIA phospholipase A2. *Lipids* 36: 689-700, 2001.
44. Darnay BG and Aggarwal BB: Early events in TNF signaling: a story of associations and dissociations. *J Leukoc Biol* 61: 559-566, 1997.
45. Heyninc K and Beyaert R: Crosstalk between NF-kappaB-activating and apoptosis-inducing proteins of the TNF-receptor complex. *Mol Cell Biol Res Commun* 4: 259-265, 2001.
46. Strater J and Moller P: Expression and function of death receptors and their natural ligands in the intestine. *Ann NY Acad Sci* 915: 162-170, 2000.
47. Trump BF, Berezsky IK, Chang SH and Phelps PC: The pathways of cell death: oncosis, apoptosis, and necrosis. *Toxicol Pathol* 25: 82-88, 1997.
48. Bromme HJ and Holtz J: Apoptosis in the heart: when and why? *Mol Cell Biochem* 164: 261-275, 1996.
49. Vaux DL and Strasser A: The molecular biology of apoptosis. *Proc Natl Acad Sci USA* 93: 2239-2244, 1996
50. Kanuka H, Hisahara S, Sawamoto K, Shoji S, Okano H and Miura M: Proapoptotic activity of *Caenorhabditis elegans* CED-4 protein in *Drosophila*: implicated mechanisms for caspase activation (programmed cell death). *Proc Natl Acad Sci USA* 96: 145-150, 1999.
51. Kerr JF, Wyllie AH and Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26: 239-257, 1972.
52. D'Amours D, Sallmann FR, Dixit VM and Poirier GG: Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. *J Cell Sci* 114: 3771-3778, 2001.
53. Kelly KJ, Plotkin Z and Dagher PC: Guanosine supplementation reduces apoptosis and protects renal function in the setting of ischemic injury. *J Clin Invest* 108: 1291-1298, 2001.
54. Uezono T, Maruyama W, Matsubara K, Naoi M, Shimizu K, Saito O, Ogawa K, Mizukami H, Hayase N and Shiono H: Norharman, an indoleamine-derived beta-carboline, but not Trp-P-2, a gamma-carboline, induces apoptotic cell death in human neuroblastoma SH-SY5Y cells. *J Neural Transm* 108: 943-953, 2001.
55. McLaughlin R, Kelly CJ, Kay E and Bouchier-Hayes D: The role of apoptotic cell death in cardiovascular disease. *Ir J Med Sci* 170: 132-140, 2001.
56. Fishelson Z, Attali G and Mevorach D: Complement and apoptosis. *Mol Immunol* 38: 207-219, 2001.
57. Rana A, Sathyanarayana P and Lieberthal W: Role of apoptosis of renal tubular cells in acute renal failure: therapeutic implications. *Apoptosis* 6: 83-102, 2001.
58. Koukoulis GK, Shen J, Karademir S, Jensen D and Williams J: Cholangiocytic apoptosis in chronic ductopenic rejection. *Hum Pathol* 32: 823-827, 2001.
59. Denecker G, Vercammen D, Declercq W and Vandenebeele P: Apoptotic and necrotic cell death induced by death domain receptors. *Cell Mol Life Sci* 58: 356-370, 2001.
60. Martin LJ: Neuronal cell death in nervous system development, disease, and injury. *Int J Mol Med* 7: 455-478, 2001.
61. Balla A, Toth B, Timar G, Bak J and Krajcsi P: Molecular targets for pharmacological cytoprotection. *Biochem Pharmacol* 61: 769-777, 2001.
62. Renvoize C, Biola A, Pallardy M and Breard J: Apoptosis: identification of dying cells. *Cell Biol Toxicol* 14: 111-120, 1998.
63. Altmann HW and Bannasch P: Die intravitale Karyorrhexis der exokrinen Pankreas-Zelle im elektronenmikroskopischen Bild. *Z Zellforsch* 71: 53-68, 1966.
64. Catchpole DR and Stewart BW: Etoposide-induced cytotoxicity in two human T-cell leukemic lines: delayed loss of membrane permeability rather than DNA fragmentation as an indicator of programmed cell death. *Cancer Res* 53: 4287-4296, 1993.
65. Janicke RU, Sprengart ML, Wati MR and Porter AG: Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. *J Biol Chem* 273: 9357-9360, 1998.
66. Paddenberg R, Wulf S, Weber A, Heimann P, Beck LA and Mannherz HG: Internucleosomal DNA fragmentation in cultured cells under conditions reported to induce apoptosis may be caused by mycoplasma endonucleases. *Eur J Cell Biol* 71: 105-119, 1996.
67. Efferth T, Fabry U and Osieka R: Induction of apoptosis, depletion of glutathione, and DNA damage by extracorporeal photochemotherapy and psoralen with exposure to UV light *in vitro*. *Anticancer Res* 21: 2777-2783, 2001.

68. Wilkins RC, Wilkinson D, Maharaj HP, Bellier PV, Cybulski MB and McLean JR: Differential apoptotic response to ionizing radiation in subpopulations of human white blood cells. *Mutat Res* 513: 27-36, 2002.
69. Lum JJ, Pilon AA, Sanchez-Dardon J, Phenix BN, Kim JE, Mihowich J, Jamison K, Hawley-Foss N, Lynch DH and Badley AD: Induction of cell death in human immunodeficiency virus-infected macrophages and resting memory cd4 t cells by trail/apo2l. *J Virol* 75: 11128-11136, 2001.
70. Lieberman MW, Verbin RS, Landay M, Liang H, Farber E, Lee T-N and Starr R: A probable role for protein synthesis in intestinal epithelial cell damage induced *in vivo* by cytosine arabinoside, nitrogen mustard, or X-irradiation. *Cancer Res* 30: 942-951, 1970.
71. Verbin RS, Diluio G, Liang H and Farber E: The effects of cytosine arabinoside upon proliferating epithelial cells. *Cancer Res* 32: 1476-1488, 1972.
72. Verbin RS, Diluio G and Farber E: Protective effects of cycloheximide against 1-beta-D-arabinosylcytosine-induced intestinal lesions. *Cancer Res* 33: 2086-2093, 1973.
73. Lockshin RA and Zakeri Z: Programmed cell death and apoptosis: origins of the theory. *Nat Rev Mol Cell Biol* 2: 545-550, 2001.
74. Erickson GF and Schreiber JR: Morphology and physiology of the ovary. In: *Principles and Practice of Endocrinology and Metabolism*. Becker KL (ed). JB Lippincott, New York, pp776-787, 1990.
75. Rebar RW, Kenigsberg D and Hogden GD: The normal menstrual cycle and the control of ovulation. In: *Principles and Practice of Endocrinology and Metabolism*. Becker KL (ed). JB Lippincott, New York, pp788-795, 1990.
76. Kanduc D, Rossiello MR, Aresta A, Cavazza C, Quagliariello E and Farber E: Transitory DNA hypomethylation during liver cell proliferation induced by a single dose of lead nitrate. *Arch Biochem Biophys* 286: 212-216, 1991.
77. Haddow A: Cellular inhibition and the origin of cancer. *Acta Unio Intern Contra Cancrum* 3: 342-352, 1938.
78. Levin S, Bucci TJ, Cohen SM, Fix AS, Hardisty JF, Le Grand EK, Maronpot RR and Trump BF: The nomenclature of cell death: recommendations of an ad hoc Committee of the Society of Toxicologic Pathologists. *Toxicol Pathol* 27: 484-490, 1999.