Age-Specific Morphofunctional Changes in Cambial Cells and Their Derivatives in Human Skin

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The count of cambial cells decreases with age in the total morphofunctional zone of the skin. However, their number determines the stretching of the daughter cells, which, in turn, activates the Src domain of protein SH2. Twelve cambial cells is the optimal quantity, at which the SH2 domain (responsible later for full-value stretching and looping of chromosomes near telomeres in the region of epithelial cells formation) is sufficiently active, which is characteristic of the age of 20-40 and 41-59 years. If the number of cambial cells is less than 12, stretching of the nuclei and expression of SH2 domain reduce, this leading to looping of lesser sites of chromosomes, modulating the transcription; the expression of Src and RhoA proteins is imbalanced, which leads to reduction of cell proliferation, modification of the direction of the cell's basic axis, and to hyperdesquamation. This is characteristic of the age of over 60 years. After 75 years of age the number of cambial cells in both subunits of the epidermal basal layer decreased to 7 and is close to the threshold level (6 cells), when interactions between fields of the two subunits are weak or zero. Therefore, despite the involvement of both subunits (14 cells), cell stretching and expression of the Src protein SH2 domain in them drop sharply, while activity of RhoA protein increased, which leads to looping of chromosomes closer to the centromeres, that is, to active development of fibroblasts, which is characteristic of subjects over 75 years of age.

Key Words: *skin cambial cells; skin cambial cell derivatives; age-associated aspect*

Specific changes in organs and tissues, including the skin, develop with aging. These processes are now attributed to common proliferation and differentiation processes and to specific structure of the epidermal proliferative units in humans of different age [2-4]. However, the problem of skin aging received little attention, considering the role of cambial cell (CC) functioning in the system of morphofunctional zones.

We studied the distribution of CC and their descendants in the epidermis at different age and the relationship between their functioning and expression of some enzymes.

MATERIALS AND METHODS

Skin specimens of 60 healthy men aged 20-90 years, dead from cardiovascular diseases or injuries, were studied. According to the classification of the World Health Organization, the material was distributed into 4 groups, including 15 specimens each. Group 1 included specimens from subjects aged 20-40 years, group 2 were specimens from subjects of 41-59 years, group 3 were specimens from subjects aged 60-74 years, and group 4 consisted of specimens from subjects aged over 75 years. Total planar preparations of the epidermis and skin of the abdomen (1×1 cm) were studied. Tissue fragments were plunged in Hanks' solution (pH 7.4) with gentamicin and incubated for 12 h in a thermostat at 37°C. After this treatment the epidermis was completely separated from the derma. The

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epidermis and derma were stained with iron hematoxylin after Heidenhein.

Morphometric analysis was carried out using a Video-Test-3.2 image analyzer. Cell area (S) and ellipticity degree (ED) were calculated. The content of each of the cell subpopulations was calculated from these parameters: daughter cells (S=52.4 μ^2, ED 0.61), reserve (S=43.7 μ^2, ED 0.58), elongated (S=43.0-47.4 μ^2, ED 0.26-0.42), oval (S=51.7-89.0 μ^2, ED 0.62-0.78). The synthetic period of CC is very short [9], which impedes estimation of their number by automated morphometry, and hence, the functional activity of CC was evaluated by the number of daughter cells, whose quantity was equal to that of CC. A total of 2000 cells were studied in each case. In addition, the number of CC per 120 cells was evaluated, which corresponded to their content in a subunit of a functional zone. Since cell distribution by DE is identical in mouse and human skin epidermis [8], we compared the specific features of cell distribution in humans of different age and in animals in order to evaluate the degree of proliferation, as the maximum and minimum proliferative activity could be evaluated only experimentally.

RESULTS

We previously found [6,8] that reserve cells, forming a sort of a stock (25-30%) spent for physiological regeneration of the layer (Fig. 1), constitute the greatest portion of the epithelial population. Activation of these cells leads to reduction of their percentage to 6-8%, because they are gradually transformed into elongated cells, of which 5% divide. The count of reserve cells, in turn, is replenished at the expense of proliferation of CC functioning in the morphofunctional zones,

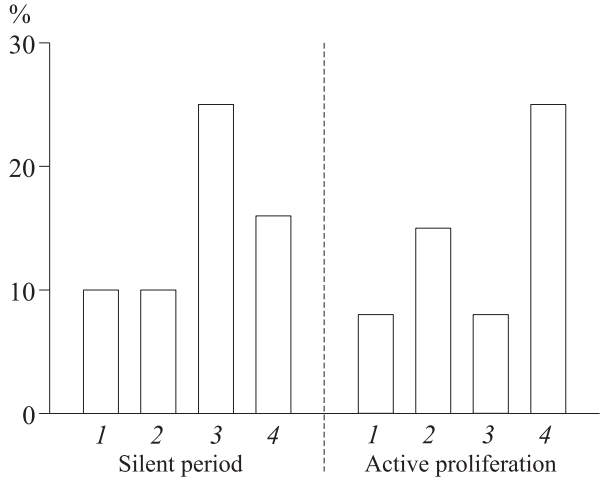

Fig. 1. Distribution of mouse epidermal cells by their shape in low and high proliferation. Here and in Fig. 2: 1) daughter cells; 2) oval cells; 3) reserve cells; 4) elongated cells. Abscissa: cell DE.

each consisting of two subunits (with 12 CC). CC of subunit 1 are the first to divide and form 12 pairs of maternal and daughter cells creating an electric field, in which the daughter cells differentiate. A similar process takes place in subunit 2. When the summary number of daughter cells in both subunits of the zone reaches 24, 12 of them (which have formed earlier) and then the remaining 12 start transforming into oval and then into reserve cells.

High proliferative activity in the epidermis was retained in groups 1 and 2, which was seen from reduction of the share of reserve cells to 11.6-11.9% and an increase in the percentage of their derivatives (elongated cells) to 22.6% (30 and 16% at rest, respectively), which directly started mitosis. The percentage of daughter cells decreased to 7.7% (10% at rest) at the expense of their gradual transformation into oval cells, which were then transferred into reserve. The increase of the share of oval cells from 10 to 15% in these groups indicated that these cells were going to replenish the reserve pool, and another subunit was to start working (Figs. 1, 2).

The percentage of reserve cells in group 3 decreased to 10.3% in comparison with groups 1 and 2 (11.6 and 11.9%). The percentage of elongated cells remained high (22.9%), similarly as in groups 1 and 2. However, the percentage of daughter cells dropped to 7.3% in comparison with groups 1 and 2 (7.7%). That is why the reserve cell pool in humans aged over 60 is reduced not because of intensification of transition of these cells into elongated ones, but because of a decrease in the number of CC, which was seen from a lesser count of daughter cells (indicator of their activity). At this age the two subunits function in turn, because reduced count of CC can still create a sufficient pool of reserve cells, which is seen from a sufficiently high content of oval cells (15%).

In group 4, the percentage of reserve cells increased to 11.6% in comparison with group 3 (10.3%). The percentage of daughter cells also increased in comparison with group 3 (8.2 *vs.* 7.3%). In addition, an increase in the percentage of oval cells in group 4 to 17.4% (maximum elevation to 15%) indicates the involvement of subunit 2 simultaneously with subunit 1 for creation of the necessary depot of reserve cells for providing the physiological regeneration of the population. Hence, at the age of 75 years and more the percentage of CC and the proliferative activity reduce still more, which is seen from a reduction of elongated cell percentage to 20.7% (22.6% in the rest groups).

Hence, the count of CC decreases with age, and therefore, the number of daughter cells in group 3 is 1.1 times lower than in groups 1 and 2. Further reduction of CC percentage in group 4 led to simultaneous proliferation of the two CC subunits, and therefore

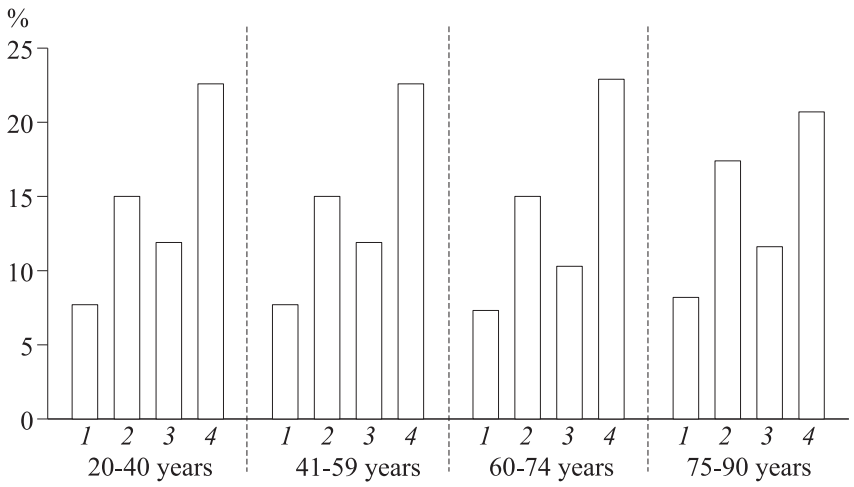

Fig. 2. Distribution of human epidermal cells by their shape during active proliferation at different age.

the percentage of daughter cells increased 1.2 times in comparison with group 3. Hence, 12 CC proliferate simultaneously in the epidermal zone at the age of 20-40 and 41-59 years, about 11 CC at 60 and older, while after 75 years of age 14 CC proliferate in the epidermal zone.

We previously showed that the derma, located on the opposite side of the basal membrane, contains the same number of CC as the epidermis, the epidermis and derma exhibiting opposite effects towards each other [6,7]. The growth factors of 12 epidermal daughter cells have a spastic effect on the cells sensitive to them because of RhoA activation (RhoA is a minor G protein regulating the actin cytoskeleton), while the factors of 12 dermal CC are characterized by a relaxing effect, because they activate Src kinase in dermal cells, which inactivates RhoA in them. The Src is one of the main regulators of cell proliferation and differentiation. It has a catalytic and two other domains (SH2 and SH3) at the expense of which it can be activated. Only after inactivation of RhoA the epidermal daughter cells can be stretched by the electric field force. But before stretching, at the end of mitosis, the chromosomes have an orderly orientation, as they are pooled away by means of microtubules attached to the centromeres (anatelophase orientation) [5]; that is why the centromeres in daughter cells with a vertical division axis are located in the upper poles of the cells, while the telomeres are in the lower poles; the location in the maternal cells is the opposite. Hence, the main axis of CC and the daughter cell coincide with the orientation of chromosomes.

Electric field of the needed force sufficient for stretching the daughter cells and their nuclei is generated in each subunit of 12 CC at the age of 20-40 and 41-59 years. The stretching activates in them the Src SH2 domain, which initiates the formation of microtubules and intermediate filaments and stress fibrils, which rotate the lower pole of the daughter cell nuclei and stretch them parallel to the basal membrane. Hence, telomeres fixed to the nuclear membrane and the centrosome also rotate from vertical into horizontal direction and shift towards the leading edge of the epidermal cell. Chromosomes which have certain binding sites to the nucleus periphery and a package in the form of loopy rosette-like structures [1,5] will stretch near the telomeres, which will lead to untwisting of the loops and chromatin decondensation in these sites and will thus create the probability of its transcription. In addition, due to the direction of the main axis of the daughter cells, parallel to the basal membrane at the age of 20-40 and 41-59 years, the cells gradually mature in the basal layer till their transition to the upper layers.

At the age of 60 the daughter cells are exposed to electric field of a weaker force than at 20-40 or 41-59 years, which is generated by just about 11 (but not 12) pairs of maternal and daughter cells. This leads to a lesser stretching of daughter cells and their nuclei and a drop of the Src SH2 domain expression in them. As a result, the formation of stress fibrils, microtubules, and intermediate filaments decreases, while activity of RhoA and cell contraction increases. Hence, the chromosome stretching near the telomeres will grow weaker, which will lead to incomplete untwisting of the loops and looping of chromosomes in these sites and modification of protein transcription. In addition, the main axis of the daughter cell will be positioned under a certain axis to the basal membrane because of imbalance between Src and RhoA, which will lead to rapid desquamation of cells and hence, the skin will loose layers and become thinner. Reduction of epidermis cells will eventually lead to a drop in the production of epidermal growth factor and to a reduction of total expression of RhoA in the skin. We previously showed that RhoA promotes elimination of proliferation inhibitors from the cells and to DNA synthesis [6]. Hence, with time the reduction of its activity in

the total morphofunctional zone leads to a drop of proliferation of CC and their derivatives.

After the age of 75 years, the count of CC in one of subunits drops to 7 because of their death and is close to the threshold level (6 cells), when interactions between the fields of two subunits in the zone are the minimum or zero. Therefore, despite simultaneous work of two subunits, stretching of the epidermal daughter cells and the expression of the Src protein SH2 domain in them reduces even more than in humans aged over 60 years, which stimulates the expression of RhoA in comparison with Src in these cells. This leads to a lesser formation of stress fibrils, microtubules, and eventually stretches the daughter cell more vertically, i.e. closer to the centromeres. However, the chromosomes in the fibroblast-like cells are looping in these regions [6]. Hence, fibroblasts are actively forming instead of epitheliocytes after 75 years of age. This reduces the percentage of parenchymatous cells, production of epidermal growth factor, and expression of RhoA in the layer, which still more reduces the proliferation of CC and their descendants.

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