Changes in Apoptosis and Cell Proliferation in Human Pineal Gland during Aging

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Immunohistochemical analysis of the expression of proapoptotic protein P53 and proliferation protein Ki-67 in human pineal gland showed that in people over 60 years pinealocyte proliferation is virtually absent. Moreover, pinealocyte apoptosis is more pronounced in elderly, whereas in senile and long-living individuals its intensity decreases.

Key Words: *pineal gland; aging; apoptosis; cell proliferation*

Pineal gland (PG, epiphysis) is the main biological rhythm coordinator involved in homeostasis control via secretion of hormones regulating the functions of the pituitary gland, hypothalamus, and thymus [5,8,9]. In addition, PG participates in immune response regulation, which involves its hormone melatonin, the key biological rhythm regulator [1,4]. Multifunctionality of PG allows regarding it as one of the organs regulating neuro-immuno-endocrine interaction [4].

Uniqueness of PG lies in the fact that it undergoes minor morphological changes during aging, whereas functional involution of PG is highly pronounced and affects the functions of ageing body. Morphological changes in PG in people over 50 years include connective tissue hyperplasia, appearance of calcifications and cysts, and pronounced fibrosis [2,6]. However, the number of pinealocytes and their fine structure in old people, including long-living individuals, slightly differ from that in young people.

For evaluation of the functional state of PG, many authors used histological and electron microscopy data, which showed that pinealocyte nuclei significantly shrinks after 40 years [3,7], while the size of cells remains unchanged. This leads to reduction of nucleus/cytoplasmic ratio attesting to a decrease in PG activity with age. The absence of drastic morphological and functional changes in PG reflects preserved functional activity of PG even in old and senile individuals.

Immunohistochemical approach allowing observation of involution changes in PG at the molecular level provides new possibilities in investigation of the mechanisms of PG aging. Here we studied the expression of main signal factors of cell renewal, proapoptotic protein P53 and proliferation protein Ki-67, in PG of individuals over 60.

MATERIALS AND METHODS

PG specimens obtained during autopsy from people over 60 were divided into 3 groups according to patient age: group 1 (n=6) included old individuals (60-74 years), group 2 (n=6) senile individuals (75-90 years), and group 3 (n=5) long-livers (≥90 years).

Standard one-step protocol with high-temperature antigen retrieval in citrate buffer (pH 6.0) was used for immunohistochemical reaction with antibodies against proapoptotic protein P53 (1:50, Dako) and proliferation marker Ki-67 (1:50, Dako). Biotinylated anti-mouse immunoglobulins (Dako) from universal kit were used as secondary antibodies. Avidin complex with horseradish peroxidase was used to visualize the reaction; diaminobenzidine (ABC-kit, Dako) was used for staining development.

Morphometric evaluations were performed using the system for computer microimaging processing (microscope Nikon Eclipse E400, digital camera Nikon

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DXM1200 and software VideoTesT-Morphology 5.0). In each case, 5 fields of view were analyzed at ×400. Expression area of the studied markers was calculated as the ratio of the area occupied by immunopositive cells to the total cell area in the field of view and expressed in percents. Optical density of expression was expressed in arbitrary units.

Statistical processing of the data was performed using two-way Student's test and Spearman's rank correlation coefficient. Spearman's coefficients were used to assess correlation ratio between samples of the values of the expression of investigated markers and age values; statistically valid hypotheses on the character of the correlation between investigated samples were produced.

RESULTS

Proapoptotic marker P53 was detected in all examined PG preparations. In group 1, area of P53 expression was 1.18±0.32%, *i.e.* 3.5-fold higher than in group 2 (0.36±0.09%, $p<0.05$), and by 40 times higher than in group 3, where P53 expression area was 0.030±0.009% ($p<0.05$; Fig. 1, *a*). In addition, P53 expression area in PG in group 2 was 3 times higher than in group 3 (Fig. 1, *a*). Optical densities of P53 expression in group 1 and group 2 did not significantly differ (0.42±0.12 and 0.38±0.12 arb. units., respectively; Fig.1, *b*). Optical density of P53 expression in group 3 was 2-fold lower than in group 2 (0.18±0.04 arb. units; $p<0.05$; Fig. 1, *b*).

Correlation coefficient between P53 expression area values and patient age values was -0.95, which attested to a strong correlation between the studied parameters. The dependence between P53 expression area in PG and age can be approximated by an equation $y=0.1x^2-9x+235$ ($R^2=0.76$), where x is age, y is P53 expression area, %; R^2 is approximation reliability (Fig. 2). The obtained relationship showed that P53 expression in pinealocytes sharply decreases at the

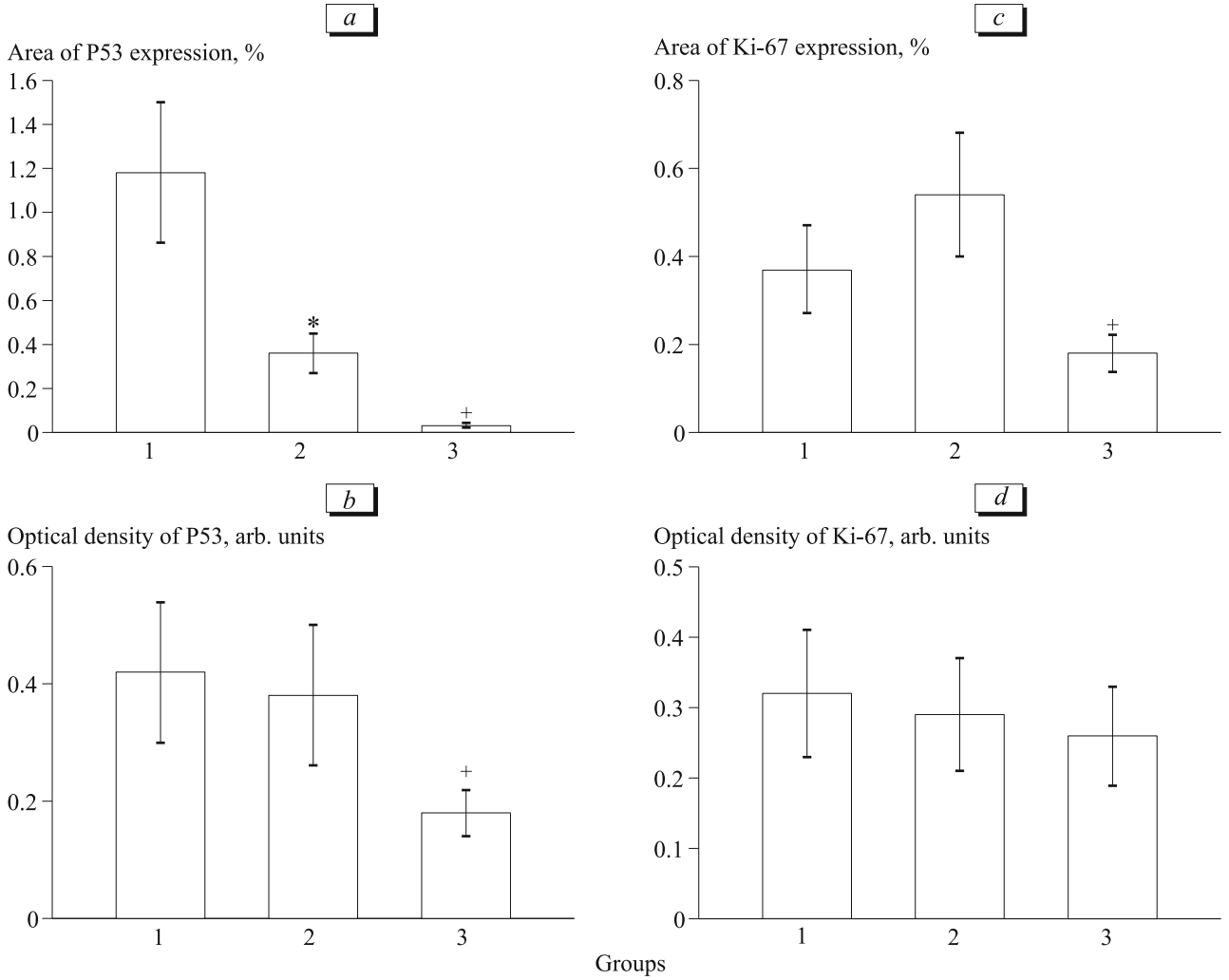

Fig. 1. Expression of proapoptotic protein P53 (*a*, *b*) and proliferation protein Ki-67 (*c*, *d*) in PG in individuals of different ages. $p<0.05$ compared to: *group 1, +group 2.

age of 60 to 70 years and after 70 this process slows down. Spearman's coefficient for optical density of PG53 expression in PG and age was -0.27, which indicates independence of changes in optical density of P53 expression on patient's age.

The obtained data indicated that the synthesis of P53 protein significantly decreases at the age of 60 to 70 years, but is not completely terminated even in long-living individuals. Apparently, reduction of its synthesis by PG cells with aging is associated with reduction of pinealocyte number. Preserved expression of P53 in long-livers shows that activity of PG is present even in individuals over 90 years, in spite of involution of the organ.

Expression of proliferation marker Ki-67 in PG was low in all age groups. Areas of Ki-67 protein expression in groups 1 and 2 did not significantly differ: 0.37±0.10 and 0.54±0.14%, respectively (Fig. 1, *c*). In group 3, the area of Ki-67 expression was 0.18±0.04%, *i.e.* 3-fold lower than in group 2 ($p<0.05$; Fig. 1, *c*). There were no significant between-group differences in optical density of Ki-67 expression (Fig. 1, *d*).

Spearman's coefficients between samples of age values and Ki-67 expression area values, and between age and Ki-67 optical density were -0.22 and -0.03, respectively, which indicates the absence of significant correlation between these parameters.

These findings suggest that proliferative activity of pinealocytes is low in people over 60 years and then did not virtually change with age. In long-livers, Ki-67 expression is weak.

Results of immunohistochemical investigation of PG indicated that P53 expression by pinealocytes dramatically decreases at the age of 60 to 70, and remains virtually unchanged after the age of 70. However, Ki-67 expression in pinealocytes of old, senile, and long-living individuals remains unchanged.

Our findings suggest that PG involution in old age is determined by intensification of pinealocyte apoptosis against the background of weakened proliferative activity. In senile age and in long-livers, cell renewal processes in PG are reduced, but not totally terminated, due to which PG performs certain regulatory function during aging.

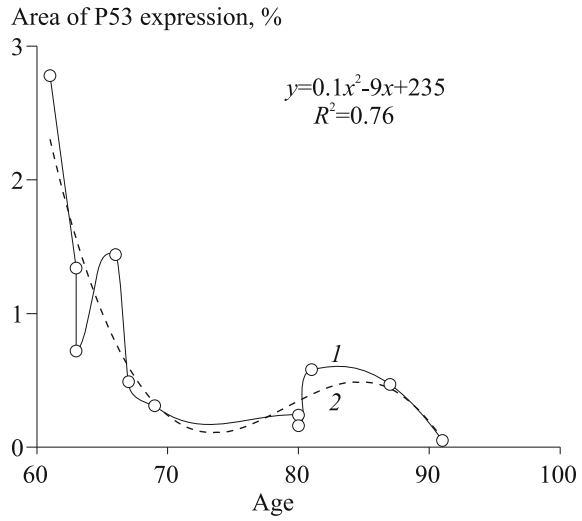

Fig. 2. Correlation between area of expression of proapoptotic protein P53 and age. *1*) experimental data, *2*) approximating function.

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