BIOCHEMICAL MARKERS OF SKIN AGEING

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As the ageing process develops, gradual and subtle changes take place in the structure and functions of different systems, including the skin. The skin ages along with the whole organism and it is impossible to restrain this process only in the skin, without affecting the whole body. There are a lot of instrumental methods of studying age-related changes in skin elasticity and resilience. Hard work is being done on creating this type of devices based on data concerning mechanical, optical, acoustical and electrical parameters of skin obtained with non-invasive methods. However, these methods do not provide quantitative assessment and do not examine biochemical changes developing in tissues. It is attributed to the fact that the issue of interaction between the device and the skin has been little explored, besides, the skin – the research object – consists of several layers (the upper layer – epidermis, the main layer – derma, and subcutaneous fat) having their own specific characteristics which determines heterogeneity of its properties. It all makes difficult to interprete the results in exploring the characteristics of the skin. So, the aim of this work is to find the most adequate indices of skin ageing markers excluding the use of biopsy and confirmation with instrumental methods.

Keywords: skin, aging, PCNA, Ki67, p53, hyaluronic acid

Ageing is a universal and natural process which is characterized by graduality, irregularity, and steady progress, and it inevitably affects all the levels of a biological organization to a certain extent. The development of skin ageing processes leading to disorders in metabolic processes in derma and subcutaneous fat is contributed by many factors, such as life style, nutrition, associated diseases, environmental factors, etc. As a result of ageing process, significant changes in skin cells (as well as in the cells of the whole organism) take place: mitotic activity of skin cells goes down, synthesis processes degrade gradually, modified proteins are accumulated, and so on. This all causes an impact on a person's skin and appearance which plays an important role in the process of elder people's social adaptation. It is remarkably difficult to slow down natural processes of ageing. It is accounted to the fact that skin ageing is directly linked to ageing of the whole body.

Lately, along with integral biological age which reflects the degree of age-related changes in biological capabilities of the organism at each stage of the ontogenesis and determines the life expectancy or death probability in a certain period, it has also been proposed to determine the biological age of systems, organs, and cells. In this connection, the term 'biomarker of ageing' is used and defined as a complex of parameters reflecting the functional ability of a tissue, an organ or a cell in the absence of a disease. And it is evident that biomarkers of ageing should carry information about the functional condition of an organ or a tissue and metabolic processes taking place in them, it should reflect fundamental biological processes, and correlate with physiological age. To sum up the information on the proposed criteria for selecting biomarkers of ageing, a list of main requirements for them can be formed:

1. age-related changes of a marker should develop at a speed reflecting the ageing pace [4, 9];
2. the marker should correlate with physiological age [4, 9];
3. the marker should carry information about the functional condition of an organism, metabolic processes system and about regulatory specifics [4];
4. the marker should reflect fundamental biological processes [9, 13];
5. the marker should predict the life expectancy or serve as a retrospective ageing parameter [9];
6. the marker should reflect changes taking place in a relatively short period of time [9, 13];
7. the marker should be able to forecast the parameter for an elder age [13].

It is evident that it is rather difficult to choose an index that would meet all the requirements [1, 17]. Nevertheless, the task of searching and defining the complex of parameters that can be used as biomarkers of an organism's ageing, systems or cells remains urgent.

Defining different markers of ageing is a pressing problem of gerontology and dermatology. It is connected with the fact that the skin, exposed to external influences, the most important of which is ultraviolet radiation, often undergoes regressive changes at a younger age in comparison with other organs, and timely application of effective prophylaxis measures can prevent development of early and accelerated involutive changes. The index most often used in dermatology as a morphological marker of skin ageing is the content of collagen

and elastic fibers in derma as it is believed to integrally reflect a complex of changes in the main elements of derma, in particular, changes in fibroblasts' synthesis of matrix proteins and in the activity of the enzymes, participating in their polymerization and degradation; this index is also believed to reflect the morpho-functional condition of derma. However, a considerable drawback of this biomarker is its inability to reflect the state of epidermis. In this connection, a more perspective solution should work out a wider complex of morpho-functional skin parameters which will take account not only of the content of collagen and elastic fibers in derma, but the state of epidermis as well. It has been found that the main fundamental processes that determine stability of structural and functional organization of a tissue and skin are cellular differentiation, cell renewal and cell death. Coordination of biosynthesis, metabolism processes and reproduction of genetic information in different cell populations of epidermis and derma is carried out by cells (presented in skin) of traditional regulatory systems – nervous, immune, and endocrine – which form a local neuro-immune-endocrine system of skin. It is known that the skin in different body parts ages at a different speed which is determined by its exposure to external influences. On this basis, two types of skin ageing are identified: natural or chronologic ageing which includes displays of normal maturity, typical for all people, and photoageing which includes a complex of changes caused by UV.

Mechanical properties of skin are determined to a great extent by its structure which is a complex system of three-dimensional interweaving of collagen fibers in protein-polysaccharide matrix [10]. In old age, the most pronounced changes are the ones in skin connective tissue structures which show themselves in flattening of papillae situated in thinned reticular layer, basophilic degeneration of collagen with fibers breaking and turning into amorphous lumps and granules. In subepidermal layer, elastic fibers disappear, collagen fibers disperse to basal substance, the contact line between derma and epidermis gets smoother. Derma atrophies with age, besides, dystrophic changes take place in fibrous structures, the number of cellular elements such as fibroblasts, labrocytes, and blood vessels goes down, and capillary loops shorten. As collagen ages, its structure acquires additional intra- and intermolecular links containing chromophores which results in an increase in the structural stability of collagen, a change in color characteristics of this protein, a rise in absorption and fluorescence [5]. As an organism ages, extracellular matrix proteins undergo considerable modification which decreases the content of I, II, VII type collagen in derma. With age, unlike non-fibrillar proteins, the structure of collagen changes to become more firm and resistant to proteinases [20].

The quantity of fibroblasts in derma progressively falls with age. Age-related decrease in the number of these cells in derma is, probably, the most important factor for signs of skin ageing appearing. Fibroblasts produce all the components of the intercellular substance including collagen and elastic fibers and the amorphous component [15]. Most probably, it is a decrease in the quantity of fibroblasts that causes disorders in the renewal process of the derma intercellular substance. It is a well-known fact that appearance of skin ageing signs is connected with changes in the state of intercellular substance.

An important role in regulating of an organism's homeostasis is played by the hormone melatonin, also known as N-acetyl-5-methoxytryptamine, serotonin derivative (in its turn, serotonin is an L-tryptophan derivative).

It is known that epidermis cells are characterized by expression of melatonin components and serotonin biosynthesis system. Research of the affects caused by these biogenic amines on skin cells has revealed its considerable variability depending on the type of cells and conditions of their cultivation. For example, melatonin inhibits apoptosis of HaCaT keratinocytes cultivated without serum and their proliferation in environment supplemented with serum. Besides, it stimulates growth of melanocytes in environment poor in growth factors and, on the contrary, suppresses their growth in the presence of these factors.

To add, in vivo the variability of affects caused by melatonin can be explained by fluctuations in sensitivity of receptors to external influences. It can be regulated by melatonin itself as well as by other regulatory peptides, for example, estrogens. Besides, sensitivity and distribution density of melatonin receptors in human skin changes during the day; it depends on lighting environment and is not connected with the content of this hormone in blood.

Exogenous serotonin in physiological concentration stimulates proliferative activity of quiescent cells of L929 and L-41 lines and does not exert significant influence on proliferating cells.

During the process of skin ageing, the index of melatonin and serotonin expression in

epidermis and derma cells remains stable till an age of 50-55. A decrease in the expression of these biogenic amines is caused by reduction in the number of melatonin and serotonin producing cells. The PCNA (Proliferating Cell Nuclear Antigen) protein is polyfunctional and it is a part of DNA polymerase δ which is necessary for DNA synthesis and repair. This protein is found in cells which are in G1, S, G2, and M phases of the cell cycle [19]. Expression of PCNA is seen in the lower part of epidermis, mainly in the basal layer. Skin ageing was associated with both relative (index) and absolute number of PCNA immunopositive cells. That is why age-related changes in quantitative indicators of their expression can reflect age-associated changes in DNA-repair system and proliferative activity of cells. Consequently, PCNA may be regarded as a marker of proliferating cells and cells that are in active phases of cell cycle, and not in G0-phase [19]. That is why age-related decrease in the number of fibroblasts in derma is partly explained by a decrease in the number of cells in the cell cycle. Besides, age-related changes were also found in the expression of other markers of the cell cycle (Ki-67, p53, p21) in derma fibroblasts [12]. Age-related decrease in the number of fibroblasts in derma can also be caused by a slower renewal speed of this cell population or by less active formation of new fibroblasts from their poorly differentiated predecessors [18]. Age-related reduction in the number of stem cells in skin is illustrative in this regard [11]. In addition, a fall in derma fibroblasts in the course of ageing process may result from activation of apoptosis [14, 16]. The found close connection between PCNA and Ki67 indices reflecting the content of antigen positive epidermis cells can serve as evidence of the fact that in covered skin areas the expression of PCNA is typical, first of all, for proliferating cells.

The results of serial sections' analyzing which were colored by PCNA, Ki67 and p53 antibodies, support this assumption as well [6]. It has been found that the distribution density and localization of cells expressing PCNA coincide more frequently, though not always, with those of Ki67 antigenically responsive cells. Ki67 is referred to cancer tumor antigen. Expression of Ki67 allows marking out cells that are in an active phase of the cell cycle all over (G1, S, G2, M-phases) [20]. Immunocoloring of keratinocyte nuclei by anti-Ki67 antibodies was seen in the basal layer, and more rarely in the lower parts of the spinous layer of epidermis. Analysis of dependence between morphometric indices of Ki67 expression and the patients' age revealed a decrease in the quantity of immunopositive cells per 1 mm and a decline in Ki67 index which shows that proliferative activity of epidermis cells decreases as a body ages. Statistically significant changes in these two indicators were seen even in cases of patients aged 49-55, and over 60 the absolute and relative (Ki67 index) number of dividing cells fell almost twice [6]. Decline in proliferative activity of basal layer cells was associated with epidermis thinning and a decrease in the number of cell rows in it.

Thanks to its high biocompatibility and biological activity, absence of antigenicity, hyaluronic acid (HA) has been used in different medical spheres for many years [2]. HA accelerates epithelization, stimulates microcirculation, migration and proliferation of fibroblasts, plays a certain role in transporting a list of cytokines and growth factors, reduces endogenous intoxication by blocking lipid peroxidation, activating bactericidal factors, and increasing sensitivity to antibacterial substances [3]. As a polyanion, HA is able to bind and hold water molecules with the use of hydrogen bonds. This ability does not change event if water concentration in the environment decreases. Negative charges formed due to dissociation of its carboxylic groups begin to attract cations, in particular, osmotically active sodium ions, which leads to an increase in intradermal pressure providing turgor of skin [7].

HA content in the skin is maintained through autoregulation mechanisms on the feedback principle. Increased HA content HA in the skin, intensifies dissociation or supply from outside promote production of catabolite enzymes leading to step-by-step transformation, decrease in the length of the initial chain and formation of fragments with their own biological activity. Biological characteristics of HA are, first of all, connected with its size (molecular mass). Probably, fragmentation of HA resulting from enzymatic degradation or free radical oxidation is another mechanism of homeostasis regulation that optimizes migration and proliferation of cells depending on changes in environmental conditions [8]. For example, an increment in the activity of hyaluronidases is one of the factors stimulating an increase in the activity of fibroblasts participating in synthesis of new HA molecules which is used in aesthetic medicine in the biorevitalization techniques and results in recovering the changed homeostasis and reconstruction of quality intracellular matrix.

Speaking about chronological ageing and photoageing of skin, there is a close connection

between expression of melatonin, Ki67 antigen, and p53 protein in the skin. As the secretion of melatonin having cytostatic and antioxidant effects goes down, p53 protein characterized by proapoptotic effects begins to accumulate in cells, which reflects a complex of adaptive changes aimed at maintaining the homeostasis of ageing skin. P53 protein is a transcriptional factor regulating the cell cycle acting as a suppressor of malignant tumors development. The gene of this protein is normally considered an antioncogene and is located on the short arm of chromosome 17 (17p13.1). In epidermis of young patients (19-27 years old) immunocoloring of keratinocyte nuclei by p53 antibodies was seen in the cells of the lower and middle sections. Moreover, the more keratinized the cells were, the less dense was distribution of immunopositive nuclei. Epidermis thinning associated with skin ageing was characterized by levelling the gradient of immunocoloring of their nuclei depending on the differentiation of keratinocytes and by a tendency for an increase in p53 index. Apparently, an insignificant decrease in the absolute number of immunopositive keratinocyte nuclei is a consequence of epidermis thinning and the age-related fall in the total number of cells in it.

Expression of p53 protein was also found in derma cells. The absolute number of immunopositive cells and p53 index tended to grow as patients got older.

Conclusion

To sum up, age-related specifics of morpho-functional state of ageing skin are most fully reflected by indices of PCNA and p53 proteins expression – among all the studied parameters – which means that these can be used for assessing the biological age of skin. All the markers mentioned above are easily identified in immunohistochemical reactions, reflect fundamental processes and provide information about the functional state of skin. Their changes intimately correlate with patients' physiological age, and have peculiar age-related dynamics properties as well.

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