

## REPARATIVE REGENERATION OF SEBACEOUS GLANDS AS A MODEL FOR STUDYING STEM CELL BIOLOGY

<sup>1</sup>Kinzhibatova R.Zh., <sup>2</sup>Dyban P.A.

<sup>1</sup>FGBCEHE "St. Petersburg State Pediatric Medical University" MH RF, St.Petersburg. e-mail: kinro501@mail.ru

<sup>2</sup>FSBSI "Institute of Experimental Medicine", St.Petersburg

**Summary.** The work was performed on the sebaceous glands unassociated with hair – the glands of the external auditory duct. The rats were sacrificed on 1-30 day after the operation. The peculiarities of regeneration of the thermally damaged acinar epithelium of the sebaceous glands were studied, with a special attention payed for proliferative processes. It has been established that sebocytes lining the acini are replaced by keratinocytes. In this paper the underlying mechanisms of metaplasia and the role of stem cells in this process are discussed.

**Key words:** sebaceous gland, external auditory duct, metaplasia, regeneration.

Sebacious glands representing glands, secreting by the holocrine type, can be divided into two groups: associated and unassociated with hair. The rats' sebaceous glands unassociated with hair are represented by several glands including the complicated 4-lobar branched alveolar gland (Zymbal's gland) of the external auditory duct and the simple branched alveolar tarsal (meibomean) gland located in the eyelid tarsal plate, which has, in contrast to the former one, the single gradually narrowing common excretory duct. The secerning terminal sections (acini) open into it directly or through very short lateral ducts.

**The aim of our study was** to investigate the thermally damaged rats' sebacious glands unassociated with hair, with a special attention payed for proliferative processes.

**Materials and methods.** The work was performed on the sebaceous glands of the external auditory duct in 52 adult males of Wistar rats. The animals were sacrificed on 1-30 day after the operation at 16.00 in March. The material was fixed in Bouin's fluid. Acini were counted on serial 7-  $\mu$ m histological sections stained with hematoxylin and eosin, Heidenhain's azan staining, and the quantity of sebocytes on different stages of differentiation and mitotically dividing basal level cells was determined. Mitoses were counted on 3000 cells. In order to find lipids the material was fixed in 10% formalin, and 5-7  $\mu$ m sections were prepared by means of the cryotome and stained with Sudan III and with Scharlach-Rot. The quantitative determination of mitotically dividing cells was performed on 3000 cells. The statistical treatment of the results obtained was performed according to Fisher-Student.

**Obtained results and discussion.** The acinar part of the intact sebaceous glands of the external auditory duct of intact rats is represented by numerous acini, ending by the excretory duct and comprising sebocytes on different stages of the cell cycle. The acinar basal cells adjacent to the

basal membrane form the peripheral epitheliocyte layer in the acinar part of the gland. Their nuclei have small sizes, dark colour and stringer form. Proliferative processes can be observed particularly in this layer. The quantity of mitotically dividing cells is  $11,0 \pm 2,0 \text{ ‰}$ . The sebocytes localized above the basal layer in the differentiation process significantly increase in size attaining maximal parameters in the site where alveolus connects with the excretory duct. During the adipose metamorphosis sebocytes die.

The peculiarities of reactive changes in sebaceous glands are characterized by the presence of all typical patterns which can be observed after different damages to organs. This work will describe only the reactive changes in the epithelial lining of acini and their excretory ductules both in the damaged site and in the distal part of the gland, focusing on proliferative processes in sebocytes and keratinocytes, respectively. The most interesting are the acini of the glands which have no apparently differentiating sebocytes, and the acini lined with the reactively modified basal cells. The differentiated sebocytes die already after 1 day of the damage compared with the control ( $11,0 \pm 2,0 \text{ ‰}$ ), and the mitotic activity of the acinar basal cells increases both near the damaged site and apart from it 3- and 2-fold, respectively ( $34,7 \pm 7,0 \text{ ‰}$  и  $20,3 \pm 2,0 \text{ ‰}$ ). After 3 days epithelial proliferative processes in excretory ducts as well as in the acinary part reach their maximum. After 1 day the basal cells in such acini are typically localized in a single layer. Further, these cells form a multilayer epithelial sheet, and metaplasia of the glandular epithelium of terminal parts into the stratified squamous epithelium occurs. The presence of another type of acini wherein nearly all cells died should be noted. The histological analysis showed that such structures are being filled with the reactively modified cellular sheet growing from the stratified keratinizing squamous epithelium of ductules connecting the gland with the common excretory duct.

It should be noted that inflammatory enlargements grow both from the stratified squamous epithelium of excretory ducts and from the formed similar lining of acini towards necrotic patches.

Further, the acini lined with the stratified squamous epithelium form either closed lumens or inflammatory enlargements. In other acini separate round cells similar to differentiating sebocytes by their nuclear and cytoplasmic structure can be also observed.

It is established that in treated rats the well-defined anomalies of the sebaceous glands can be observed. Conspicuous is a large number of cysts lined with the stratified keratinizing squamous epithelium. The analysis of serial slides showed that such cyst-like lumens can contact each other as well as communicate with their environment, i.e. form a part of specifically modified enlarged excretory ducts. The modification in the relationship between the acinar part and the excretory ducts of the gland is developing. The similar pattern is also observed in the development of acne in pathologic processes [1].

The interpretation of obtained results and literature data on our finding of stereotype sebaceous glands' response to different types of impact, in particular, is unavailable without the data of the development of specialized sebaceous cells both in phylogenesis and in embryogenesis and considering the concepts of stem cells' hierarchical status.

It is known that in vertebrates sebaceous cells, being absent in fishes, amphibians and reptiles, had occurred for the first time in birds as the paired coccygeal gland. Therefore, specialized cells appear in the phylogenetic tree much later than stratified squamous epithelium do.

The establishment periods of an eyelid sebaceous (meibomean) gland in ontogenesis were detailed both for humans and laboratory rodents. It is established that the formation of meibomean glands in humans occurs in the fourth month of the embryonic development in the form of a protrusion of the basal epithelial layer – an eyelid brazing epithelial lamina. Up to the 14<sup>th</sup> week an embryonic gland looks like a chord wherein numerous lateral protrusions – future secretory alveoli — are developing [2,3,4]. The stepwise establishment of an eyelid sebaceous (meibomean) gland was described in rats [5]. It was shown that in 2-day-old pups it can be observed that the meibomean gland starts to form. This can be expressed in that cellular chords grow from the lateral wall of closed eyelids inward the eyelid, which after 6 days form protrusions analogous to the meibomean gland. In 8-day-old pups also the first sebocytes occur [5]. It should be noted that regardless of pre- (human) or postnatal (rat) development of eyelid sebaceous (meibomean) glands, their analogous chords start to grow from the earlier forming structure - stratified squamous epithelium. Therefore, in our opinion, in the most severe impact on sebaceous glands the cell death of the differentiated sebocytes and the cellular dedifferentiation occur, the basal cells being the source of the metaplastic portions – the stratified squamous epithelium (phylogenetically earlier), from which, in turn, i. e. during the pre- and postnatal ontogenesis, the sebocytes had been differentiated.

These processes can be also explained considering the current data on stem cells being the source of epidermocytes and sebocytes. As the majority of authors of those papers consider, there are three independent stem cell populations in the epidermis of the definite skin: multipotent (hair-bulb), sebocyte differentiation-committed and interfollicular epitelial keratinocyte differentiation-committed[1,2,3,6].. There is a limited number of works on sebaceous gland sebocyte stem cells unassociated with hair [ 4 ]. In the authors' opinion, there are only rapidly renewing TA progenitor cells in the basal layer of the meibomean gland acini, which cells are able to pass the all further stages of differentiation of the lipid type, while not being slowly renewing. As a candidate stem (multipotent) cell had been proposed objects outside the acinus itself but located in its excretory ductules which cells are slowly renewing and comprising keratohyalin granules [ 6]. According to several hypotheses, the multipotent cells being displaced from the ductile to the acinar part of the

gland may be the future source of the sebocyte line. In other words, the regeneration of the committed cell line probably occurs from the multipotent stem cells located outside from the meibomean gland. It is similar to the processes observed in the sebocyte cell death in the glands associated with hair – a new cell generation can be formed from the multipotent cells located in the thickened area of the hair follicle and being committed into the progenitor sebocyte line [6]. The regenerative process of the sebocyte pool of the acinar part of sebaceous glands unassociated with hair in the reparative regeneration process, i. e. involving any stem cell lines, currently remains not fully understood and needs further investigations.

**Conclusion.** It's necessary to underline, that in any impact on sebaceous glands unassociated with hair (the hormonal exposure, thermal exposures) there are stereotype modifications of the excretory part of sebaceous glands unassociated with hair, reflecting in hyperkeratosis of excretory ducts, wall thickening, lumen enlarging and so apparent organ enlargement, unrelated to hypersecretion. Our results obtained in that in the reparative regeneration process the replacement of epithelial lining – sebocytes for keratinocytes — occurs, are discussed considering the information on stem germinative cells of sebocytes, multipotent cells and metaplasia.

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