EXPRESSION OF APOPTOSIS IN THE DEVELOPING HUMAN CEPHALIC REGION DURING THE EMBRYONIC AND EARLY FETAL PERIOD

Drahomíra Černochová*, Eva Pospíšilová, Václav Lichnovský

Department of Histology and Embryology, Medical Faculty, Palacký University, 775 15 Olomouc, Czech Republic

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The aim of our work was to study the expression of apoptosis in the cephalic region in human embryonic and fetal material.

Twelve human embryos and fetuses were processed by routine histological technique and then studied using the Boehringer – Mannheim Company kit for TUNEL technique.

Rich appearance of apoptotic cells was typical for mesenchyme and for tissues of the mesenchymal origin. Dispersed or focal occurrence of apoptotic cells was described in nervous system and some orofacial structures during the embryonic period. During the early fetal period occurrence of apoptosis decreased and only rare apoptotic nuclei were visible in studied areas. Often they failed at all.

INTRODUCTION

Apoptosis is a complex of processes which lead to the physiological cell death, usually called programmed cell death.

It is one of the mostly studied biological processes at present. It is associated with normal differentiation and formation of organs during organogenesis[1], with growth defects and potentially with the origin of tumors.

The aim of our work was the study of cranial parts of human embryos and fetuses with special attention paid to the developing nervous tissue and orofacial region during embryonic and early fetal period.

METHODS

Tissue samples of 12 human embryos and fetuses aged 6–14 weeks of intrauterine life, all from normal pregnancies, 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-d UTP).

TUNEL technique detects DNA strand breakes occuring in the early stages of apoptosis by terminal deoxynucleotidyl transferase, mediated labelling of the free-3'-OH termini with fluorescein – modified nucleotides. Apoptotic nuclei are visualised by antifluorescein antibody conjugated with alkaline phosphatase which dissociates the yellow – coloured substrate (NBT/BCIP) to blue – coloured precipitate. Intact nuclei are labelled by nuclear fast red[2].

RESULTS

In all studied areas we noticed relatively rich appearance of apoptotic cells in mesenchyme and in the tissues of the mesenchymal origin (cartilages and bones of the head skeleton).

In tissues differentiating from ectoderm and entoderm apoptotic cells appear especially in the embryonic period.

In embryonic material apoptotic nuclei are dispersed in germ and mantle zones of the telencephalic wall, they are present in the retina and in the anterior epithelium of the lens, as well. Many apoptotic nuclei were found in the epithelium of the respiratory region in the nasal cavity. In the olfactory epithelium apoptosis failed to be proved.

In the fetal material in all the localizations mentioned above rare apoptotic nuclei were present.

In the embryonic material the focal occurence of apoptotic nuclei was present in the surface covering epithelium of the lingual anlage. Only single positive nuclei were visible on the surface of lingual anlage in the 13- and 14-week-old fetuses.

Apoptotic nuclei were not found in the ectodermal and mesodermal anlage of the tooth germ in the 6-week-old embryo. Single positive nuclei were present in 7- to 13-week-old tooth germs.

Solitary apoptotic nuclei in the salivary gland anlages were found in the embryonic period. In the fetal material they failed to be proved.

In the superficial ectoderm diffuse expression of apoptotic nuclei was present in the 6-week-old embryo.

Massive presence of apoptotic nuclei was visible in this localization in the 9th week.

In the fetal period (13-week-old fetus) we found diffuse expression of apoptotic nuclei. In the older material (14-week-old fetus) apoptotic nuclei were not visible.

Massive expression of apoptotic nuclei was found in the lining membrane of the primitive oral cavity during embryogenesis. Later the expression decreased and in the 13- and 14-week-old fetuses apoptotic nuclei were not present at all.

DISCUSSION

Apoptosis and proliferation play an important role in morphogenetic processes in early developmental stages of the human intrauterine development. In the present paper we observed distinct apoptosis first of all in the organs which undergo complicated development in early fetal period (kidney, limbs, myocardium)[3].

Occurrence of apoptosis in these localizations supports the importance of this process during the differentiation.

In the cephalic region presence of apoptosis was not so frequent as in the above mentioned organs. We studied expression of proliferative antigens PCNA and Ki-67 in all mentioned localizations, as well. We described the cell proliferation in the 6 th week of the intrauterine life followed by an increase with the age of the fetus[4,5]. Expression of apoptosis was higher in the embryonic period, in the early fetal period it gradually disappeared.

We suppose that higher presence of apoptotic cell nuclei in embryonic tissues developing from the ectoderm is connected with the differentiation of the primary simple ectoderm into anlages of the respiratory epithelium, epidermis and stratified squamous epithelium of the oral cavity. Higher appearance of apoptotic nuclei in periderm proves that these cells are gradually eliminated during the development.

Presence of apoptosis correlates positively in CNS with that of p53[6]. Similar findings in some organs, e. g. mesonephros, neogenous zone of metanephros or spongious layer of myocardium support the idea of Donehower et al.[7] who suggest that after detection of DNA damage p53 induces block of the cell cycle which gives time for the cell to repair its DNA. If it fails to do this it is assumed that apoptosis is triggered by p53. The mechanisms which account for the occurence of apoptosis in the absence of p53 remain unsolved.

Our findings agree with the hypothesis that the normal human embryogenesis is under multiple level control[8].

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Apoptotic nuclei are distinctly dark.

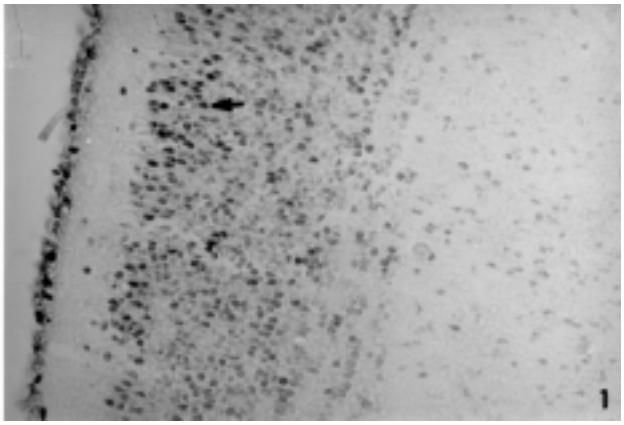

Fig. 1. Apoptosis in cell nuclei in the telencephalic wall in the 7-week-old embryo. Positive nuclei (arrow) are present in the mantle zone.
Magn. x 600.

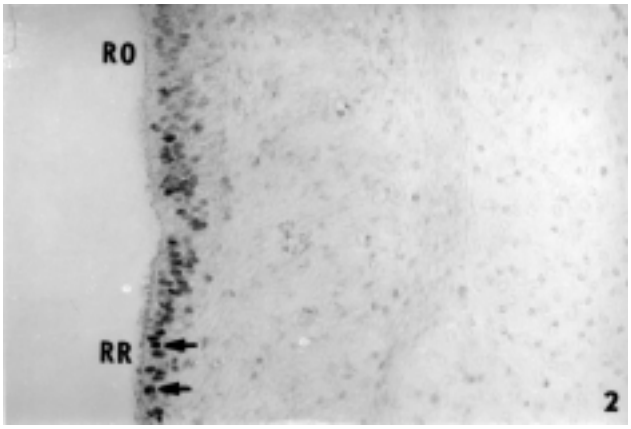

Fig. 2. Massive occurrence of apoptosis (arrows) in the respiratory region of the nasal cavity mucosa of the 7-week-old embryo. Boundary between the respiratory region (RR) and the olfactory region (OR).
Magn. x 600.

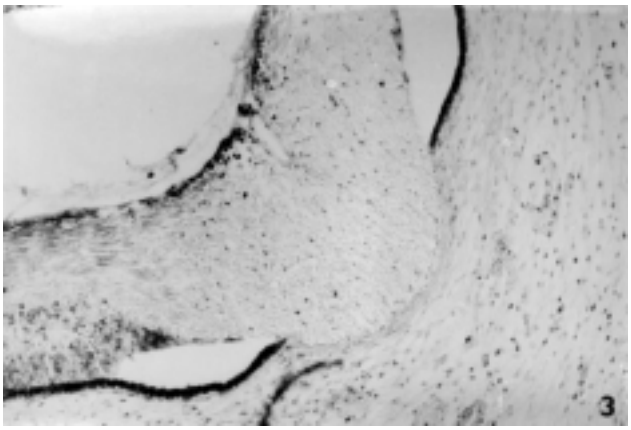

Fig. 3. Apoptotic nuclei in the retina anlage near the blind spot of the 7-week-old embryo.
Magn. x 300.

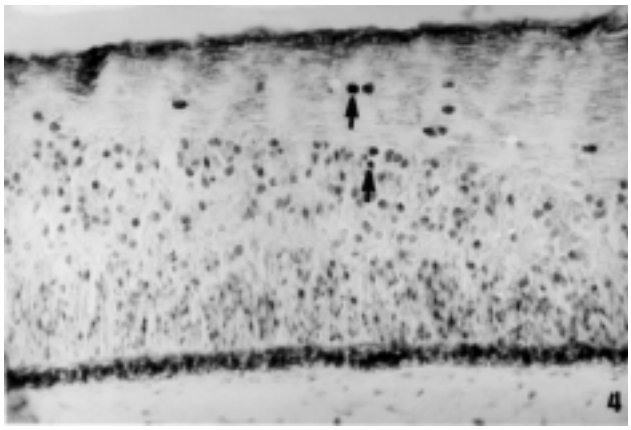

Fig. 4. Appearance of apoptosis (arrows) in the neuroectodermal eye anlage of the 7-week-old embryo.
Magn. x 600.

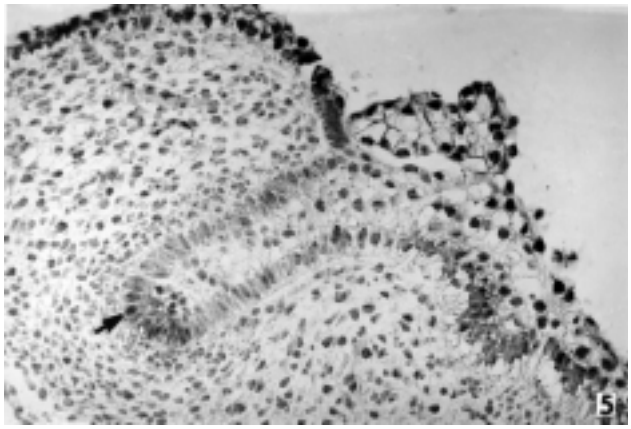

Fig. 5. Solitary occurrence of apoptotic cells (arrow) in the tooth anlage of the 9-week-old fetus. Massive apoptosis in the lining membrane of the primitive oral cavity.
Magn. x 600.

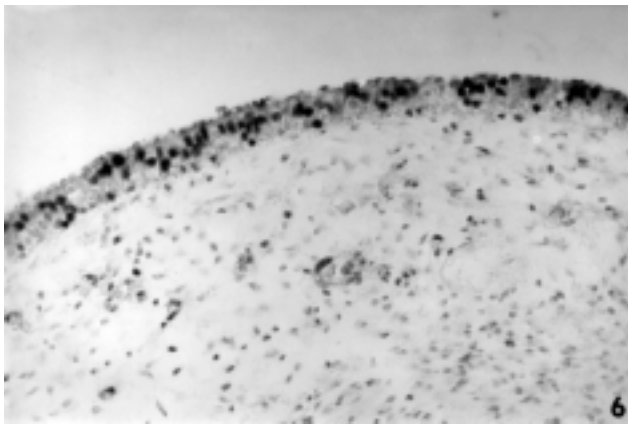

Fig. 6. Focal occurrence of apoptotic nuclei in the covering epithelium of the tongue anlage in the 7-week-old embryo.
Magn. x 600.

REFERENCES

1. Sanders E. J., Wride M. A. (1995) Programmed Cell Death in Development. Int. Rev. Cytol. *163*, 105–173.
2. In Situ Cell Death Detection Kit, POD (1995) Boehringer Mannheim, 1–12.
3. Lichnovský V., Macháčková L., Hlobilková A., Kolář Z., Černochová D., Pospíšilová E., Nepožitková D. (1998) Apoptosis and Bcl-2 expression during human embryogenesis. Histochem. J. *11*, 843.
4. Černochová D., Pospíšilová E., Lichnovský V., Havelka P. (1998) Expression of PCNA and Ki-67 in orofacial region of human embryonic and fetal material. Histochem. J. *11*, 893.
5. Pospíšilová E., Černochová D., Lichnovský V., Nepožitková D. (1998) PCNA and Ki-67 expression in the developing CNS. Histochem. J. *11*, 844.
6. Černochová D., Pospíšilová E., Lichnovský V., Burda L. (1997) Expression of p 53 protein in CNS of human embryos. Histochem. J. *29*, 715.
7. Donehower L. A., Harvey M., Slagle B. L., et al. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. Nature *356*, 215–221.
8. MacCallum D. E., Hupp T. R., Midgley C. A. et al. (1996) The p53 response to ionising radiation in adult and developing murine tissues. Oncogene *13*, 2557–2558.