MORPHOLOGY AND PATHOMORPHOLOGY

Structural Organization of the Basal Layer and Morphofunctional Characteristics of Mouse Epidermal Cambial Cells

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The basal layer of mouse skin epidermis consists of special structures - rosettes functionally arranged in zones including about 20 rosettes. The rosette contains a central cambial cell; after division of this cell the mother cell remains in the center of the rosette and the daughter cell migrated to the center of a free rosette. The mother and daughter cells are dipole cells (sources of electric field). If the disposition of mother cells is unidirectional and they predominate over daughter cells, the latter cells transform into other cells, *i. e.* are differentiated.

Key Words: *cambial cells; functional zones; dipole cells*

Abnormal organization of epithelial tissue leads to various pathological stated [6,7,9]. Both mono- and multilayer epithelium consists of elementary morphofunctional units - epidermal proliferative units (EPU). Indirect data suggest that the stem cell is located in the center of EPU basal layer (8,12). Maturing cells formed after division of the stem cell migrate from the center to the periphery of EPU and pass 2-3 mitotic divisions during this migration, *i. e.* cells at the periphery of EPU are mainly proliferating. Hence, these tissue units are self-renewing. However, there is a hypothesis that the epidermis is compartmentalized only in its differentiated part, while the basal layer is common for the entire layer [11]. A close relationship between the basal parts of the neighboring EPU was later detected [8]. Based on these data, we studied morphology and function of central cells of EPU and the relationship between the basal parts of EPU during proliferation.

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MATERIALS AND METHODS

Cambial elements of the skin epidermal basal layer were stimulated with growth factor neupogen (Hoffman La Roche). The study was carried out on 8 intact male Balb/c mice (20 g). Experimental animals (n=5) received a single subcutaneous injection of neupogen in a dose of 120 μg/kg, corresponding to 10 μg/kg for humans. Neupogen was diluted with 5% glucose solution and subcutaneously injected to animals at 10.00. Controls (n=3) received no neupogen.

Ear tissue samples from experimental and control animals were collected daily for 5 days after neupogen injection. Tissue fragments were placed into Hanks' solution (pH 7.4) with gentamicin and incubated at 37°C for 12 h. After this treatment the epidermis was completely separated from the derma. One portion of total epidermal preparation was stained with hematoxylin after Heidenhain.

For investigation of cell behavior in electric field (EF) the other portion of the epidermis was put into methylene blue solution in normal saline (1:10,000)

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incubated for 10 min at 37°C. After incubation wet preparations were transferred onto slides and covered with coverslips. Two parallel electrodes were placed at the edges of the slide at a distance of 0.5 cm from the coverslip (distance between the electrodes was 2 cm) and constant voltage (300 V) was applied for 2 min, after which the image was recorded. Then the poles of electrodes were altered and the exposure was repeated (2 min). Cell image was recorded using a Video-Test-3.2 image analyzer.

RESULTS

The study showed that the basal layer of mouse ear skin consists of rosette-like formations with 6-7 cells at the periphery and 3-4 cells in the center. Other authors observed a vertical column of cells above each rosette, this column consisting of one pricky cell, 2 granular cells, and a bar of 6-12 completely hornified cells. These structures were called EPU [4-6,8,15]

Small round or oval cells with large round dark-stained nuclei surrounded by a narrow cytoplasmic rim were detected in all experimental mice on days 2 and 3 after neupogen injection (Fig. 1). Each cell occupied the center of a rosette. These cells were seen in some rosettes (about 20) and were absent in others (also about 20), that is, alternating zones with and without central dark cells were observed over the entire surface of the sample. All cells within each zone were in the same phase of the mitotic cycle (mainly in the telophase). Interestingly, that mitosis of central cells was characterized by certain specific features: two cells formed as a result of division did not part, but closely adhered to each other (Fig. 1, *b*). One of these cells was located closer to the basal membrane, the other at some distance from it.

Central dark cells were rarely seen on days 4 and 5 of the experiment and during the entire period of observation in the control (0-1 per visual field).

Analysis of changes in the central cells in each zone showed a dynamic picture of their functioning.

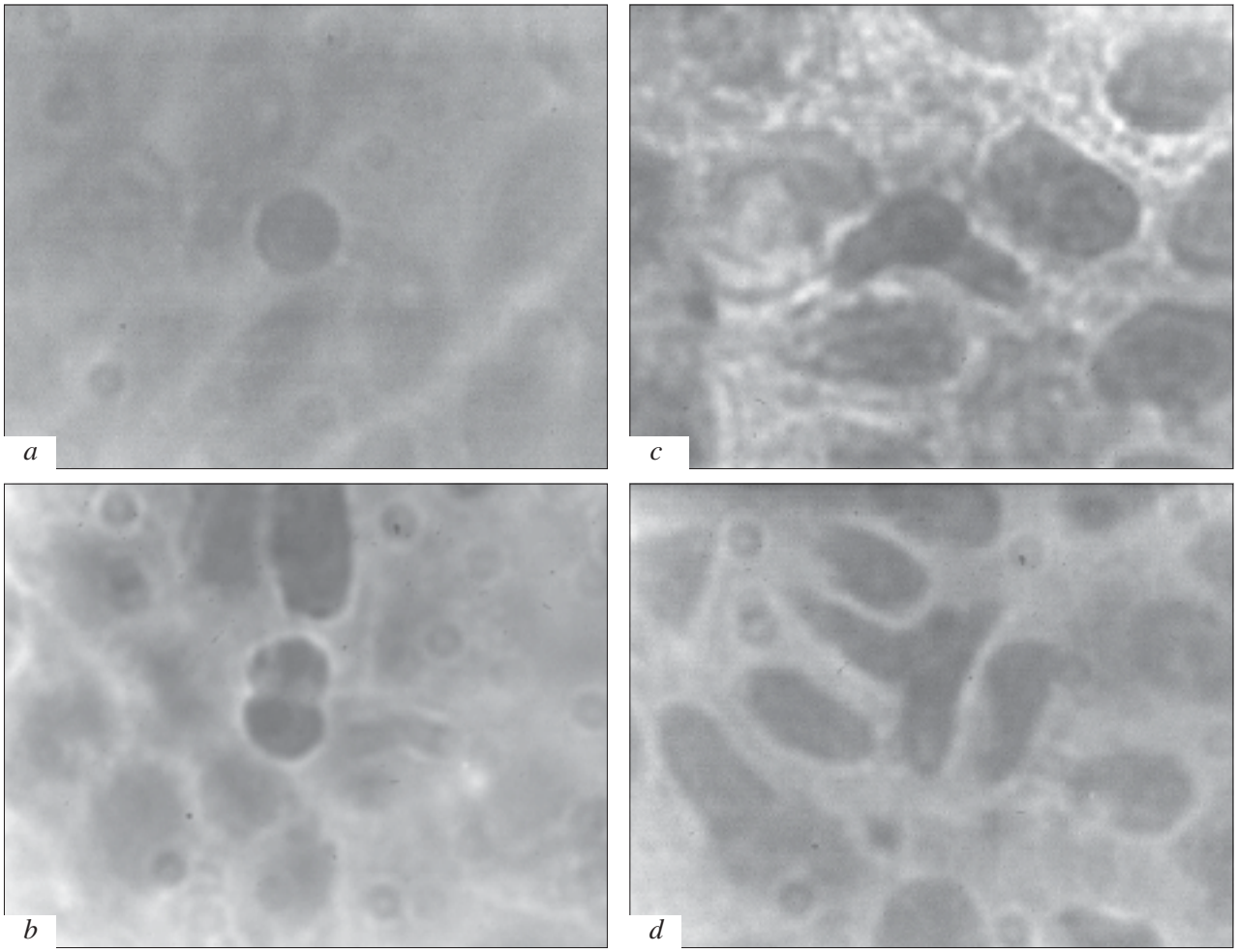

Fig. 1. Morphological changes in the rosette central cells in the course of their functional activity. Ferric hematoxylin staining after Heidenhain, ×900. *a*) central cell nucleus is round before division; *b*) cells resultant from division of the central cells are not separated but remain in close contact with each other; *c*) one of the cells, more distant from the basal membrane, gradually stretches acquiring the elongated ellipsoid shape; *d*) after both daughter cells were elongated, they part.

Two cells formed as a result of central cell division gradually changed their shape. The cell more distant from the basal membrane changed first, acquiring the shape of a elongated ellipse (Fig. 1, *c*). This cell had a large clear nucleus with large dark chromatin lumps. After the first cell was elongated, the other cell gradually stretched along the surface of the first cell. This cell had a dark nucleus with finely dispersed chromatin; the second cell was situated in the immediate proximity from the basal membrane and was connected to it by 1-2 hyperchromatic compact processes. Later, as the second cell elongated and reached the size of the first cell, it forced out the first cell (situated above it) from the rosette center, occupying its place in the center of the rosette (Fig. 1, *d*). The first cell migrated to the center of another rosette not occupied with such cells. As a result, the centers of about 20 rosettes were occupied, forming functional zones. It seems that these zones are formed at the expense of cambial cells of the same type. Study of embryogenesis of free nematodes revealed that cells originating from a certain type of stem cells possessed a synchronous rhythm of cell division [10].

Hence, rosettes in the skin epidermal basal layer are arranged in functional zones. The presence of these zones containing a stable number of rosettes indicates that the number of cambial cells, and hence, the total number of cells, is strictly regulated in these zones. Strict regulation of the number of stem cells is vitally important [9,12], because each extra stem cell can produce 60-120 extra cells in the crypt.

The appearance of central dark cells in the epidermal basal layer after stimulation of proliferation, their small size, position strictly in the center of the rosette, specific features of their mitosis, and the fact that only one of two cells tightly bound to the basal membrane remains in the focus of cell multiplication suggest that these cells are cambial cells of the epidermal basal layer. The fact that stem cell is situated in the center of EPU is confirmed by other scientists [12,15].

Our previous studies by automated quantitative morphometry confirmed that division of the central rosette cell results first in the appearance of two round cells tightly adhering to each other (0.93 ellipticity) [7]. These cells were tentatively called shape I cells, one of these cells was denoted as the mother cell and the other as daughter cell. After elongation of these cells and their separation, shape II mother and daughter cells with 0.61 ellipticity formed, *i. e.* shape I cells transformed into shape II cells. As mother cells are oriented mainly similarly within the same zone, let us introduce the notion of the basal direction charac-

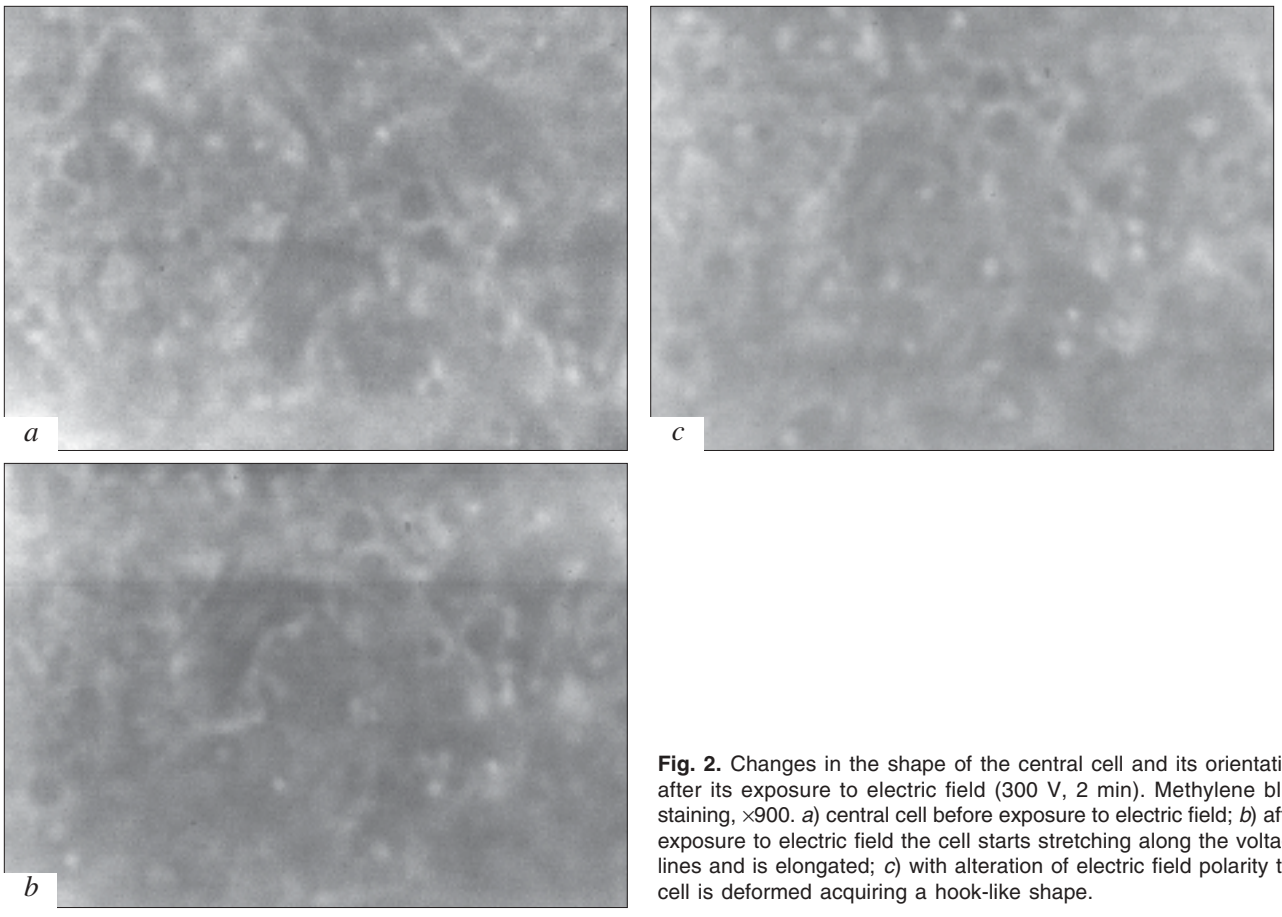

Fig. 2. Changes in the shape of the central cell and its orientation after its exposure to electric field (300 V, 2 min). Methylene blue staining, ×900. *a*) central cell before exposure to electric field; *b*) after exposure to electric field the cell starts stretching along the voltage lines and is elongated; *c*) with alteration of electric field polarity the cell is deformed acquiring a hook-like shape.

terizing orientation of the mother cells. It was shown that the orientation of the daughter cells of shapes I and II was perpendicular to the basal direction of mother cells (evaluated by the angles between the greater axes of ellipses). When mother cells start predominating over daughter cells during transition of shape I cells into shape II ones, the daughter cells are transformed into cells of other forms (differentiated). Hence, differentiation of daughter cells starts at the moment of predominance of universally oriented mother cells over daughter cells, their orientation being mutually perpendicular. It is known that integration of similarly oriented electrically polarized epithelial cells can be a source of endogenous electric field (EF) [2,13]. Presumably, mother and daughter cells are sources of EF or dipole cells. In order to confirm this hypothesis, elongated cells in the rosette center (Fig. 2, *a*) were exposed to EF. In some cases 2-min exposure to EF led to cell stretching along EF voltage lines, and the cell was elongated, with one end directed towards the cathode and the other turning towards the anode (Fig. 2, *b*). After alteration of EF polarity the cell was deformed, acquired a hook-like shape, and shrank in size (Fig. 2, *c*). In other cases exposure of the cell to EF at first resulted in its deformation and shrinking, while alteration of polarity led to cell elongation and direction of its ends along the EF voltage line. Hence, in the former case the cell polarity at its terminals did not coincide with EF polarity, which led to attraction of opposite poles of the cell and field and the resultant stretching of the cell along the EF voltage lines. If EF polarity was altered, *i. e.* the polarity of the cell and EF coincided, this led to repulsion of similarly charged poles of the cell and field, which resulted in cell shrinkage and deformation. Hence, the charges were redistributed in these cells before exposure to EF (depolarization and hyperpolarization of cell membrane at opposite ends of the cell). Therefore, such cells possess the characteristics of dipole cells.

The shape and position of other neighboring cells during exposure to EF and polarity alteration were unchanged. 10 min after exposure to EF was over the central cells resumed their initial position.

If the number of mother and daughter cells at the moment of differentiation were equal, the fields generated by two types of these cells would have been compensated [2]. According to our data, predominance of mother cells over daughter cells (2:1) permits the mother cells generate an EF. The central cambial cells of each zone were in the same phase of the mitotic cycle, which can indicate a positive correlation of their electrical activity. Other authors also noted the significance of positive correlation in electrical activity of cells for the generation of endogenous electricity [2, 13,14]. Moreover, the daughter cells are differentiated only during transition from shape I into shape II (during elongation), and hence, the cell shape is essential for this process. It is known that cell shape is significant for stimulated cells. In many cases the shape of these cells and fibers is elongated [1]. This geometrical structure is characterized by axial symmetry with respect to the longitudinal axis, which permits the creation of unidirectional laminae. In addition, the degree of cell elongation is essential for their dipole quality and hence, EF generated by them [3].

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