doi 10.18699/bgrs2024-9.1-18

Distribution of epidermal proliferating cells in the model of human skin xenograft

Cherkashina O.[1]*, Tsitrina A.[2], Kosykh A.[3], Vorotelyak E.[1], Kalabusheva E.[1]

[1] *Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia*

[2] *Ilse Katz Institute of Nanoscale Science, Beer Sheva, Israel*

[3] *Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Pirogov Russian National Research Medical University, Moscow, Russia*

** olgalcher@gmail.com*

Key words: skin regeneration; epidermal proliferation; skin stem cells; skin xenograft

Motivation and Aim: The study of proliferation is of great importance for the comprehension of the processes underlying skin regeneration in the context of wound healing and pathological conditions, as well as for the understanding of normal skin functioning. A plethora of models has been developed to predict the pattern of cell divisions in the skin. However, despite this, the question of the precise pattern of epidermal proliferation remains controversial. Some models propose the existence of a single-progenitor whose asymmetrical divisions result in the generation of other progenitor cells or differentiating cells. In the initial studies, epidermal proliferation units were described, comprising approximately 10 basal cells with a clonogenic cell in the center and suprabasal cells located above them [1, 2]. Later, it has been proposed that cells in the epidermis are evenly distributed, with a more random division pattern. In this scenario, cell localization at epidermal rete ridges or inter-ridges may play a pivotal role. It has been shown that human rete ridges are transcriptionally similar to mouse tail scales, which are characterized by a higher cell division rate [3]. However, epidermal cell proliferation in human skin is less well-studied than in mice due to the difficulty of utilizing vital labels in humans, which is complicated by bioethical considerations.

The objective of this study was to analyze the dynamics of cell proliferation using a xenotransplantation model, which allows for the modeling of the regeneration process.

Methods and Algorithms: To investigate the process of skin regeneration, we developed a model of human skin xenograft transplantation into mice with immunodeficiency. Biopsies were obtained at 40, 75, and 110 days post-transplantation, fixed in optimal cutting temperature (O.C.T.) compound, and cryosectioned. To study cell proliferation, a bromodeoxyuridine (BrdU) label was injected intraperitoneally seven days prior to sacrifice. The cryosections were then immunohistochemically stained with Ki67 and BrdU, imaged at high resolution, and analyzed by the QuPath-0.5.0 program.

A QuPath algorithm was developed to study the distribution of proliferating cells. The StarDist extension was used for nuclei segmentation based on the DAPI channel, with subsequent classification of detected nuclei based on Ki67/BrdU labeling. Nuclei were classified as negative, Ki67+BrdU-, Ki67-BrdU+, or Ki67+BrdU+. Additionally, BrdU+ cells were classified based on BrdU fluorescence intensity. All cells were divided by their localization in the basal or suprabasal epidermal layer. To analyze the cell distribution pattern, the epidermis was segmented into annotations containing two to four basal cells and located above them suprabasal cells. K-means cluster analysis was

performed to classify these annotations. The output data was analyzed with Microsoft Excel 2016 and Origin 2022.

Results: Following xenografting, the structure of human skin is gradually restored, and by day 110, epidermal morphology and marker distribution is close to normal skin, thus rendering it suitable for use as a healthy skin model. To analyze cell proliferation, Ki67 staining is a common marker of cells in the active phase of the cell cycle. The introduction of BrdU prior to the sacrifice allows for labeling of cells that have divided following the BrdU injection. High intensity of the BrdU staining is indicative of cells that have divided only once. During subsequent divisions, the intensity of the BrdU label decreases. Based on these data, cells may be classified by their division rate and the data may be used to analyze cell proliferation patterns. In order to conduct a cluster analysis of dividing epithelial cells, image annotations were created that included 2-4 basal and overlying suprabasal cells. The number of cells included in each annotation corresponds to an approximate size of an epithelial proliferation unit. The analysis of these annotations and their clustering will provide valuable insights into the distribution of proliferating cells. The Ki67+BrdU+ cells were observed in both rete-ridges and inter-ridges, with a higher density in the rete ridges. Our observations are consistent with previous studies that have identified the rete ridges as a site of active epidermal proliferation. The distribution of Ki67-BrdU+ cells, which had undergone one or more divisions and entered the G0 phase of the cell cycle, was observed to differ from that of other cell groups in that these cells were distributed at a greater distance from the basal membrane. At the early stage of regeneration, the xenograft displays a thickened hyperproliferating epidermis. Following xenografting, the epidermal layers exhibited a thickening with a delayed differentiation of basal cells. The number of proliferating Ki67+ and BrdU+ cells increased significantly, with Ki67+BrdU+ cells being distributed over a larger distance from the basal membrane. As the skin xenograft gradually restores its structure, it may serve as a model for studying skin regeneration, with late stages corresponding to normal skin morphology.

Conclusion: An algorithm has been developed for the analysis of epidermal proliferation patterns. As previously demonstrated, human rete ridges exhibit a closer transcriptional relationship with mouse scales, which are distinguished by their elevated proliferation rates [3]. The use of human skin xenografts permitted the investigation of cell division rates in human epidermis through the use of BrdU tracking. The presence of Ki67+BrdU+ cells was observed in both rete ridges and inter-ridges, with a higher density observed at the former. This finding supports the functional similarity of these human and mouse epidermal domains.

Funding: The study is supported by the Russian Science Foundation (project No. 21-74-30015, https://rscf.ru/en/project/21-74-30015/).

References

1. Roy E., Neufeld Z. et al. Bimodal Behaviour of Interfollicular Epidermal Progenitors Regulated by Hair Follicle Position and Cycling. *EMBO J.* 2016;35:2658-2670. doi 10.15252/embj.201693806
2. Sada A., Jacob F., Leung E. et al. Defining the Cellular Lineage Hierarchy in the Interfollicular Epidermis of Adult Skin. *Nat Cell Biol.* 2016;18:619-631. doi 10.1038/ncb3359
3. Ghuwalewala S., Lee S.A., Jiang K. et al. Epidermal Basal Domains Organization Highlights Skin Robustness to Environmental Exposure. *bioRxiv*. 2022. doi 10.1101/2022.02.23.481662
4. Schmidt U., Weigert M., Broaddus C., Myers G. Cell Detection with Star-Convex Polygons. In: Medical Image Computing and Computer Assisted Intervention – MICCAI 2018. MICCAI 2018. Springer, 2018;265-273. doi 10.1007/978-3-030-00934-2_30
5. Bankhead P., Loughrey M.B., Fernández J.A. et al. QuPath: Open Source Software for Digital Pathology Image Analysis. *Sci Rep.* 2017;7:16878. doi 10.1038/s41598-017-17204-5