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The Role of the Apoptosis and the Genes Regulating Apoptosis in the Early Differentiation of Human Embryo

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Abstract. Apoptosis (programmed cell death) is an important process participating in the formation of organs and tissues during embryogenesis Our aim of the work is studying the role of the apoptosis during the human embryonic differentiation We tend to give acquired findings into the correlation with expression of proteins Bcl-2 and Bax (products of genes regulating apoptosis) Detection of the apoptosis was carried out on 25 routinely processed human embryos by means of TUNEL technique The level of expression of Bcl-2 and Bax was determined using standard three-step immunohistochemical procedure Results were achieved by the comparison of apoptoic index and the level expression of Bcl-2 and Bax was semiquantitatively evaluated The low value of apoptic index was mostly accompanied by the high expression of Bcl-2 and the Bax expression was not proportionally related to the value of apoptic index

Key words: Apoptosis — Bax — Bcl-2 — Embryo — TUNEL

For every cell, there is a time to live and a time to die There is one mode of cell death that occurs under the normal physiological condition so orderly that it is often called programmed cell death (Kerr *et al* 1972) This process (also called apoptosis) plays an important role in the maintenance of homeostasis (e g hormone-dependent involution in the adult, death of immune cells after cytokine depletion or deletion of autoreactive T-cells in the developing thymus) as much as is needed for proper development of organs and tissues during embryogenesis (e g resorption of the tadpole tail during its metamorphosis into a frog, removal of the cells in interdigital parts of primitive autopodia, extinction of

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mesonephrotic structures) and for destroying cells that represent a threat to the integrity of the organism (e g cells infected with viruses, immunologically autoreactive cytotoxic T-lymphocytes, cells with DNA damage, cancer cells after the effective chemotherapy)

The cascade of activating reactions (involving caspases and Ca^{2+} ions) is triggered by inner or outer stimuli (hormones, irradiation) and it results in the activation of endonucleases which cleave aggregated chromatin into mono- and oligonucleosomal fragments Though plasma membrane remains intact, the semipermeability is impaired Therefore, cell is shrinking, condensed cytoplasm and nucleus are parted into membrane-bound vesicles called apoptotic bodies, which contain morphologically intact mitochondria, ribosomes and fragmented nuclear material *In vivo,* these bodies are rapidly phagocytosed and due to this efficient mechanism for the removal of apoptotic cell no inflammatory response is illicted

We traced to contribute to the elucidation of a control mechanism of apoptosis during the human embryonic differentiation and we tend to give the acquired findings into the correlation with the expression of proteins Bcl-2 and Bax (Lichnovský *et al* 1998) (products of genes regulating apoptosis and members of the family of Bcl-2-related proteins) Bcl-2 is the plasma membrane associated protein with antiapoptoic and antioxidative effect and it can regulate intracellular concentration of Ca^{2+} Another cytoplasmatic protein, Bax, partially homologous to Bcl-2, is involved in the regulation of apoptosis as well and its effect is proapoptoic In addition to it, another members of Bcl-2 family participate in apoptoic regulation – e g Bcl-X_L prevents apoptosis and Bcl-X_S is a Bcl-2 antagonist (Reed 1996) Whether a cell lives or dies may depend on the ratio of the level of expression of these proteins Naturally, there are other factors involved in the regulation of apoptosis

25 human embryos of 4–28 weeks of i u d , processed in routine way (fixed in methacarn or formalin and embedded in paraffin), pre-treated by exposing to microwaves or Proteinase K were studied using the kit from Boehringer-Mannheim Company for TUNEL technique (TdT-mediated X-dUTP nick end labeling) and immunohistochemical assay

TUNEL technique detects DNA strand breaks occuring in the early stages of apoptosis by terminal deoxynucleotidyl transferase-mediated labeling of a free 3′-OH terminus with fluorescein-modified nucleotides Apoptoic nuclei are visualised by anti-fluorescein antibody conjugated with alkaline phosphatase, which dissociates the yellow-coloured substrate (NBT/BCIP) to blue-coloured precipitate Intact nuclei are labeled by nuclear fast red

Standard three-step method was proceeded by using commercially accessible antibodies anti-Bcl-2 (Biogenex) and polyclonal antibodies P-19 (Biogenex) for the detection of Bax for the immunohistochemical assay of Bcl-2 and Bax

For quantitative evaluation of the apoptoic level the intestine, kidney, axial skeleton and limbs were selected from 17 embryos, because these structures undergo considerable rearrangement in the course of differentiation The apoptoic index (AI = number of apoptoic cells/all cells in the observed field or structure) was determined using computer image analysis system LUCIA M/Comet v 3 51ab (Laboratory Imaging Ltd) Semiquantitative evaluation of immunohistochemical assay was performed and compared visually with results acquired by the TUNEL technique

Sections of younger embryos were observed qualitatively in series, especially we were concerned with liver and heart (Figs 1–3)

In the majority of structures examined we proved the occurrence of cells ceased by apoptosis which are substituted by cells of higher developmental level The other are protected by effects of antagonists of apoptosis (Bcl-2 etc)

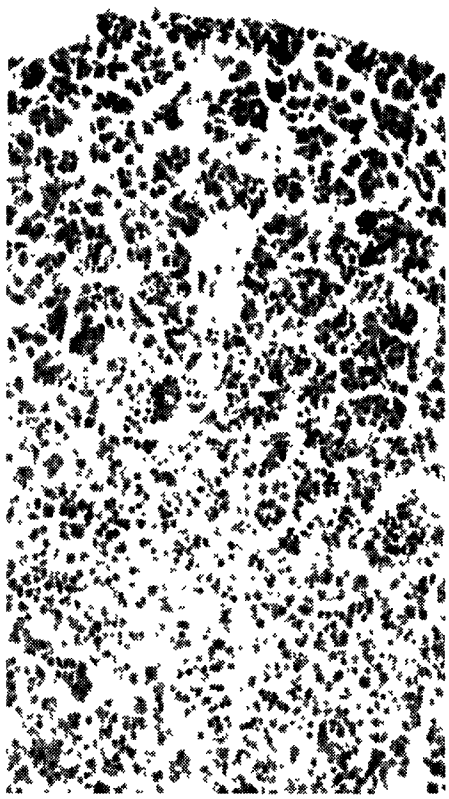

Figure 1. A large part of left liver lobe becomes extinct by apoptosis during differentiation Massive occurrence of apoptoic cells (arrow) (8-week old embryo) Magnification ×240

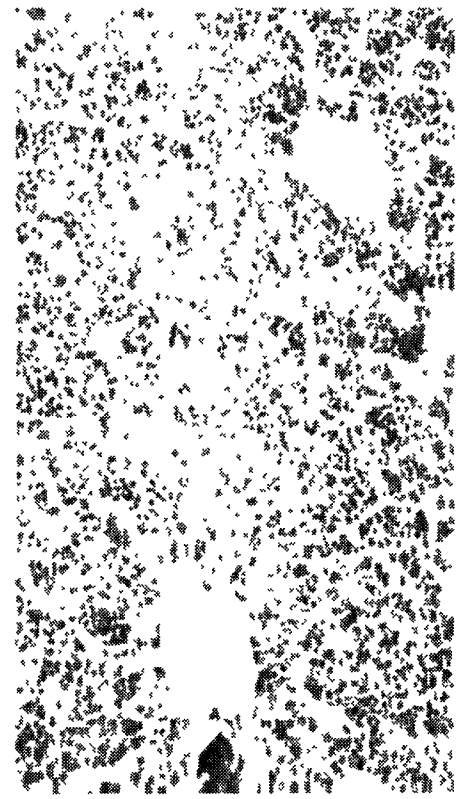

Figure 2. There are no apoptoic cells in the right liver lobe as this part of liver quickly proliferate (8-week old embryo) Magnification ×240

The highest level of apoptosis was found out in tissues and organs undergoing regression or becoming extinct during i u d (mesonephros, spongious layer of primitive myocardium, interdigital parts of primitive autopodia and left lobe of liver) In these observed structures we also demonstrated the reverse proportion between the level of Bcl-2 expression and localization of apoptosis and whether the occurrence of apoptosis is directly proportional to high expression of Bax gene

In neogenic zone of metanephrons apoptosis appeared more frequently in primitive tubules than in renal corpuscles what reflects quite abundant exchange of epithelial cells In the observed time period (5 -28 week of i u d) the lowest level of apoptosis was in the 14th week of i u d

In the wall of primitive intestine, the level of apoptosis conspicuosly prevails in mesothelium of serosis Apoptosis sequentially increases in villi and Lieberkuhn's crypts In other layers of the intestine wall the level of apoptosis remains without marked changes

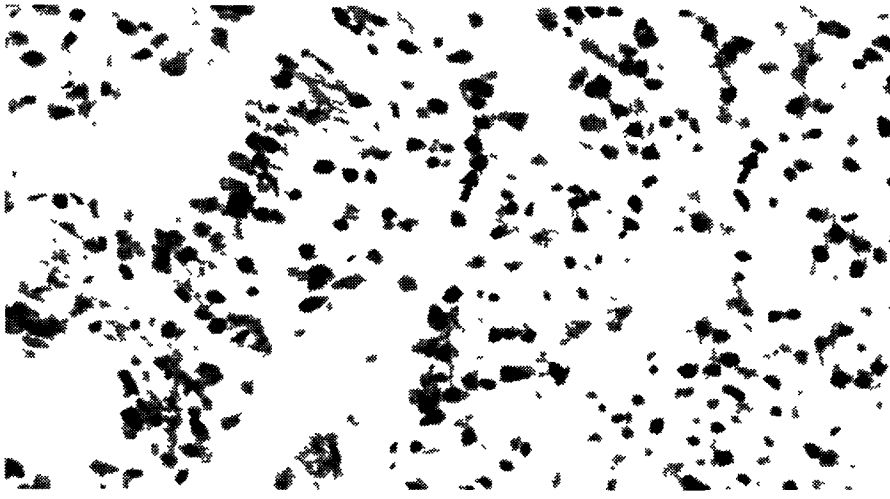

Figure 3. Abundant apoptoic cells (arrows) in the disappearing spongious layer of the embryonic heart (7 week old embryo) Magnification ×480

The level of expression of Bcl-2 and Bax accorded with the level of apoptosis in structures mentioned above

The level of apoptosis is predominant in the marginal zone of cartilaginous anlage of limb bone and does not present any pronounced changes between 7–14 week of i u d what may be related to rise and growth of the collar bone Our observation confirmed the one-week acceleration of the upper limb development

There is a high level of apoptosis in the part of anlage of axial skeleton which is going to ossify into vertebra On the other hand, there is practically no apoptosis in the area of future intervertebral disc

In the anlage of axial skeleton and limbs, there is approximately the same level of expression of Bcl-2 and Bax protein in all structures examined This discrepancy between the occurrence of apoptosis and expression of Bcl-2 and Bax genes might have been caused by other factors involved in this multilevely regulated process We have the intention to study these points at issue in the future

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