REVIEW ARTICLE

APOPTOSIS AND CELL DEATH (MECHANISMS, PHARMACOLOGY AND PROMISE FOR THE FUTURE)

Ján Franko[1], Mikuláš Pomfy[2], Terry Prosbová[2]

Šafárik University Hospital: Department of Surgery[1]; Šafárik University Košice, Faculty of Medicine: Department of Histology and Embryology[2]

Summary: Rapidly growing body of evidence on cell death mechanisms and its disorders during last five years has replaced old paradigms and opened new horizons in medicine. Identification of different morphological and signaling aspects, as well as variances in requirement for energy enabled us to construct a theory of three main types of cell death: necrosis, apoptosis, and lysosomal cell death. Mitochondria, certain oncoproteins such as Bcl-2 family, and special catabolic enzymes participating in cellular demise might serve as targets for pharmacological manipulation. Upregulation or downregulation of programmed cell death has been implicated in ischemic, neurodegenerative, and autoimmune disorders, as well as in oncology and chronic inflammation. This minireview brings a short overview of genesis and development of theories on programmed cell death and apoptosis, summarizes basic relevant facts on apoptotic mechanisms and draws a new hypothesis on possible implication in medicine and surgery.

Key words: *Cell death; Apoptosis; Oncoprotein; Mitochondria; Caspase; Calcium*

Introduction

Loss of cellular populations is a key limiting factor in many medically and socially high-impact diseases. Refinement of scientific technology in recent decades made detection of various forms of physiological cell death possible. Physiological cell death is very distinct from necrosis. It occurs in two distinct models, and perhaps a whole spectrum between these two. Classic apoptosis, in which most of the early morphological changes occur in the nucleus, and a lysosomal or cytoplasmic cell death, in which the early alterations take place in the cytoplasm (6,46).

Apoptosis, a morphologically defined cellular death, is implicated in removal of cells during ontogenesis, physiological cellular turnover in adults, cytokine-induced tumor involution, clonal selection of T-lymphocytes, gerontopathology, neurodegenerative disorders, immunopathologic states, and even myocardial or cerebral ischemia (6,7,22,70). However, a loss of cell's susceptibility to inducers of physiological cell death is likely an important component in development of malignancy and maintenance of drug resistance (4,68,69). Therefore, after years of academic ignorance, processes of programmed cell death have become a hot issue in scientific world.

Pathways of Cellular Demise

The term 'programmed cell death' appeared in 1960s in works on cell elimination in metamorphosing insect (30,31, 50). This refers to a genetically invoked form of death (6). Morphologically it may assume a picture of either apoptosis or lysosomal cell death. Although morphological descriptions consistent with apoptosis have been present in literature since the 19th century (32), it has not been sooner than in 1970s, when Kerr and associates (22) unequivocally defined its histological appearance. Apoptosis serves as a one of three models of cell death during ontogenesis and its microscopical appearance is less marked than that of necrosis (6). It includes distinctive chromatin condensation, formation of cytoplasmic „finger-like" projections, which detach from the cell body and form apoptotic bodies, and heterophagocytosis of cellular remnants by surrounding tissue. Absence of inflammation, cellular edema and significant subcellular damage is characteristic, but is observed in necrosis. Histological appearance tends to be conserved with respect to type of cell and damage (65). Prompt phagocytosis as well as the absence of inflammatory reaction persuades pathologist to greatly underestimate the contribution of apoptosis in cell removal from population (6,7).

Cytoplasmic cell death may be seen in cells possessing large cytoplasm and is heralded by early and vast changes in cytoplasm. These involve increase in a number of lysosomes along with their redistribution, development of large autophagic vacuoles, and specific sequence of organelle removal. Very late in this process nuclear alterations similar to apoptosis take place (46). Energy resources probably remain generous until the very late stage.

Tab. 1: Principal differences between necrosis and apoptosis.

	Necrosis	Apoptosis
Histopathology	Edema Damage to organelles Membrane discontinuity	Cellular shrinkage Chromatin condensation Formation of apoptotic bodies Membrane continuity preserved
DNA cleavage	Random, diffuse	Internucleosomal cleavage
Reaction of surrounding tissue	Inflammation	Phagocytosis, no inflammation

Necrosis is opposed to physiological cell death (Tab. 1). It represents a morphological counterpart of energy resources loss, membrane penetrations, ruined control of ion flow and osmolysis resulting in uncontrolled loss of cellular content (14,61,62).

Using classical staining procedures it is almost impossible to quantify apoptosis due to a very short lifetime of morphologically evident apoptosis, which is believed to be at the level of tens of minutes. Classical microscopic analysis was the first method used to define apoptosis, and it should be kept in mind, that none of modern methods has replaced it. However, a myriad of new methods suitable for detection of certain apoptotic features has emerged, among them annexin V immunohistochemistry (66), multiparameter flow cytometry (11), TUNEL (TdT-mediated dUTP-biotin nick end labeling) staining, and diphenylamine assay of DNA fragmentation. Apoptosis can be in most instances identified by characteristic breakdown of DNA into oligonucleosomal fragments, which give so called laddering appearance on electrophoresis (2). Pioneer observation of this feature should be probably granted to Czech researchers (55). Chemical asymmetry of plasma membrane is a characteristic feature of normal cells. However, early during apoptosis cells export phosphaditylserine residues normally confined to the inner leaflet of the plasma membrane to the outer leaflet, thus flagging apoptotic cell to phagocytes (35). This feature has been recently employed to identify apoptotic cells using immunolabeling techniques against annexin V, cell membrane confined phospholipid binding protein with a high affinity for phosphatidylserine (66).

Morphological manifestation of apoptosis is linked to its terminal stage. Only these latter stages of the whole process are heralded by cell rounding, cytoplasm blebbing, and nuclear condensation and fragmentation. Acquisition of typical apoptotic morphology is dependent on caspase-mediated and energy-dependent rearrangements of cytoskeleton.

Nuclear pyknosis and karyorrhexis are near-to-definite morphological features of apoptosis. Factors responsible for chromatin condensation and pyknosis include DNases, Acinus and AIF (Apoptosis Inducing Factor). Caspase-activated DNase (CAD) is a cytosolic protein inactivated by heterodimerization with its inhibitor ICAD. This heterodimer splits by action of caspase-3 on ICAD and CAD translocates into the nucleus, where it exerts typical internucleosomal chromatin cleavage (12). Acinus (apoptotic chromatin condensation inducer in the nucleus) is newly described chromatin-condensation factor involved in apoptosis. For full activation it requires double caspase cleavage and features an unique peculiarity as it exerts its chromatin-condensing action without any detectable DNase activity (49). Both Acinus and CAD lead to histological appearance of karyorrhexis. Yet another factor, mitochondrial AIF, participates in nuclear changes. However, it produces large scale DNA fragmentation into pieces around 50 kb in length and gives a picture of peripheral chromatin condensation (58).

Caspases have been found to mediate cleavage of many cytoskeleton-associated proteins, among them Gas2 (3), gelsolin (25), and fodrin (35). Detachment of apoptotic cells from plate or from other tissue cells was found to be a consequence of calpain-mediated cleavage of cytoplasmic domain of integrin β3 subunit (40), which is required to maintain cellular adhesion and cytoskeletal association. On the other hand, studies employing microtubule-damaging drugs such vincristine suggest that microtubule damage is an important event in Bcl-2 inactivation via hyperphosphorylation and induction of apoptosis (57). This means that upstream intracellular mediators of apoptosis initiate cytoskeletal rearrangements, which in turn potenciate apoptotic cascade via inhibition of anti-apoptotic function of Bcl-2 oncoprotein. Moreover, initiation of apoptotic cascade at any point may cause self-amplification and inevitable cell death.

Interesting association between inhibition of apoptosis and a gain of metastatic capability was found in cells lacking expression of cytoskeleton-bound Death Associated Protein (DAP) kinase (20). Restoration of normal DAP kinase expression in high-metastatic tumor cells suppressed their metastatic ability. Links among suppression of apoptosis, cytoskeleton rearrangements, and neoplastic immortality and metastatic capability need further elucidation.

Susceptibility of cells to suicide varies significantly. Genetic control of apoptosis is mediated through several gene products. Some of them promote apoptosis (*p53*, *TNF*, *Fas/CD95*, *bax*, *bak* and *bad*), while the others (e.g. *bcl-2*, *bcl-*X_L) block apoptosis and promote cell survival (41, 71).

Subcellular Mechanisms of Apoptosis

Apoptosis seems to be an old and conserved reaction based on a self-scarifying anti-viral defense originally deve-

loped in primitive eukaryotes. Execution of this process involves inhibition of protein synthesis at the level of translation initiation, proteolysis specifically involving degradation of DNA repair mechanisms, and polynucleotide degradation. This complex molecular signaling system includes feedback mechanisms tending toward activation of all elements of the execution platform if only one element is initially engaged (67).

Because morphological alteration of nucleus was first observed in apoptotic elements, it was considered a coordinator of whole process. However, studies on enucleated cells have shown they can die with apoptosis as well (52). Although cellular suicide program is genetically encoded, its translation immediately before execution of apoptosis is not required. Apoptosis in certain cellular populations can be prevented with transcriptional and/or translational inhibitors (29,43,60). However, similar approach in different settings may provoke or accelerate apoptosis (5,10). The effector tool of apoptosis is a class of cysteine proteases localized in cytoplasm – caspases, also termed ICE-like enzymes (Interleukin-1β-Converting Enzyme) according to the first discovered member of this family. Two pathways of cell suicide exist. One triggered by signals created within the involved cell (unbalanced oxidative stress, calcium overload) and the other one initiated by signals generated from outside of the cell (TNF, Fas ligand, NO, glucocorticoids, actinomycin D). The intrinsic signals activate caspase-9, while extrinsic engages caspase-8. Both modes converge to sequential activation of other caspases, all process thus resembling limited proteolysis seen with blood clotting. Activated caspases digest structural proteins and degrade chromosomal DNA leading to death of the cell. Cell's decision to commit suicide is driven by death activators and is counterbalanced by the action of trophic factors, among them nerve growth factor, basic fibroblast growth factor, interleukin 2, insulin, and others.

Classical studies have been carried out in a worm *Caenorhabditis elegans* model, which features extensive removal of cell population during ontogenesis. Genes responsible for programmed cell death were identified as cell death genes (ced), and their corresponding proteins were termed CED. The genes ced-3 and ced-4 are essential for cell death; ced-9 antagonizes the activities of ced-3 and ced-4, thus resembling *bcl-2* family in mammals. CED-3 is a counterpart of mammalian caspases, while the function of CED-4 resembles that of mammalian Apaf-1 (Apoptosis Protease-Activating Factor 1). In mammalian cells, Bcl-2 protein drags caspases to mitochondrial membrane and prevents their activation, and blocks release of cytochrome C, which is a potent activator of pro-caspase-3.

Indeed, recent studies provided evidence on rate limiting behavior of mitochondria in early stages of apoptosis (16,41). These have given the experimental basis for three-step model of apoptosis (58):

1. Premitochondrial phase - activation of apoptotic signaling pathways, including so-called upstream caspases (Fig. 1),
2. Mitochondrial phase – loss of mitochondrial inner membrane potential accompanied by a release of proteins activating apoptotic effectors (Fig. 1,2),
3. Postmitochondrial phase – activation and action of apoptotic effectors (catabolic proteases – downstream caspases, nucleases) leading to microscopical appearance of apoptosis (Fig. 1).

Fig. 1: Sequence of main events after injurious stimulus leading to cellular death.

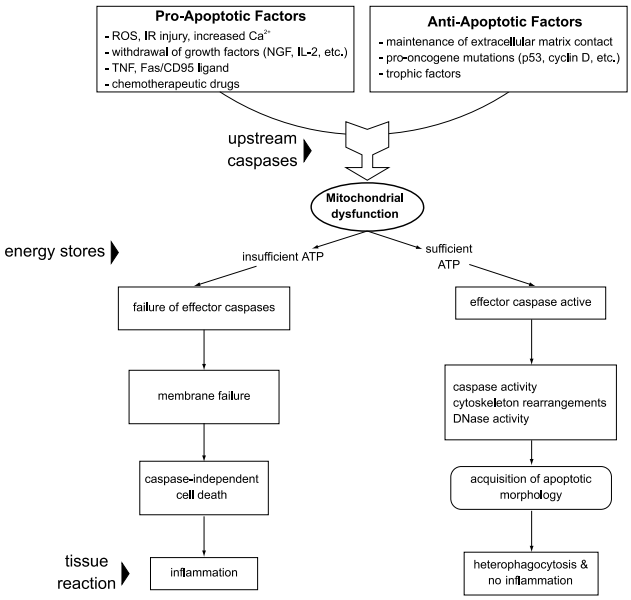
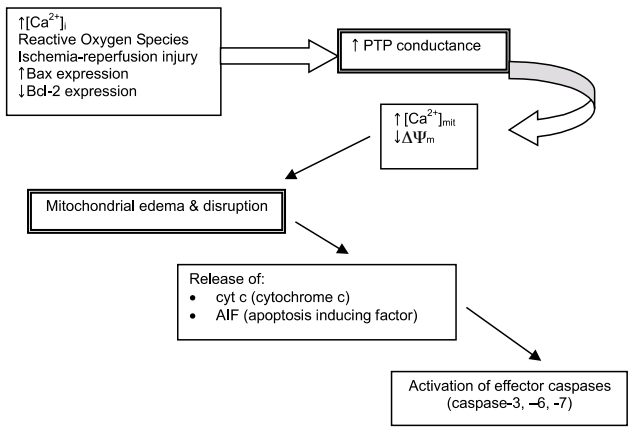


Fig. 2: Mitochondrial dysfunction and opening of permeability transition pore (PTP).



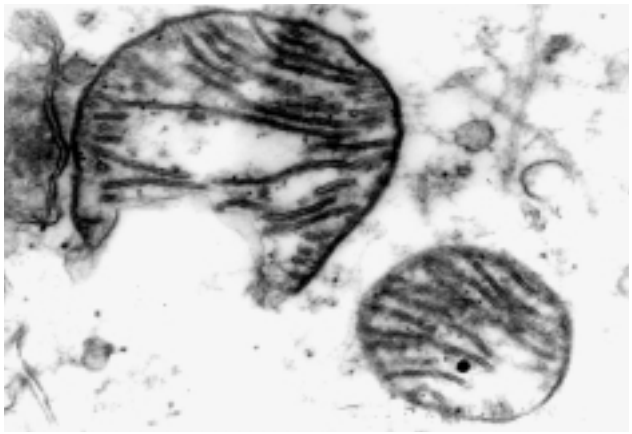
Data published by Zamzami and associates (31,72) document, that lowering of mitochondrial transmembrane potential $\Delta\Psi_m$, and subsequent intramitochondrial production of reactive oxygen species are triggers of apoptosis. Apoptosis has been observed in those cells only, in which depolarization of inner mitochondrial membrane occurred.

It has been reported that disturbances of mitochondrial function and integrity paralleled by disruption of intracellular calcium homeostasis preceded apoptotic changes of nucleus (37,42). Mitochondrial calcium overload initiates opening of a special mitochondrial megachannel and is prerequisite of all further steps of apoptosis (31,42,44).

Loss of electrochemical gradient on inner mitochondrial membrane is mediated by a sudden increase in its permeability. This permeability transition creates a shunt for protons, lowers protonmotive force $\Delta\Psi_m$, and results in a cessation of mitochondrial ATP synthesis. In all likelihood, permeability transition is caused by opening of special proteinaceous multiple conductance channel, or „megapore" or „megachannel". This nonselective channel is probably permeable to any atomic ion as well as water and forms at the junction of inner and outer mitochondrial membranes (45). Its opening gives rise to massive ion movement accompanied by water with resulting edema and rupture of outer mitochondrial membrane. Intermembrane proteins capable of inducing caspases (cytochrome c and apoptosis inducing factor AIF) are thus released to cytosol (16,56,74). Moreover, caspases induce liberation of intermembrane proteins from other mitochondria (36), hence engaging in self perpetuating cycle leading to coordination of proapoptotic behavior among all mitochondria in a given cell. For that reason permeability transition of inner mitochondrial membrane takes on a chain reaction profile and spreads as outbreak affecting entire mitochondrial population (53). Loss of mitochondrial potential $\Delta\Psi_m$, is a common trait of necrosis and apoptosis (72). End result of the mitochondrial dysfunction leads to biological catastrophe culminating in disintegration of plasmatic membrane (necrosis), or to activation of apoptotic proteases with subsequent activation of endonucleases and manifestation of apoptosis. Cell's decision on which morphological presentation will be preferred depends on intensity of initiating factor and energetic charge of the cell (27,28,39). Cells low in energy undergo uncoordinated process of necrosis, yet cells with sufficient energy stores experience apoptosis. Other explanation may be that cells mainly dependent on anaerobic glycolysis (leukocytes) undergo apoptosis, while cells reliant on aerobic glycolysis tend to suffer from necrosis. This is consistent with our findings of necrotic neuronal death in our model of transient seven-minute global cerebral ischemia in dogs, where we repeatedly failed to morphologically identify apoptosis (15,47). However, mitochondrial damage consistent with apoptosis has been observed (Fig. 3).

Either preventive or postinsult application of immunomodulant agent cyclosporine A or tacrolimus (FK-506) in settings of disrupted blood-brain barrier protects neurons against apoptosis induced by ischemia-reperfusion injury (18,58,72). These compounds inhibit formation of mitochondrial megapore (54), similarly to performance of intracellular calcium chelators (63,64) and natural inhibitors of apoptosis - some of protein products of *bcl-2* gene family. Employment of free radical scavengers can only retard terminal phase of apoptosis – reduction of cellular volume (73), but not cellular death itself.

Fig. 3: Electronogram of ruptured mitochondria after cerebral ischemia-reperfusion injury in canine neocortical neuron. Original magnification 32 000x.



Immunosuppressant actions of cyclosporine A, tacrolimus and rapamycine are mediated by the drug binding intracellular target - immunophillin. Drug-immunofillin complex binds to and inhibits the phosphatase calcineurin, thus resulting in modification of special proteosynthesis. Immunophillin ligands, including nonimmunosuppressants that do not inhibit calcineurin, stimulate regrowth of damaged peripheral and central neurons (48). Furthermore, tacrolimus inhibits the activity of nitric oxide synthase. Nitric oxide is capable of inducing apoptosis via direct opening of mitochondrial megapore (19).

Cell death activators, both apoptotic and necrotic, may be identical (54). Cell's fate is likely to be defined by the intensity and duration of exposure to initiating event. This is supported by reported succession of necrosis and apoptosis in glutamate-induced model of excitotoxic neuronal death. Early survival of necrotic phase was determined by recovery of mitochondrial energy-producing machinery, and neurons surviving the necrotic phase underwent apoptotic transformation (1).

Conclusion

Known associations of apoptosis controlling and cellular growth gene mutations include familial adenomatous polyposis (APC gene), hereditary malignant melanoma (regulators of cyclin-dependent kinases), Lynch syndromes (microsatelit DNA mutations) and others (8). Mutations in the p53 tumor suppressor gene are amongst the most frequent genetic abnormalities identified in human solid neoplasms. Besides malignancy, downregulation of apoptosis controlling mechanism is seen with inability to handle some forms of chronic inflammation (26,51), e.g. ulcerative colitis and rheumatic arthritis. Upregulation of apoptosis has been implicated in many autoimmune, neurodegenerative, and ischemic disorders (21,23,67).

Current immediate clinical applications of apoptosis-related research constitute estimation of anticancer chemotherapy effectiveness (33), survival prediction in acute leukemia (13), or reduction of allograft reperfusion injury after transplantation (24). Basic research has also recently questioned usage of lactated Ringer solution for acute shock therapy (9).

New knowledge of cellular death control is to be conceived by basic research. Thereafter, applied research should extend our pharmacological armamentarium with new approaches for therapy of ischemic disorders, malignant diseases, chronic inflammation and many others. Sufficient understanding of apoptosis and cell growth regulation yet requires more years of investigation. Nevertheless, new millenium may bring a significant breakthrough in treatment of many incurable and incapacitating diseases.

References

1. Ankarcrona M, Dypbukt J, Bonfoco E et al. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 1995;15:961-73.
2. Arends M, Morris R, Wyllie A. Apoptosis. The role of the endonuclease. Am J Pathol 1990;136:593-608.
3. Brancolini C, Benedetti M, Schneider C. Microfilament reorganization during apoptosis: the role of Gas2, a possible substrate for ICE-like proteases. EMBO J 1995;14:5179-90.
4. Carder P, Wyllie A, Purdie C et al. Stabilised p53 facilitates aneuploid clonal divergence in colorectal cancer. Oncogene 1993;8:1397-401.
5. Clarke A, Purdie C, Harrison D et al. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 1993;362:849-52.
6. Clarke P. Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol (Berl) 1990;181:195-213.
7. Cotran R, Kumar V, Robbins S. Robbins pathologic basis of disease. 5 ed. Philadelphia: WB Saunders, 1994.
8. Daly J, Bertagnolli M, DeCosse J, Morton D. Oncology. In: Schwartz S, Shires G, Spencer F et al., eds. Principles of Surgery. 7 ed. New York: McGraw-Hill, 1999:2162.
9. Deb S, Martin B, Sun L et al. Resuscitation with lactated Ringer's solution in rats with hemorrhagic shock induces immediate apoptosis. J Trauma 1999;46:582-8.
10. Deshpande J, Bergstedt K, Linden T, Kalimo H, Wieloch T. Ultrastructural changes in the hippocampal CA1 region following transient cerebral ischemia: evidence against programmed cell death. Exp Brain Res 1992;88:91-105.
11. Dive C, Gregory C, Phipps D et al. Analysis and discrimination of necrosis and apoptosis (programmed cell death) by multiparameter flow cytometry. Biochim Biophys Acta 1992;1133:3275-85.
12. Enari M, Sakahira H, Yokoyama H et al. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature 1998;391:43-50.
13. Estrov Z, Thall P, Talpaz M et al. Caspase 2 and caspase 3 protein levels as predictors of survival in acute myelogenous leukemia. Blood 1998;92:3090-7.
14. Farber J, Kyle M, Coleman J. Mechanisms of cell injury by activated oxygen species. Lab Invest 1990;62:670-9.
15. Franko J, Pomfy M, Nováková B, Beneš L. Stobadine protection against ischemia-reperfusion induced morphological alteration of cerebral microcirculation in dogs. Life Science 1999;65:1963-7.
16. Green D, Kroemer G. The central executioners of apoptosis: caspases or mitochondria? Trends Cell Biol 1999;8:267-71.
17. Hirsch T, Marchetti P, Susin S et al. The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. Oncogene 1997;15:1573-81.
18. Hirsch T, Susin S, Marzo I et al. Mitochondrial permeability transition in apoptosis and necrosis. Cell Biol Toxicol 1998;14:141-5.
19. Hortelano S, Dallaporta B, Zamzami N et al. Nitric oxide induces apoptosis via triggering mitochondrial permeability transition. FEBS Lett 1997;410:373-7.
20. Inbal B, Cohen O, Polak-Charcon S et al. DAP kinase links the control of apoptosis to metastasis. Nature 1997;390:180-4.
21. Kajstura J, Cheng W, Reiss K et al. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab Invest 1996;74:86-107.
22. Kerr J, Wyllie A, Currie A. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26:239-57.
23. Kiess W, Gallaher B. Hormonal control of programmed cell death/apoptosis. Eur J Endocrinol 1998;138:482-91.
24. Kohli V, Selzner M, Madden J, Bentley R, Clavien P. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation 1999;67:1099-105.
25. Kothakota S, Azuma T, Reinhard C et al. Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. Science 1997;278:294-8.
26. Ksontini R, MacKay S, Moldawer L. Revisiting the role of tumor necrosis factor alpha and the response to surgical injury and inflammation. Arch Surg 1998;133:558-67.
27. Leist M, Nicotera P. The shape of cell death. Biochem Biophys Res Commun 1997;236:1-9.
28. Leist M, Single B, Castoldi A, Kühnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. J Exp Med 1997;185:1481-6.
29. Linnik M, Zobrist R, Hatfield M. Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. Stroke 1993;24:2002-8.
30. Lockshin R, Williams C. Programmed cell death - I. Cytology of degeneration in the intersegmental muscles of the pernyi silkmoth. J Insect Physiol 1965a;11:123-33.
31. Lockshin R, Williams C. Programmed cell death. 3. Neural control of the breakdown of the intersegmental muscles of silkmoths. J Insect Physiol 1965b;11:601-10.
32. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 1995;146:3-15.
33. Mancini M, Sedghinasab M, Knowlton K et al. Flow cytometric measurement of mitochondrial mass and function: a novel method for assessing chemoresistance. Ann Surg Oncol 1998;5:287-95.
34. Marchetti P, Castedo M, Susin S et al. Mitochondrial permeability transition is a central coordinating event of apoptosis. J Exp Med 1996;184:1155-60.
35. Martin S, Finucane D, Amarante-Mendes G, O'Brien G, Green D. Phosphatidylserine externalization during CD95-induced apoptosis of cells and cytoplasts requires ICE/CED-3 protease activity. J Biol Chem 1996;271:28753-6.
36. Marzo I, Susin S, Petit P et al. Caspases disrupt mitochondrial membrane barrier function. FEBS Lett 1998;427:198-202.
37. McConkey D, Orrenius S. The role of calcium in the regulation of apoptosis. Biochem Biophys Res Commun 1997;239:357-66.
38. Mehmet H, Edwards AD. Hypoxia, ischaemia, and apoptosis. Arch Dis Child Fetal Neonatal Ed 1996;75:F73-75.
39. Mehmet H, Yue X, Squier MV, Edwards AD. The relationship between impaired cerebral energy metabolism and apoptosis in the cingulate gyrus of newborn piglets following transient hypoxia-ischaemia. UCL/RPMS Perinatal Brain Research Group. Biochem Soc Trans 1994;22:421s.
40. Meredith J, Jr, Mu Z, Saido T, Du X. Cleavage of the cytoplasmic domain of the integrin beta3 subunit during endothelial cell apoptosis. J Biol Chem 1998; 273:19525-31.
41. Mignotte B, Vayssiere J. Mitochondria and apoptosis. Eur J Biochem 1998;252:1-15.
42. Nicotera P, Orrenius S. The role of calcium in apoptosis. Cell Calcium 1998 Feb-Mar 23:2-3 173-80 1998;23:173-80.
43. Oppenheim R, Prevette D, Tytell M, Homma S. Naturally occurring and induced neuronal death in the chick embryo in vivo requires protein and RNA synthesis: evidence for the role of cell death genes. Dev Biol 1990;138:104-13.
44. Orrenius S, Nicotera P. The calcium ion and cell death. J Neural Transm Suppl 1994;43:1-11.
45. Petit P, Goubern M, Diolez P et al. Disruption of the outer mitochondrial membrane as a result of large amplitude swelling: the impact of irreversible permeability transition. FEBS Lett 1998;426:111-6.
46. Pilar G, Landmesser L. Ultrastructural differences during embryonic cell death in normal and peripherally deprived ciliary ganglia. J Cell Biol 1976;68:339-56.
47. Pomfy M, Maršala J, Bona M. Study of critical damage to the neurons in dog cerebral cortex in the presence of different degrees of ischaemia and after subsequent recirculation. Folia Morphol Praha 1985;33:322-7.
48. Sabatini D, Lai M, Snyder S. Neural roles of immunophilins and their ligands. Mol Neurobiol 1997;15:223-39.
49. Sahara S, Aoto M, Eguchi Y et al. Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. Nature 1999;401:168-73.
50. Saunders J, Jr. Death in embryonic systems. Science 1966;154:604-12.
51. Savill J, Wyllie A, Henson J et al. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest 1989;83:865-75.
52. Schulze-Osthoff K, Walczak H, Dröge W, Krammer P. Cell nucleus and DNA fragmentation are not required for apoptosis. J Cell Biol 1994;127:15-20.
53. Siesjö BK, Kristián T. Cell calcium homeostasis and calcium-related ischemic damage. In: Welch K, Caplan L, Reis D, Siesjö B, Weir B, eds. Primer on Cerebrovascular Diseases. San Diego: Academic Press, 1997:172-8.
54. Siesjö BK, Siesjö P. Mechanisms of secondary brain injury. Eur J Anaesthesiol 1996;13:247-68.
55. Skalka M, Cejková M, Matyáová J. The sensitivity of chromatin from thymuses and spleens of irradiated mice to alkaline solutions. Folia Biol (Praha) 1976;22: 335-42.

56. Skulachev V. Cytochrome c in the apoptotic and antioxidant cascades. FEBS Lett 1998;423:275-80.
57. Srivastava R, Srivastava A, Korsmeyer S et al. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol Cell Biol 1998;18:3509-17.
58. Susin S, Lorenzo H, Zamzami N et al. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999;397:441-6.
59. Susin S, Zamzami N, Kroemer G. Mitochondria as regulators of apoptosis: doubt no more. Biochim Biophys Acta 1998;1366:151-65.
60. Tata J. Requirement for RNA and protein synthesis for induced regression of the tadpole tail in organ culture. Dev Biol 1966 1966;13:77-94.
61. Trump B, Berezesky I. The role of cytosolic Ca2+ in cell injury, necrosis and apoptosis. Curr Opin Cell Biol 1992;4:227-32.
62. Trump B, Berezesky I. Calcium-mediated cell injury and cell death. FASEB J 1995;9:219-28.
63. Tymianski M, Spigelman I, Zhang L et al. Mechanism of action and persistence of neuroprotection by cell-permeant Ca2+ chelators. J Cereb Blood Flow Metab 1994;14:911-23.
64. Tymianski M, Wallace M, Spigelman I et al. Cell-permeant Ca2+ chelators reduce early excitotoxic and ischemic neuronal injury in vitro and in vivo. Neuron 1993;11:221-35.
65. van Lookeren Campagne M, Gill R. Ultrastructural morphological changes are not characteristic of apoptotic cell death following focal cerebral ischaemia in the rat. Neurosci Lett 1996;213:111-4.
66. Walton M, Sirimanne E, Reutelingsperger C et al. Annexin V labels apoptotic neurons following hypoxia-ischemia. Neuroreport 1997;8:3871-5.
67. White B, Sullivan J. Apoptosis. Acad Emerg Med 1998;10:1019-29.
68. Wyllie A. Apoptosis: cell death under homeostatic control. Arch Toxicol Suppl 1987;11:3-10.
69. Wyllie A. Apoptosis. Death gets a brake. Nature 1994;369:272-3.
70. Wyllie A, Kerr J, Currie A. Cell death: the significance of apoptosis. Int Rev Cytol 1980;68:251-306.
71. Zamzami N, Brenner C, Marzo I, Susin S, Kroemer G. Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. Oncogene 1998;16:2265-82.
72. Zamzami N, Hirsch T, Dallaporta B, Petit P, Kroemer G. Mitochondrial implication in accidental and programmed cell death: apoptosis and necrosis. J Bioenerg Biomembr 1997;29:185-93.
73. Zamzami N, Marchetti P, Castedo M et al. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. J Exp Med 1995;182:367-77.
74. Zhivotovsky B, Orrenius S, Brustugun O, Doskeland S. Injected cytochrome c induces apoptosis. Nature 1998;391:449-50.

MUDr. Ján Franko,
Šafárik University Hospital,
Department of Surgery,
trieda SNP 1, 040 66 Košice,
Slovak Republic.
e-mail: franko@kosice.upjs.sk