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Basic Research

The Relationship between the Epidermal Melanocytes, Langerhans Cells and Epidermal Cambial Cells

Tatyana M. Yavisheva, PhD, ScD*, Sergey D. Shcherbakov, PhD, ScD, Irina S. Golubeva PhD, Ludmila A. Savluchinskaya, PhD, Natalya I. Rizhova, PhD

Blokhin Cancer Research Center of Russian Academy of Medical Sciences, Moscow, Russian Federation

Abstract

The epidermis of 60 mice ears is investigated in this study. The proliferative activity of the epidermal basal cells over 24h was observed to occur in two active phases (AP) and 2 passive phases (PP). The AP phase in turn consists of two subphases. Subphase 1, the longer of the two, is a proliferation of the descendants of the cambial cells; Subphase 2, is very short, involving a proliferation of the cambial cells. The proliferation of the latter occurs at the time of transition of the AP to PP; therefore, the number of melanocytes and Langerhans cells getting involved in the epidermal cambial cell division, increases in the epidermis. The number of Langerhans cells almost doubles compared with the melanocytes, as the latter are gradually transformed into epidermal basal cells. IJBM 2012; 2(3):228-231.
Key words: *cambial cells, Langerhans cells, melanocytes.*

Introduction

The structural organization of the epithelial tissue assumes great significance as any abnormality in structure leads to various pathological states. Now, the epithelium is known to have a more complex composition than was believed earlier. Therefore, the epidermis is seen to consist of epidermal-proliferative units (EPU) with a stem cell at the center. However, at the same time, Langerhans cells, whose basic function is to control immune response of the skin and regulation of the mitotic activity of the keratinocytes, are found in the center of the EPU [1, 2]. Besides the EPU, there is an epidermal melanin unit, in which one melanocyte controls 36 keratinocytes [3]. However, the corneocyte number is proportional to the sum of the Langerhans cells, melanocytes and keratinocytes. Therefore, these cells are presumed to enjoy a functional and close relationship. Based on these data, we studied the morphology and relationship existing between the melanocytes, Langerhans cells and epidermal cambial cells during proliferation.

**Corresponding author: Tatyana M. Yavisheva, PhD, ScD, Leading Researcher, Blokhin Cancer Research Center of Russian Academy of Medical Sciences, 29/33, Building «B», Valovaya str., apt. #50, 115054, Moscow, Russian Federation.*

Tel: 7-905-7354590 (mobile)

E-mail: javisheva@rambler.ru

Materials and methods

Experiments were performed on male CBA mice weighing 20 g (n=60). Specimens of ear tissues were taken every 2h over a 24h period (n=5 for every time point). All the film preparations of the ear epidermis were examined. The tissue specimens were placed in an EDTA buffer solution and incubated at 37°C for 4h. After this procedure, the epidermis became completely detached from the derma. The first part of epidermal preparations was treated to identify the presence of melanocytes. To achieve this, an incubation solution (solution of 0.3% DOPA+the phosphatic buffer) was prepared and the sheets of epidermis were placed in it at 37°C for 2h. Brown-black granules appeared showing the presence of tyrosinase. In addition, the specimens were stained with Heidenhain iron-hematoxylin.

In the second part of the preparations, the epidermal sheets revealed the presence of Langerhans cells, defining the

ATPase activity within them. To achieve this, the epidermal sheets were fixed in 5% neutral formalin at 4°C for 20 minutes. Then they were incubated at 37°C for 1h in 1.32×10-3M ATP incubation mixture. Next, they were placed in 1% ammonium sulfide for one minute. The appearance of black granules, revealed the cells with ATPase activity. The Langerhans cells and melanocytes were counted in 20 randomly chosen fields, and the cell populations were expressed as the average number of cells per square millimeter of epidermis. Morphometric analysis was next carried out using a Video-Test-3.2 video analyzer to define the proliferative activity of the basal cells [4]. This enabled us to identify the content of the epidermal cells of a certain size and form.

Results

We earlier reported that the proliferation of the cambial cells occurs in the morphofunctional zone consisting of 2 subunits with the formation of mother and daughter cells. The latter are gradually transformed into the cells of peak 2, representing the earliest cambial cells descendants, which on maturation, are transformed into «narrow», and then into «oval» cells. The latter fill up the number of «reserve» cells (30%), presenting the greatest portion of the population, providing the physiological regeneration of a layer [4, 5]. On intensification of the proliferation, these cells become transformed into «transitional», and then into «elongated» cells, which directly undergo mitosis. As a result, «round» cells appear which are gradually transformed into the final cells and eliminated from the population.

In the present study, the basal layer of the epidermis revealed the presence of two active phases of proliferation (AP): from 2 AM to 8 AM and from 12 AM to 6 PM; and two passive phases (PP): from 8 AM to 12 AM and from 6 PM to 2 AM.

AP is characterized by an intensification of the «elongated» cells initiation into mitosis, and therefore, their number decreases to 20.6-20.7% compared with PP (22.0-23.4%). Thus, the portion of the «round» cells formed by their division increases to 8.2-8.3% in AP compared with PP (7.2%). The number of «narrow» cells, which are the early cambial cells descendants, decreases to 7.1-7.5 % compared with PP (8.2-8.8%) because they are transformed into the «oval» cells whose portion increases to 22.8 % (in PP 19.8 %). Hence, in the AP phase there is an initiation into mitosis and a transformation of the «elongated» and «narrow» cells, which are initially accumulated in PP. We earlier reported [5] that on functioning of each of the two morphofunctional zone subunits, the pool of the «elongated» cells actively enters into mitosis for layer regeneration. Two AP phases in the epidermis apparently reflect the work of two zone subunits.

Interestingly, in AP and PP the number of «transitional» cells practically does not vary and fluctuates from 22.4 to 22.8%. These findings argue for a decrease in the proliferative activity in PP at the expense of the delay of the «elongated» cells' entry into mitosis.

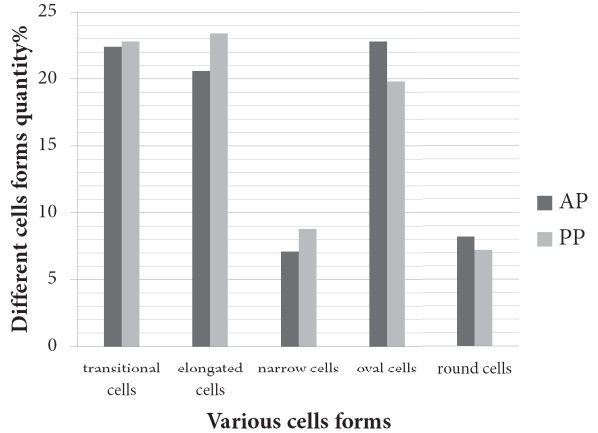
The number of melanocytes in the epidermis was noted to sharply increase at the time of transition of the active phase into the passive phase, i.e. in 8 AM and 6 PM and consists of 269.2-307.7 cells/mm². Then, during the PP there is a gradual depression of the melanocyte number to 223.1-200.0 cells/mm²; thus the number of «narrow» cells increases to 8.2-8.8% (in AP 7.1-7.5%) and the portion of the «elongated» cells which then enter into the mitotic process also increases to 22.0-23.4% (in AP 20.6-20.7%). Further, on transitioning into the AP phase, during the active proliferation of the «elongated» cells, the number of melanocytes decreases even more and reaches 119.2-150 cells/mm². Thus, the melanocytes participate in the maintenance of the proliferative pool of the epidermal basal cells, and their numbers steadily decrease to the end of proliferation.

Research on the Langerhans cells has shown that their numbers increased a little at the time of transition of AP into PP (8 AM and 6 PM) to 480.8-500 cells/mm² and becomes approximately double the number of melanocytes. Further, their quantity decreases to 384.6-400 cells/mm² and remains more or less constant throughout the entire proliferative period. Other authors also argue for the stability of the quantity of these cells in the epidermis, and therefore, the ratio of the Langerhans cells to melanocytes varies due to fluctuations in the melanocyte number [3, 6].

The increase in the number of melanocytes and Langerhans cells, at the time of transition of the AP into the PP phase, gives evidence for the functional relationship between these cells. The histochemical methods, determining the tyrosinase activity in the melanocytes and ATPase activity in the Langerhans cells, reveal that these cells represent the portion of the circular structures formed by the mother and daughter cells, which appeared at the first epidermal cambial cells division. In the cytoplasm of the daughter cells, using the DOPA-reaction, black-brown granules were seen to appear, identifying the localization of tyrosinase in these cell; a dense network of thin processes was also revealed (Fig. 2). Therefore, the daughter cells, which are a part of the circular structures, become transformed into melanocytes. After disbanding from the mother cells, the melanocytes settle down between the basal membrane and basal cells in the

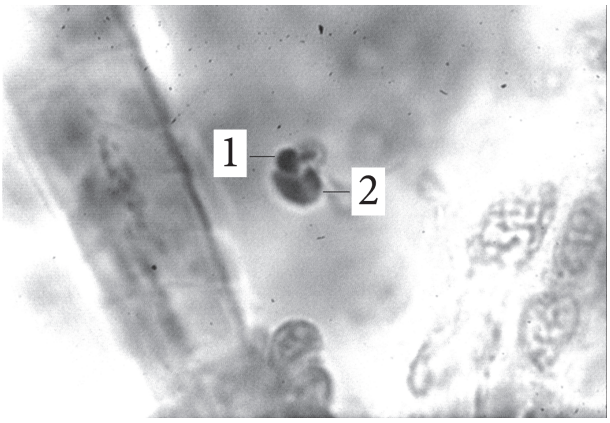
Figure 1

The comparative quantitative characteristic of various epidermal cell forms and passive in the active phases of proliferation.



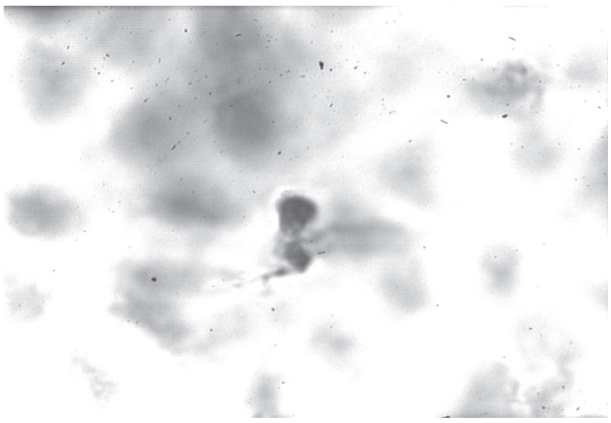
Notes: *AP - active phase, PP - passive phase.*

Figure 2



In the cytoplasm of the daughter cells, black-brown granules become visible, which specify the tyrosinase localization in these cells; also, a dense network of thin processes is revealed. Histochemical DOPA-reaction, x1000.

Figure 3



The cell situated at the basal membrane remains constricted and is not stretched although its lateral edges are tightened up, i.e. the cell becomes canoe-like in shape. Histochemical staining reveals ATP-ase, x1000.

form of the dark extended cells, which later on gradually get a more rounded form.

We earlier reported that the differentiation of the daughter cells is identified by a distention in the electric field, which is excited by 12 mother and daughter cell pairs in the circular structures. The daughter cells stretch along their major axis, which corresponds to the chromosomes in the anatelophase orientation. The stretching activates Src kinase, which participates in the formation of stress fibers and microtubules, which causes the cell nucleus distention and unwinding of the loops of certain chromosome loci between the points of their nucleus envelope fixation. Growth factors in the nearest microenvironment correct the differentiation, according to a certain tissue type. In reality, various factors activate Src kinase to different degrees; therefore, the chromosome loci will be stretched either closer by their telomeres on strong Src kinase activation or closer by their centromeres at moderate activity or else occupy a mediate position between them. Based on this, fibroblast-like or epithelial cells are formed. In the epidermis, where growth factors with moderate Src kinase activity and strong RhoA activity prevail, causing cell spasms, the daughter cells stretching at the circular stricture will be closer at the centromeres and provide the epithelial differentiation. Conditions for activation of melanogenesis, where all the precursors of melanin have increased the RhoA expression, are thus created.

Further study of the circular structures showed that the mother cells possess ATPase activity. Thus the cell body at the basal membrane remained constricted and was not stretched, and the lateral edges were tightened up, i.e. the cell became canoe-like in shape (Fig. 3). Then the stretched lateral edges were joined closely with each other with a groove formation between them. The roundish cell with a wrinkled nucleus was formed as a result, because it was not stretched in the electric field. Further, this cell became extended and moved into the suprabasal layer. Thus, the Langerhans cells in the basal layer, formed by the mother cell, obviously, emerge at epidermal cambial cell divisions, adjacent to the basal membrane. The growth factors, accumulating in the basal membrane and possessing strong spastic action, thus play the main role. Other authors also argue that the epidermal Langerhans cells have their own epidermal precursors with other sequences of proliferation, than the manner formed in the bone marrow [7, 8].

The number of Langerhans cells mentioned above, in the AP and PP phases, decreases slightly and reaches a stable number; however, their ratio always exceeds the number of melanocytes. This is apparently dependent upon the gradual transformation of the melanocytes into basal cells at cambial cell division in one subunit, while in the other subunit the melanocytes are not present at this time. The Langerhans cells, at the same time are present in the other subunit as they do not become transformed into other epidermal cells.

Thus, both melanocytes and Langerhans cells are formed at epidermal cambial cell division and are indicators of the proliferative activity of these cells. Therefore, the rise in the number melanocyte and Langerhan cells at the time of transition from AP into PP phase argues for the intensification of the cambial cell proliferation at this time. Therefore, the active phase actually includes two subphases: Subphase 1 is the proliferation of the descendants of the cambial cells; Subphase 2 involves cambial cell proliferation. However, between these two subphases there is an attenuation of mitotic activity in the cells that is expressed in the slowing down of the «elongated» cells' entry into mitosis and the depression of «round» cell formation. This is the fundamental characteristic of the cell cycle [9]. We earlier reported, that the DNA synthesis in basal cells occurs when the influence of the derma on the epidermis weakens, which leads to an intensification of the contraction of the basal cells and difficulty hindrance for the working of the transcription mechanisms [5]. Thus, the accumulated ribonucleotides are gradually transformed into deoxyribonucleotides, which involve into DNA synthesis. Due to the stretching of the daughter cells accompanied by the unwinding of the

chromosomal loops and the appearance of euchromatin under the influence of the electric field, the DNA synthesis in these cells and that of their descendants first takes place in relation to the cambial cells, where heterochromatin is present. But as the deoxyribonucleotides need to be replenished at the expense of the ribonucleotides, which require time for their transformation, it can cause attenuation of the mitotic activity of the Subphase 1 cells and a delay in the synthetic period of the Subphase 2 cambial cells. Duration of Subphase 1 (from 2 AM to 8 AM and from 12 AM to 6 PM) exceeds beyond the time taken for the prolongation of Subphase 2 (8 AM to 6 PM), which reveals a very short cambial cell mitotic cycle; therefore, the circular structures are seldom found in the epidermis, which complicates further research on them.

Thus, the research conducted in this study has revealed, that it is the epidermal cambial cells that give rise to both the Langerhans cells and melanocytes. It is the latter, which become gradually transformed into the epidermal basal cells.

References

1. Merad M, Manz M, Karsunky H. Langerhans cells renew in the skin throughout life under steady-state conditions. Nat Immunol 2002; 3:1135-1141.
2. Semkin V. Morphofunctional changes of differons at ageing. Klinicheskaya gerontologiya 2001; 9:37-31.
3. Bauer J, Bahmer F, Worl J. A strikingly constant ratio exists between Langerhans cells and other epidermal cells in human skin. A stereologic study using the optical dissector method and the confocal laser scanning microscope. J Invest Dermatol 2001; 116:313-318.
4. Yavisheva T, Yagubov A. Quantitative morphology of mouse cornea multilayered epithelium basal layer cells and human lung cancer. Ontogenez 1996; 27: 95-99.
5. Yavisheva T, Shcherbakov S. Characteristic features of proliferation and differentiation of cambial and daughter cells in morphofunctional zones in normal epithelium and cancer in age aspect. Uspekhi gerontologii 2009; 22:605-613.
6. Hoath S, Leahy D. The organization of human epidermis: functional epidermal units and phi proportionality. J Invest Dermatol 2003; 121:1440-1446.
7. Chorro L, Sarde A, Li M. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expantion of the epidermal LC network. J Experimental Medicine 2009; 206:3089-3100.
8. Romani N, Holzmann S, Tripp C. Langerhans cells – dendritic cells of the epidermis. Apmis 2003; 111:725-740.
9. Chagin V, Rozanov Yu, Tomilin N. Multiple retardation of DNA synthesis during S-phase of cell cycle: research by a method of flow cytometry. Doklady Akademii Nauk 2004; 394:123-126.