Involvement of p53 and Bcl-2 Family Proteins in Regulating Programmed Cell Death and Proliferation in Human Embryogenesis

J. Prochazkova[1], V. Lichnovsky[1], D. Kylarova[1], B. Erdosova[1] and P. Vranka[2]

[1] *Department of Histology and Embryology, Medical Faculty of Palacky University Hnevotinska 3, 775 15 Olomouc, Czech Republic*
[2] *Department of Zoology and Ecology, Faculty of Science of Masaryk University Kotlarska 2, 611 37 Brno, Czech Republic*

Abstract. Homeostasis and development in vertebrates are regulated by cell proliferation, differentiation and death. Permeability of mitochondrial membranes, a decisive feature of apoptosis, is regulated by Bcl-2 family regulators. Protein p53 is able to reduce bcl-2 and promote bax expression. This study focused on the immunohistochemical detection of the expression levels of Bcl-2 family regulators (anti-apoptotic Bcl-2 and Bcl-X_L, pro-apoptotic Bcl-X_S and Bax), p53, and PCNA as a marker of proliferation, together with the evaluation of the level of apoptosis in human embryos (anlage of limbs, axial skeleton, metanephros, and intestine). Expression of observed proteins was assessed by a three-step immunohistochemistry and evidenced by the double-staining technique. Apoptosis was detected by the TUNEL technique.

This study provided circumstantial evidence of the exclusive role of Bcl-2 and Bcl-X_L proteins in the inhibition of apoptosis – only rarely were the Bcl-2/Bcl-X_L positive cells stained by TUNEL. The role of pro-apoptotic members of Bcl-2 family remains ambiguous, as TUNEL positive cells are both Bax/Bcl-X_S positive and negative. This study provided substantial evidence that expression patterns of observed proteins are neither fully explainable by "rheostat" theory, nor are the findings obtained from animal model tissue or cell culture commonly applicable to human embryos.

Key words: Apoptosis — Bcl-2 proteins — p53 — Proliferation — Double-staining technique

Correspondence to: Vaclav Lichnovsky, Department of Histology and Embryology, Medical Faculty of Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic
E-mail: lichno@tunw.upol.cz

cell division and cell death (according to our observation – apoptosis appears in intestinal villi after the 10^{th} week of IUD) the development of epithelium can continue by formation of crypts and their specific Paneth cells and also of the layer of connective tissue.

We have observed the cells double-stained for TUNEL+Bcl-2 or for Bax+ Bcl-2 outside the regions where the cells have to remain viable to proliferate (e.g. cartilage of limbs and axial skeleton). The immunohistochemical staining of murine tissues has also revealed that the expression of bax and bcl-2 overlap in some tissues (Krajewski et al. 1998). One possible explanation for this peculiar observation is the principle of so-called "rheostat". According to this model, when Bcl-2 is in excess, Bcl-2–Bax heterodimers predominate and cell death is inhibited. Conversely, when Bax is in excess, Bax homodimers predominate and the cell becomes susceptible to cell death induction (Bruckheimer et al. 1998). Good example for interaction between pro-apoptotic and anti-apoptotic Bcl-2 proteins is the stratification of future epidermis (see Results).

Developing cartilage is an intriguing system. Although the ossification centers first appear in the central part of cartilage, the level of apoptosis was higher in its marginal parts. This may be caused by signal conflict coming from the inner layer of perichondrium, its differentiation to chondrocytes, and beginning of ossification to collar bone. The predestination to die was evidenced by extremely high apoptotic index and intense expression of Bax protein since the 8^{th} week of IUD, but these cells express also anti-apoptotic Bcl-2 protein and even PCNA.

Although the processes of cell renewal and cell death appear to be opposing, substantial evidence now indicates that the two are linked. There are potential oncogenes with both pro-mitotic and pro-apoptotic functions (e.g. c-Myc, E1A, E2F, Rb) (Evan and Littlewood 1998) and p53 protein was also linked to both processes. p53 can act as a direct transactivator of PCNA promoter in dose-dependent manner. PCNA as a clamp molecule of DNA polymerase delta is involved in the DNA damage repair induced by p53 (Morris et al. 1996). The role of p53 protein in morphogenesis of embryo is ambiguous. Inhibition of p53 activity in embryo of *Xenopus laevis* blocks the ability of *Xenopus* early blastomeres to undergo differentiation (Wallinford et al. 1997). On the other hand, homozygous p53 null mice appear to develop normally but are prone to the spontaneous occurrence of neoplasms (Donehower et al. 1992). Lichnovsky and his colleagues (1998) demonstrated by immunohistochemistry the presence of p53 protein in most of developing organs before the end of organogenesis, after which the levels gradually decrease. The same trend was observed during the embryonal development of mouse and chicken – there is a decline in the steady-state levels of the p53 protein and an equal decline in p53 mRNA caused by post-transcriptional downregulation (Louis et al. 1988). Since the majority of evaluations in this study were performed in terminal stages of organogenesis and mostly in fetal stages of IUD, the level of p53 expression was extremely low. This might be the reason for rather sceptic results obtained by any comparisons of p53 with other regulators.