Relationship of Epidermal Melanocytes and Langerhans Cells with Epidermal Cambial Cells

T. M. Yavisheva, S. D. Shcherbakov, I. S. Golubeva, L. A. Savluchinskaya, and N. I. Ryzhova

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Studies of mouse ear epidermis showed that proliferative activity of basal epidermal cells has two active and two passive phases throughout 24 h. Active phases consist of two subphases: long (proliferation of cambial cell descendants) and very short (cambial cell proliferation). Cambial cells proliferate at the boundary between active and passive phases; this results in an increase in the counts of epidermal melanocytes and Langerhans cells resultant from division of epidermal cambial cells. The count of Langerhans cells almost 2-fold surpasses melanocyte count, because melanocytes gradually transform into epidermal basal cells.

Key Words: *cambial cells; melanocytes; Langerhans cells; epidermis*

Structural organization of epithelial tissue attracts special attention, as structurization disorders can lead to disease. The epidermis consists of epidermal proliferative units, each with a stem cell in the center. On the other hand, there are also Langerhans cells in the centers of these units. The main function of a Langerhans cell is realization of the skin immune response and regulation of mitotic activity of keratinocytes [1,8]. In addition to the epidermal proliferative units, an epidermal melanocytic unit functions in the epidermis: one melanocyte controls 36 basal cells [5]. The count of corneocytes is proportional to the sum of Langerhanscells, melanocytes, and keratinocytes. Hence, these cells are united by not only functional relationships, but are related.

We studied the relationships between epidermal melanocytes, Langerhans cells, and cambial cells (ECC).

MATERIALS AND METHODS

The study was carried out on 60 adult male CBA mice (20 g). Specimens of ear tissue (5 per time point) were collected in animals every 2 h throughout 24 h.

N. N. Blokhin Russian Oncological Research Center, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** javisheva@rambler.ru. T. M. Yavisheva

Total planar preparations of the ear skin epidermis were studied. Tissue fragments were plunged in EDTA buffer solution and incubated during 4 h at 37°C. After this treatment the epidermis was completely separated from the derma. One portion of the epidermis preparation was processed for detection of melanocytes as follows. Incubation solution (0.3% DOPA+phosphate buffer) was prepared and epidermal membranes were incubated in it for 2 h at 37°C. The epidermis was poststained with ferric hematoxylin after Heidenhein. Brown-black granules showed the location of tyrosinase. Other epidermal membranes were used for detection of Langerhans cells by ecto-ATPase activity. The membranes were fixed in 5% neutral formalin (20 min, 4°C) and plunged in 1.32×10^{-3} M ATP incubation solution (1 h, 37°C), then in 1% ammonium sulfide (1 min), after which black granules indicating ATPase activity of cells were detected.

Melanocytes and Langerhans cells were counted in 20 fields of view in 1 mm^2. Morphometric analysis of the epidermal population was carried out on a Video-Test-3.2 image analyzer for evaluation of the proliferative activity of the layer [4]. The content of cells of certain area and shape in the epidermis was evaluated.

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RESULTS

The ECC proliferate in a morphofunctional zone consisting of 2 subunits, with the formation of maternal and daughter cells. Daughter cells gradually transform into peak 2 cells — the earliest descendants from ECC, which, maturing, transform into "narrow" and then "oval" cells. Oval cells are then added to reserve cells (30%), constituting the greatest part in the population and forming the depot of cells spent for physiological regeneration of the layer [3,4]. As the proliferation grows more intense, these cells transform into "transitional" and then "elongated" cells which start mitosis. As a result, "round" cells form, gradually transforming into terminal ones and eliminated from the population.

The present study showed two active proliferation phases (AP) in the basal epidermis layer (from 02:00 to 06:00 and from 12:00 to 16:00) and two passive phases (PP; from 0.8:00 to 10:00 and from 18:00 to 24:00). Active phases are characterized by more intense onset of elongated cell mitosis, which is shown by reduction of their content to 20.6-20.7% in comparison with PP (22.0-23.4%). The share of round cells, forming after their division, increases to 8.2-8.3% in AP *vs.* 7.2% in PP. The count of narrow cells (early descendants from ECC) reduces to 7.1-7.5% in comparison with PP (8.2-8.8%), because they transform into oval cells, their share increasing to 22.8% (19.8% in PP). Hence, elongated and narrow cells start mitosis and are transformed during AP, after these cells have been accumulated during PP. When both subunits of the morphofunctional zone are functioning, the pool of elongated cells actively starts mitosis in order to maintain the layer regeneration [3]. Two AP in the epidermis seem to reflect the work of two subunits of the zone.

Interestingly, the counts of transitional cells virtually do not change in AP and PP, varying from 22.4 to 22.8%. This indicates that reduction of proliferative activity during PP is primarily caused by delayed onset of mitosis in elongated cells.

Study of melanocyte activity in the epidermis has revealed that their count sharply increases at the interphase between AP and PP (at 08.00 and 18.00), reaching 269.2-307.7 cell/mm^2. The melanocyte count gradually decreases during PP to 223.1-200.0 cell/mm^2, the percentage of narrow cells (the closest descendants from ECC) increasing to 8.2-8.8% (7.1-7.5% in AP), that of elongated cells increasing to 22.0-23.4% (20.6-20.7% in AP), which then start mitotic division. The melanocyte count reduces still lower with the beginning of AP, when elongated cells actively proliferate, and reaches 119.2-150.0 cell/mm^2. Hence, melanocytes are involved in the maintenance of the proliferative pool of epidermal basal layer cells, and their count steadily decreases by the end of proliferation.

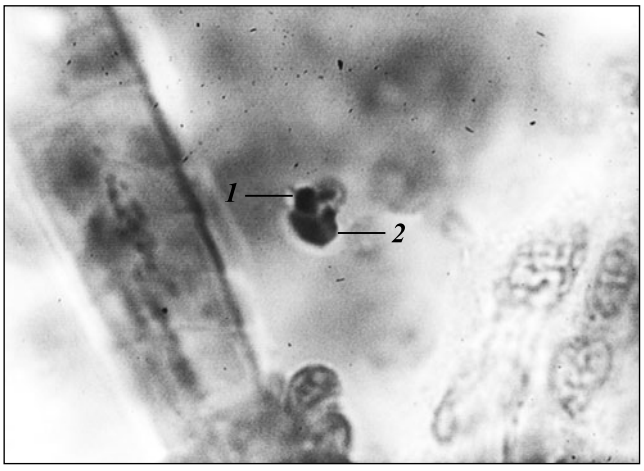

Fig. 1. Black-brown granules in daughter cell cytoplasm indicate the location of tyrosinase in these cells; there is also a dense network of fine processes. Histochemical DOPA reaction, ×1000. *1*) daughter cell (melanocyte); *2*) maternal cell (Langerhans cell).

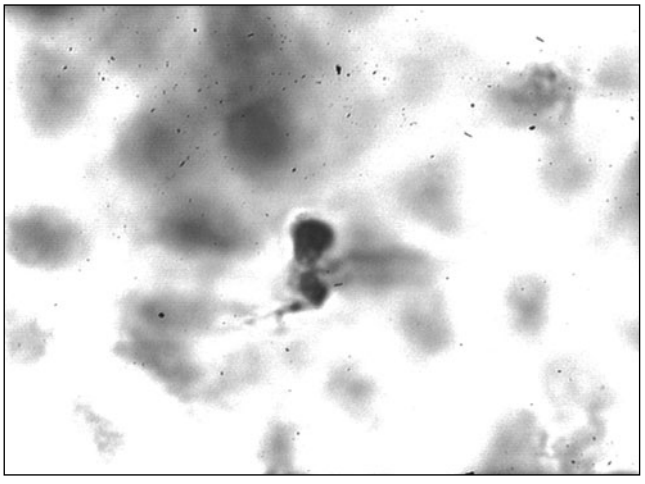

Fig. 2. The cell adjacent to the basal membrane remained spasmodic and did not stretch, while its lateral edges stretched up, that is, the cell acquired a canoe-like shape. Histochemical staining for ecto-ATPase, ×1000.

Studies of Langerhans cells have shown that their count somewhat increases at the interphase between AP and PP (08.00 and 18.00) to 480.8-500.0 cell/mm^2 and remains more or less stable, surpassing almost 2-fold the melanocyte count. Their count then reduces to 384.6-400.0 cell/mm^2 and remains more or less stable throughout the entire proliferative period. Other authors have also noted the stability of these cells' count in the epidermis; hence, changes in the Langerhans cells/melanocyte percent proportion are mainly caused by fluctuations in melanocyte count [5,7].

The increase in the counts of melanocytes and Langerhans cells at the boundary of AP and PP indicates a functional relationship between these cells. Morphological studies by two histochemical methods, detecting tyrosinase activity in melanocytes

and ecto-ATPase activity in Langerhans cells, have shown that these cells are components of annular structures formed by maternal and daughter cells which formed in the ECC first division. Black-brown granules, indicating the tyrosinase location in these cells, and a dense network of fine processes have been detected by DOPA reaction in the daughter cell cytoplasm (Fig. 1). Hence, daughter cells in the annular structures are transformed into melanocytes. After separation from maternal cells melanocytes are located between the basal membrane and basal cells as dark elongated cells, then they cleave and acquire a more round shape.

Differentiation of daughter cells is determined by stretching in electric field, created by 12 pairs of maternal and daughter cells in annular structures. Daughter cells stretch along their main axis, corresponding to the anatelophase orientation of chromosomes [4]. Stretching stimulates Src kinase, involved in the formation of stress fibrils and microtubules, this providing stretching of the cell nucleus and looping of certain chromosome sites between points of their fixation to the nuclear membrane. Growth factors in the immediate microenvironment correct the differentiation in accordance with the tissue type. Different factors differently stimulate Src kinase, and this stimulation determines stretching of certain chromosome sites - closer to telomers under conditions of stronger stimulation of Src kinase or closer to centromers in moderate stimulation, or intermediate between them. Depending on this, fibroblast-like or epithelial cells form. Growth factors with moderate Src kinase activity and strong RHOA activity, causing cell spasm, predominate in the epidermis – and daughter cell in the annular structure is stretching closer to the centromers, proving epithelial differentiation. Melanogenesis stimulation is probable under these conditions.

Further studies of annular structures have shown that maternal cells possess ATPase activity. The body of the cell adjacent to the basal membrane remains spasmodic and does not stretch, while the lateral edges stretch upward, that is, the cell acquires a canoe-like shape (Fig. 2). The stretched edges then meet and fuse, forming a sulcus between them. As a result, a round cell with a plicated nucleus forms, because it has not been tretched in electric field. Later this cell is elongated and migrates into the suprabasal layer. Hence, a Langerhans cell in the basal layer is presumably a result of ECC division, formed from the maternal cell adjacent to the basal membrane. Growth factors accumulating in the basal membrane and characterized by a strong spastic activity play an important role in this process. Other authors have also noted that Langerhans cells have a special epidermal precursor with a different proliferation rhythm [6,9].

The counts of Langerhans cells in AP and PP reduce negligibly and are rather stable, their share being always greater than that of melanocytes. This is presumably due to the fact that during ECC division the melanocytes are gradually transformed by one of subunits into basal cells, while the other subunit has no these cells at that time, and Langerhans cells are present in the other subunit as well, as they are not transformed into other epidermal cells.

Hence, melanocytes and Langerhans cells are formed during ECC division and serve as indicators of these cells' proliferative activity. Therefore, higher percentage of melanocytes and Langerhans cells during transition from AP to PP indicates more intense proliferation of ECC. That is why AP consists of two subphases: the first is proliferation of ECC descendants and the second is ECC proliferation. The mitotic activity of cells dies between two subphases, which manifests by inhibition of the mitosis onset in elongated cells and inhibition of round cells formation. This feature of the cell cycle is its basic characteristic [2]. Our previous studies have shown that DNA synthesis in basal cells starts when the dermal effects on the epidermis is attenuated, which manifests by more intense compression of basal cells and inhibited work of transcription mechanisms [3]. Ribonucleotides accumulating under these conditions are gradually transformed into deoxyribonucleotides, which are involved in DNA synthesis. As the daughter cells stretch with looping of chromosome sites and emergence of euchromatin under the effect of electric field, the synthesis of DNA in these cells and their derivatives anticipates the events in ECC, in which heterochromatin predominates. However, as the spent deoxyribonucleotides should be replenished at the expense of ribonucleotides (and their transformation takes some time), this can cause attenuation of the mitotic activity of subphase 1 and arrest the subphase 2 in ECC synthetic period. The length of subphase 1 (from 02.00 to 06.00 and from 12.00 to 16.00) is longer than that of subphase 2 (08.00 and 18.00), this indicating a very short mitotic cycle of ECC; that is whyannular structures are rarely detected in the epidermis, which impedes their studies.

Hence, Langerhans cells and melanocytes stem from ECC. Melanocytes are gradually transformed into basal cells of the epidermis.

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