DOI 10.18699/SBB-2022-443

The pattern of YAP1 distribution in the normal and pathological state of the human epidermis

Cherkashina O.L.*, Beilin A.K., Kosykh A.V., Rippa A.L., Chermnykh E.S., Kalabusheva E.P., Vorotelyak E.A.

Koltzov Institute of Developmental Biology, RAS, Moscow, Russia

** olgalcher@gmail.com*

Key words: YAP1, skin regeneration, skin xenograft, living skin equivalint

Motivation and Aim: YAP1 is a coactivator that binds to TEAD transcription factors and in the skin it controls the maintenance of homeostasis and regeneration due to interaction with the signaling pathways Wnt/b-catenin, RhoA/ROCK, NOTCH, SHH. In skin pathologies associated with hyperproliferation of keratinocytes, such as psoriasis, basal cell carcinoma, squamous cell carcinoma, there is an increase in the expression level and content of the activated form of YAP1 in the epidermis, while its reduced level and lack of activated form are associated with impaired expansion of basal keratinocytes. Currently available studies of human skin regeneration describe certain aspects of YAP1 participation in the regenerative process, however, it is necessary to conduct a comprehensive analysis of the role of YAP-signaling in the process of skin regeneration and the relationships between processes of proliferation and differentiation in normal and pathological conditions.

Methods and Algorithms: The following *in vivo* and *in vitro* models were used: xenografting of human skin to NOD/SCID mice (specimens were studied 40, 75 and 110 days after transplantation), live skin equivalents, biopsies of cutaneous papilloma, chronic wound during osteomyelitis, as well as healthy human skin. Immunohistochemycally stained cryosections were used for measurement of morphometric parameters of model objects (epidermis thickness, thickness of dermal layers, adipocyte sizes) and the distribution of markers were calculated using ImageJ and Cell Profiler programs based on data on colocalization of signals from various channels and fluorescence intensity. The expression levels of the genes of interest were studied using quantitative PCR analysis. Statistical processing was carried out using GraphPad Prism 8.

Results: To study the dynamics of skin regeneration and assess the distribution of YAP1, we have developed a unique model of human skin xenotransplantation to mice, which reproduces the structure of human skin and contains all the main components: epidermis, dermis, adipose tissue, as well as hair follicles, sebaceous and sweat glands. Morphometric analysis of the grafts revealed a number of differences from healthy skin, including thickening of the epidermis in the graft on day 40 (the average thickness reaches 100 microns, while the thickness before transplantation averaged 19 microns). By 75 and 110 days, its thickness gradually reduces, but is still increased compared to normal skin, which indicates an incomplete regenerative process.

For quantitative evaluation, an algorithm was developed for analyzing the content of the nuclear and cytoplasmic form of YAP1 in the basal and suprabasal layers of the epidermis in ImageJ based on image segmentation and determining the presence of colocalization of signals in channels with YAP1 and with DAPI. YAP1 is normally

located mainly in the basal layer of the epidermis and has a nuclear localization in single cells of the basal layer. Analysis of skin xenografts on days 75 and 110 as well as live skin equivalents obtained from primary keratinocytes showed the following pattern: YAP1 has a nuclear localization in most cells of the basal layer of the epidermis, while analysis of markers of the basal (K15, K19, Inta6) and suprabasal (K10) layers shows that keratinocytes with a basal phenotype form several layers above the basement membrane, unlike one layer in normal skin. The distribution pattern of YAP1 and markers of epidermal differentiation similar to that detected in xenograft and in skin equivalents, is observed in the case of cutaneous papilloma. These data indicate a correlation between an increase in the content of the activated form of YAP1 in the epidermis and an impairment of the normal process of keratinocyte differentiation, which may be associated with a violation of the polarization of keratinocytes or a change in the microenvironment. In a chronic wound, on the contrary, the content of YAP1 in the epidermis was low, the activated form of YAP was not detected, and there was also no expression of K10, which may be due to the inhibited differentiation process.

Conclusion: An increase in the content of activated YAP1 in the epidermis in the xenograft, skin equivalent and in the cutaneous papilloma may be associated with an active regeneration process associated with hypertrophy of basal layer of keratinocytes. A decrease in its content and the absence of an activated form in the epidermis of a chronic wound may indicate a slowdown in the regenerative process, which is accompanied by a violation of keratinocyte differentiation. Further study of YAP-signaling will allow us to analyze in more detail the signaling pathways that can be involved in the process of regeneration of human skin, and which can be therapeutically influenced to correct impairments of the regenerative process.

Funding: The work was performed with the financial support of a grant within the framework of the Russian Science Foundation, No. 21-74-30015.

Asknowledgements: We thank the Core Centrum of Koltzov Institute of Developmental Biology, Russian Academy of Sciences, for providing access to Keyence BZ-9000 and Leica DMI 6000 fluorescence microscopes and cryostat Leica CM1950.