Interaction of Cambial Dermal Cells (Fibroblasts) and Epidermis in Morphofunctional Area of Mouse Skin

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Epidermis and dermis form an integral morphofunctional area, where cambial cells proliferate and their first-division daughter cells differentiate. An important feature of this area is different rates of the development of daughter cells (fibroblasts) in the dermis and epidermis, which is greater in the epidermis. This asymmetry results in the prevalence of first epidermal daughter cells and, hence, their effect on cambial cells, and then of stromal daughter cells and their effects on cambial cells. The regulator factors of epidermal daughter cells promote unblocking of the major polarity axis of cambial (mother) cell, while stromal cells (fibroblasts) induce their polarization along the major axis and the onset of mitosis. In the dermis and epidermis, division of cambial cells is asymmetric; a prominent role in the formation of mother and daughter cells is given to the basal membrane as an elastic support. Mother and daughter cells form ring-like structures generating electric field that can promote differentiation of the daughter cells.

Key Words: *fibroblast cambial cells; unified epidermal-dermal morphofunctional area*

Proliferation and differentiation of stem cells are controlled by their specific microenvironment or "niche". In mature human or animal organisms, the stem cell niches are available in many tissues (hemopoietic, nervous, cutaneous, *etc*.). They consist of descendants of epithelial stem cells and connective tissue cells [13,15].

In the niche, there are close relationships between the epithelial and stromal elements. The subepithelial connective tissue promotes proliferation and histodifferentiation of the epithelium, while epithelial tissue in its turn initiates proliferative activity of fibroblasts [7,14].

Niche cells control proliferation and differentiation of stem cells via secretion of growth factors, which interacts with their complimentary specific receptors located on the cell surface. As a result, new regulator signals are activated that trigger DNA replication and initiate mitosis [2]. However, in many cases the role of niche is viewed only as a function of its environment cells, while its performance as an orchestrated regulatory system is shadowed. Recently, new data showed that division of stem cells in the niche is asymmetric. For example, the division axis of sex cells in drosophila is directed perpendicularly to the somatic cells. Such an orientation of maturation spindle leads to a loss of connection between a descendant cell and the niche resulting in its future differentiation [8,15]. In this process, the basal membrane (BM) participates in cell adhesion and in conduction of the signals to the stem cells. However, the important role of BM as an elastic support in the process of formation of two descendant cells during the division of a stem progenitor cell was little studied.

Our aim was to study the formation and function of the niche as the regulator system for stem cells and to reveal the character of division and possible differentiation of stem cells within the niche.

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MATERIALS AND METHODS

Experiments were carried out on male CBA mice weighing 20 g (n=60). Specimens of ear tissues were taken every 3 h over 24 h. In the period of maximal proliferative activity (3:00 to 9:00), the specimens were taken every hour (n=5 for every time point). The total film preparations of ear derma and epidermis were examined. The tissue specimens were placed into Hank's solution (pH 7.4) with gentamicin and incubated at 37°C. After this procedure, epidermis completely detached from the derma. The specimens were divided into two parts. The first parts of epidermal and dermal preparations were stained with Heidenhain iron-hematoxylin.

The morphometric analysis was carried out on a Video-Test-3.2 video analyzer. The cell area S and ellipticity factor (EF) were calculated and used to determine the content of daughter (S=52.4 μ^2, EF=0.61) and the reserve cells (S=43.7 μ^2, EF=0.58) for n=240 cells, which corresponds to cell content in one morphofunctional zone [3].

To observe cell activity in the electric field, the second parts of dermal and epidermal preparations were placed into weak solution of methylene blue (1:10,000 in physiological saline) for 10 min at 37°C. Wet specimens were placed onto a slide and coverslipped. Two electrodes were mounted at the edges of the slide at the distance of 2 cm. The specimens were stimulated with a voltage pulse of 300 V applied for 2 min. Then stimulation was repeated with the alternative polarity. The images were written with a Video-Test-3.2 video analyzer.

RESULTS

Epithelium is constructed of epidermal-proliferative units (rosettes) with a cambial cell (CC) in their centers surrounded by about 10 peripheral cells [10]. The rosettes are integrated within the morphofunctional clusters subdivided into 2 subunits each housing 12 rosettes [3,4]. A subunit maintains necessary number of daughter cells (no less than 12) resulting from the first division of CC. These daughter cells differentiate, divide, and gradually transform themselves into reserve cells (30%) that provide physiological regeneration in the morphofunctional cluster. In the evening (15:00 to 21:00), the percent of reserve (non-activated) cells in mouse epidermis remains high (25-30%). The increase in mitotic activity in the period from 3:00 to 9:00 is triggered by activation of reserve cells (their percent decreased to 6-8%) and CC of the rosettes, which attests to up-regulation of proliferative processes in epidermis [4]. Then, our study focused on the behavior of epidermal CC and dermal keloidal layer during these nocturnal hours. In the derma, the groups of small cells were revealed in close proximity to and under the clusters of epidermal CC and BM, which initiated mitosis simultaneously with CC. These cells had hyperchromatic nucleus and poorly stained cytoplasm with projections like those of the fibroblasts; they transform through the stages analogous to those of epidermal CC (Fig. 1, a) [5]. Two daughter cells of a small cell are located one under another. Thus, the division axis of these cells is directed normally to BM, so only one daughter cell faces BM. The opposite-directed traction forces disintegrating both daughter cells during cytokinesis [9,12] result in invagination of the equatorial region into the body in each cell. Therefore, the density of chromatin elevates pronouncedly, and the nuclei become intensively dark stained. As an elastic support, BM promotes flatting of the contacting cell, which assumes a canoe-like shape and remains in the reproduction focus as the mother cell. Due to the traction force exerted by the cell-cell junction that remained at the outer periphery on the one hand, and the absence of a mechanical restriction from BM, on the other hand, the daughter cell turns through 90° relative to mother cell thereby blocking mechanically the polarization of mother cell along its major axis. During this process, the daughter cell polarizes itself in parallel to BM, because the turn radically changes the direction of its major axis (Fig. 1, b). While remaining in close contact with each other, the mother and daughter cells form a circular structure (Fig. 1, c). Such structures were more frequently met in the derma than in the epidermis, which means that this stage of CC transformation is shorter in epidermis than in derma. As a result, the epidermal daughter cells finish the process of disconnection from the mother cells and leave the circular structure while similar dermal cells still continue it. Really, the rate of proliferation of epidermal cells is far greater than that in the underlying stroma [6].

Thus, morphological peculiarities of the small dark dermal cells, their appearance in the hours of most high activity of epidermal CC and their synchronous (with CC) mitosis and other identical transformation stages (with BM-bound mother cell left in the reproduction focus) indicate that these cells can be CC of the fibroblasts in the dermal keloidal layer, and they work with the epidermal cells in the integral epidermodermal morphofunctional area.

The experiments showed that daughter cells in this area directly affect proliferation of CC after disconnection from mother cells. However, the epidermal daughter cells exert their effects before the

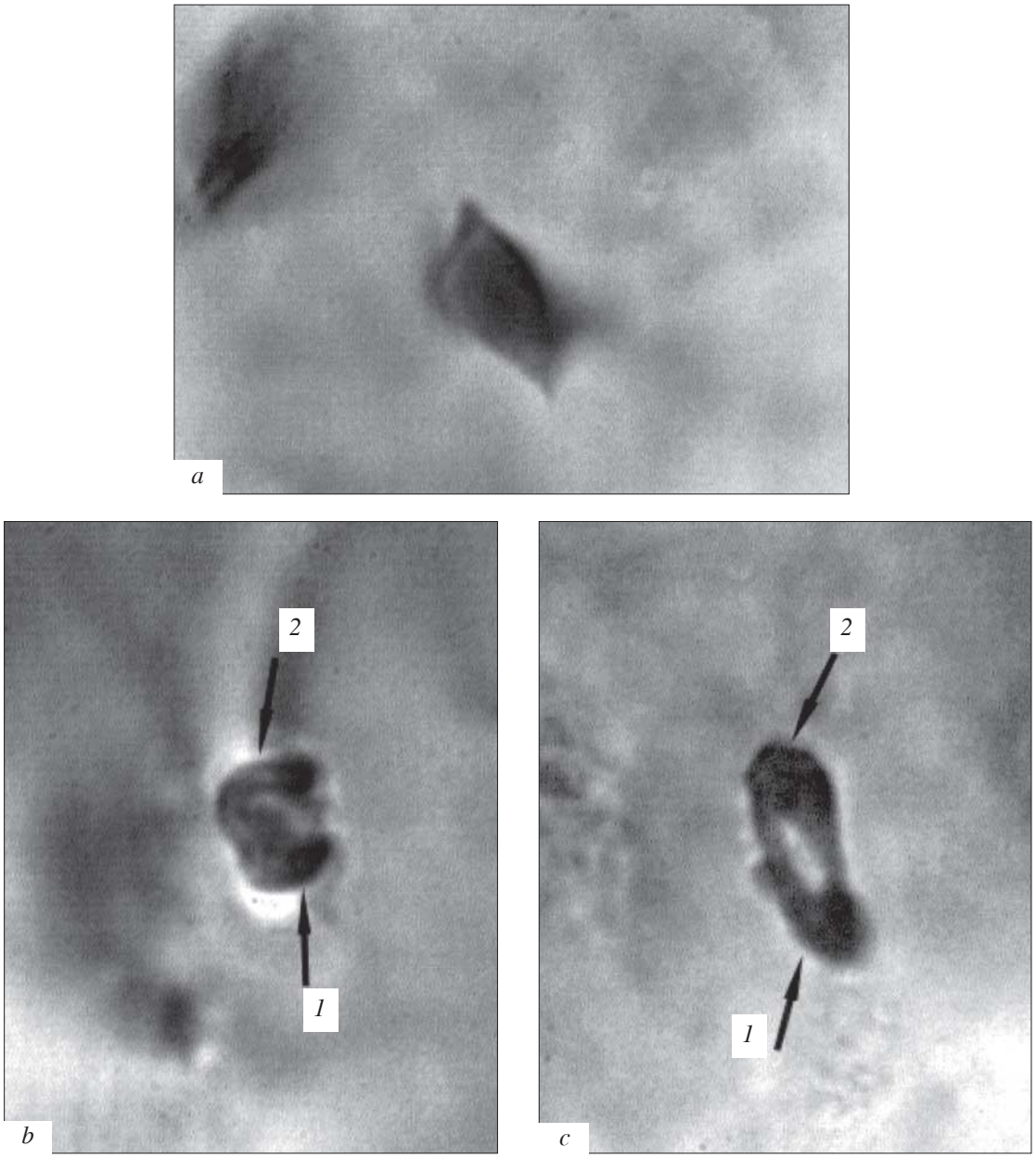

Fig. 1. Some stages of transformations of CC fibroblasts during cell division. Staining with hematoxylin, ×1000. *a*) large hyperchromic nucleus in CC and poorly stained cytoplasm with short projections; *b*) daughter cell (*2*), which polarizes in parallel to BM and gradually approaches to mother (*1*) cell; c) mother (*1*) and daughter (*2*) cells form the circular structure.

stromal cells (fibroblasts) due to their earlier formation. Really, as the number of daughter cells in the epidermodermal morphofunctional area levels 12, and they start to prevail over the stromal cells (since the most part of the daughter stromal cells are still situated within the circular structures), the epidermal and dermal mother cells change their shape from the canoe-like to a round one, which is characteristic of CC (Fig. 2, *a*, *b*). Thus, polarization of these cells along different axes and removal of block from its major axis took place due to the predominant effect of the regulator factors produced by the epidermal daughter cells. In the following, the number of stromal daughter cells equals with that in epidermis (12). This results in a balance of the regulator factors produced by the daughter cells in derma and epidermis leading to termination of the effect of epidermal daughter cells (Fig. 2, *b*). Then the epidermal daughter cells gradually transform themselves into the reserve cells, while the number of stromal daughter cells remains the same due to a lag in their formation in the derma relative to epidermis (Fig. 2, *b*). It leads to predominance of the stromal daughter cell in the common epider-

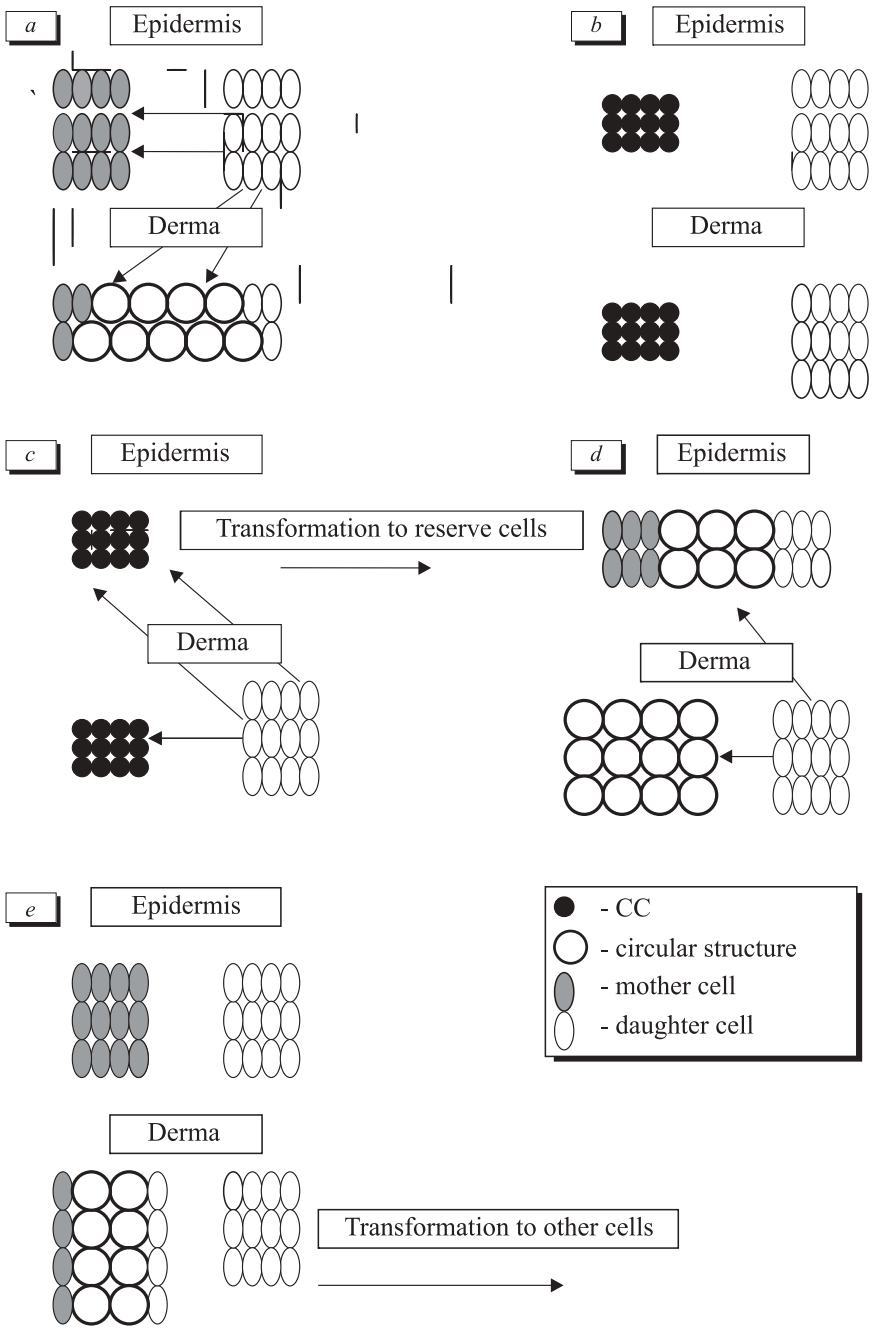

Fig. 2. Effect of epidermal and stromal (fibroblasts) daughter cells on proliferation of CC in the integral epidermodermal morphofunctional area during its function (one subunit). *a*) predominance of epidermal daughter cells in this area and their effect on mother cells in epidermis and derma; *b*) transformation of epidermal and dermal mother cells into CC leading to balance in the numbers of stromal and epidermal daughter cells and to termination of the effect of the latter on CC; *c*) predominance of stromal daughter cells and their effect on CC in epidermis and derma; *d*) simultaneous mitosis of epidermal and dermal CC due to predominance stromal daughter cells, but the formation of circular structures needs more time in the derma than in the epidermis; *e*) counterbalance in the numbers of epidermal and stromal daughter cell and termination of the effect of stromal daughter cells on CC.

modermal morphofunctional area and prevailing of their effect on CC in derma and epidermis, which start to elongate along the major axis normally to BM and finally initiate the mitosis (Fig. 2, *d*). Again, 12 epidermal daughter cells are formed, so the numbers of epidermal and dermal daughter cells are counterbalanced. Now the effect of stromal daughter cells on CC terminates (Fig. 2, *d*) arresting CC division.

When CC are transformed into mother cells, in which polarization along the major axis is blocked, they become protected from the action of proli-

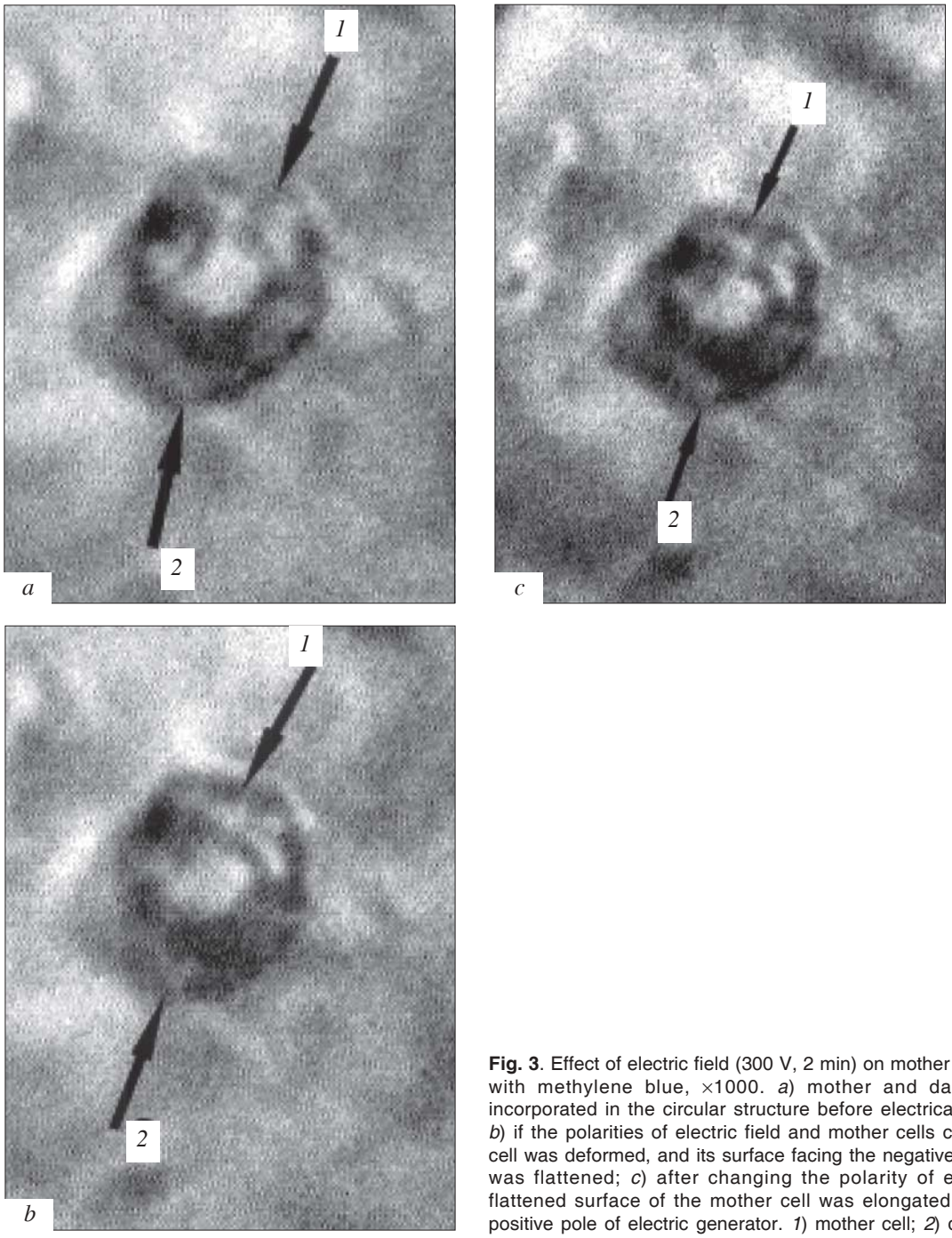

Fig. 3. Effect of electric field (300 V, 2 min) on mother cell. Staining with methylene blue, ×1000. *a*) mother and daughter cells incorporated in the circular structure before electrical stimulation; *b*) if the polarities of electric field and mother cells coincided, the cell was deformed, and its surface facing the negative electric pole was flattened; *c*) after changing the polarity of electric field, flattened surface of the mother cell was elongated towards the positive pole of electric generator. *1*) mother cell; *2*) daughter cell.

ferative stimuli. At this stage, they exert a field-like effect on the daughter cells. After invagination and polarization block, the mother cells assume the canoe-like shape with a single elongated end, so the density of chromatin carrying the negative charge [14] dramatically rises in the "head" part of the cell in comparison with chromatin density in its "tail" part or in daughter cells. This phenomenon leads to re-distribution of electric charges between the mother and daughter cells in the circular structure, which thereby could become a source of electric field. This hypothesis is supported by detection of the circular structures in the center of epidermal and dermal rosettes (Fig. 3, *a*). The changes in circular structures are indicated by deformation of mother cells only, because the daughter cells are situated in other plane preventing their analysis. When polarity of the "head" part of mother cell coincided with direction of the external electrical field applied for 2 min, the surface of mother cell facing the negative pole was flattened (Fig. 3, *b*). When the direction of the electric field was inver-

ted, the flattened surface of mother cell resumed its elongation towards the positive pole (Fig. 3, *c*).

Identical orientation of the circular structures is an important factor for positive correlation of electrical activity of the cells and generation of endogenous electricity [11]. After disintegration of these structures, the mother cells were chaotically distributed, while the daughter cells transformed themselves into the cells of other forms. Thus, electric field affects differentiation of the daughter cells only at the stage of circular structures.

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