Apoptosis and Expression of Proliferative Proteins in the Developing Nervous System and Orofacial Region of Human Embryos

D Černochová, E. Pospíšilová, D Nepožitková, P Havelka and V Lichnovský

Department of Histology and Embryology, Palacký University, Olomouc, Czech Republic

Abstract. We have studied expression of PCNA and Ki-67 in the developing nervous system, sensory organs and orofacial regions in human embryos and fetuses using monoclonal antibodies PC-10 and MIB-1 in three-step immunohistochemical method and apoptosis performed by TUNEL technique Expression of PCNA and Ki-67 increased with the age Apoptosis was rare in above mentioned regions

Key words: PCNA Ki-67 – Apoptosis Embryo — Fetus

PCNA and Ki-67 antigens represent endogenous markers and belong to markers of proliferative activity, mostly used in the world (Christensen *et al* 1993, Mollgard *et al* 1993)

PCNA represents an auxiliary protein of DNA polymerase delta and is involved in the process of DNA synthesis and reparation PCNA was demonstrated in the cell nuclei during the course of G_1, S and G_2 stages of the cellular cycle Its occurence is very low in inactive (non-dividing) cell nuclei and some consider this nuclear phosphoprotein as a reliable marker of cell proliferative activity

Ki-67 is a protein with short biological half-life time (less than 1 hour), which shows very strong expression in nuclei of proliferating cells With respect to the lenght of its biological half-life time, the labeling index of Ki-67 does not correspond to that of PCNA

Apoptosis is a complex of processes which lead to the physiological cell death, usually called programmed cell death It is one of the most studied biological processes at present (Lichnovsky *et al* 1998) It is associated with normal differentiation and formation of organs during organogenesis, with growth defects and potentially with the tumor origin

We examined cranial parts of human embryos and fetuses with special emphasis on developing nervous tissue and orofacial region

Tissue samples of 12 normal human embryos and fetuses aged 6-14 weeks of intrauterine life were fixed in methacarn and processed by the classic paraffin techniques The immunohistochemical detection of PCNA and Ki-67 antigens was performed by three-step immunohistochemical method using monoclonal antibodies PC-10 for detection of PCNA and MIB-1 for detection of Ki-67 Secondary biotin labeled anti-mouse antibody and streptavidin conjugated with horse-radish peroxidase were used in the above steps

Correspondence address V Lichnovsky, Department of Histology and Embryology Palacký University, Hnevotinska 3, 775 15 Olomouc, Czech Republic
e-mail Lichno@risc upol cz

Figure 1. Massive PCNA expression in the ectodermal and mesodermal parts of the tooth germ in the 13 week-old fetus Magn ×240 (left)

Figure 2. Ki-67 expression in the layer of cones and rods in the 8-week-old retina Magn ×480 (right)

Apoptosis was detected by the TUNEL technique TUNEL technique detects DNA strand breakes occuring in the early stages of apoptosis by terminal deoxynucleotidyl transferase, mediated labeling of the free-3'-OH termini with fluorescein-modified nucleotides Apoptotic 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

Embryonic period

In the 6-week-old embryo PCNA positive cell nuclei begin to appear in the nervous tissue of telencephalon, in epithelia of the developing nasal cavity and in the neuroectoderm anlage of the retina To the end of the embryonic period (8th week) proliferating activity increases Many PCNA positive nuclei are visible in the anterior lens epithelium and in the whole thickness of the retina anlage Distinct PCNA expression is present also in epithelia of the nasal cavity and vomeronasal organ and in the ependymal and mantle

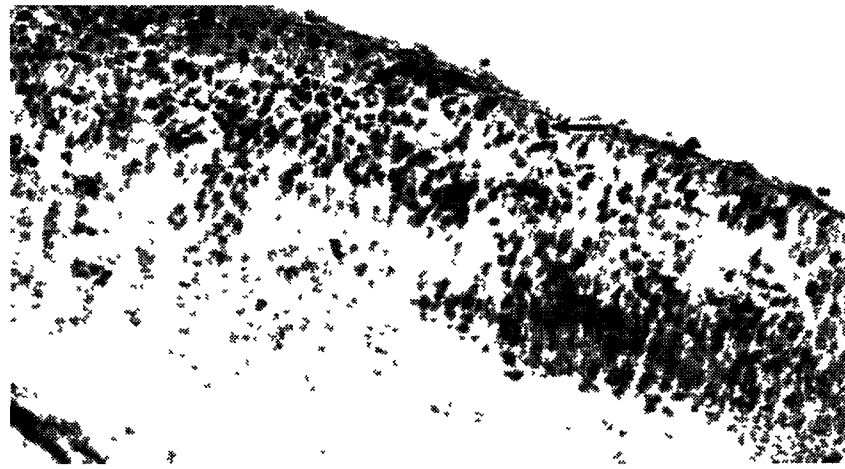

Figure 3. Apoptosis (arrow) in the germ and mantle layer of the telencephalic wall in 7-week-old embryo Magn ×480

zones of the telencephalon wall with decreasing gradient to the outer surface The amount of PCNA positive nuclei is less distinct in epithelial cells of the choroid plexus The rare PCNA positive nuclei are present in tissues of the developing tongue and salivary gland anlages Groups of PCNA positive nuclei are visible in tooth anlages and also in the superficial ectoderm and in the lining membrane of the primitive oral cavity during the whole embryonic period (6–8th week)

Ki-67 expression is minimal in the 6th and 7th weeks and only single Ki-67 positive nuclei were found in the followed localizations In the 8th week single Ki-67 positive nuclei were proved in germinative zone and on the boundary between marginal and mantle zone, in the layer of cones and rods of the retina and in the epithelium of the primitive nasal cavity In the developing orofacial region expression of Ki-67 was not found

Apoptosis was present mainly in the retina and in the epithelium of the nasal cavity respiratory region Single apoptotic nuclei are visible in the mantle and germ zones of the telencephalic wall and in the anterior epithelium of the lens A lot of apoptotic nuclei was found in the lining membrane of the primitive oral cavity, massive expression of apoptotic nuclei was seen in the superficial ectoderm in all studied material Apoptotic nuclei in the tooth germ were visible only sporadicly in the ectodermal part of the developing tooth

Fetal period

During the fetal period PCNA and Ki-67 expressions increase Abundant occurence of PCNA positive nuclei is characteristic for the epithelium of the nasal cavity and a layer of cones and rods of the differentiating retina In 14-week-old fetus many PCNA positive nuclei were localized in ependymal cells, neuroblasts and glioblasts and all the investigated organs of the orofacial region The amount of Ki-67 positive nuclei increases in the layer of cones and rods and in epithelia of the nasal cavity Positive nuclei are also in ependym, single neuroblasts and many glioblasts of the thoracic spinal cord Single Ki-67 expression appears in the 9-week-old fetuses in all the studied areas of the orofacial region Focal

positivity was found in this localization in the 13th week In older studied material (13–14th week of the intrauterine life) apoptotic nuclei failed to be proved in nervous system, eye and nasal cavity Single apoptotic cells were found in the ectodermal and mesodermal parts of the 9-week-old tooth germ and in the lingual anlage Later (in the 14th week) apoptosis disappeared in this localization The other parts of the orofacial region were negative

Expression of PCNA and Ki-67 increases with the age In correlation with expression of some other proteins it gives the information about the regulation of the cell cycle during embryonic and fetal differentiation of the studied organs Results of our study can be of some value for explanation of the proliferative activity of cells and at the same time for elucidation of the origin of anomalies in the studied regions

Detection of apoptosis was performed in the same material All studied regions were areas with high cell proliferation , appearance of apoptotic cell nuclei was rare in comparison with other organs which undergo the complicated development in early developmental period (e g kidneys, limbs) Our findings testify for hypothesis that the normal human embryogenesis is under multiple level control (Le Brun *et al* 1993)

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Detection of Ischemic Changes in the Cytoplasm of Neurocytes from Rat Brain and Spinal Cord by Densitometric Measurement of Methylene Blue Binding

L Guller, J Beňuška and P Mráz

Department of Anatomy, School of Medicine, Comenius University, Bratislava, Slovakia

Abstract. Ischemic changes in neurocytes from brain and spinal cord of rats were studied by densitometric measurement of bound basic stain – methylene blue Statistically significant differences in integrated optical density (I O D) of cytoplasm near to cell nucleus in brain and spinal cord neurocytes were detected after ischemia After 10 minutes of

Correspondence address L Guller, Institute of Anatomy, School of Medicine, Comenius University, Sasinkova 2, 813 72 Bratislava, Slovakia E-mail guller@fmed.uniba sk