Molecular Mechanisms of Human Skin Aging

Cilt Yaşlanmasının Moleküler Mekanizmaları

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ABSTRACT Skin has long been recognised to protect the organism from deleterious environmental (physical, chemical, microbiological) agents, and is crucial for the maintenance of temperature, electrolyte and fluid balance. In addition, skin is a sensory organ, a biofactory for the synthesis, processing and/or metabolism of a wide range of structural proteins, glycans, lipids and it fulfils the requirements of a classic endocrine organ. Indeed, human skin cells produce a variety of hormones including growth factors and sex steroids and essential vitamins such as vitamin D. With accelerating age skin loses its structural and morphological characteristics and as a consequence all functions mentioned above deteriorate. This deterioration is enhanced cumulatively by various environmental physical, chemical and mechanical insults. Exposed areas of the body such as the face, the neck, and the hands suffer the most by the influence of extrinsic factors and overexposure of these regions may result in premature skin ageing. Ultraviolet (UV) radiation, infrared (IR) radiation and tobacco smoking are by far the most aggressive environmental agents. In non-exposed areas ageing is mainly attributed to intrinsic factors, e.g. genetic predisposition and changes in the endocrine environment and reflects degradation processes of the entire organism. The clinical signs of ageing are more prominent in sun-exposed skin than in sun-protected regions. Typical histological features and biochemical changes accompany intrinsically and extrinsically aged skin, respectively. Although the fundamental mechanisms are still poorly understood, a growing body of evidence points toward the involvement of multiple pathways in the generation of aged skin. Several theories have been proposed including the theory of cellular senescence, decrease in cellular DNA repair capacity and loss of telomeres, point mutations of extranuclear mtDNA, oxidative stress, increased frequency of chromosomal abnormalities and gene mutations. Furthermore, hormones have been also shown to play a distinct role. In the following years, as the number of persons aged 80 and over is expected to rise, health care financing, and disease prevention will be important issues to content with.

Key Words: Skin, extrinsic factors, intrinsic factors, ageing, cellular senescence, telomeres, DNA repair capacity, mtDNA, hormones, oxidative stress, progeroid syndromes

ÖZET Derinin organizmayı zararlı çevresel (fiziksel, kimyasal, mikrobiyolojik) ajanlardan koruduğu ve sıcaklık, elektrolit ve sıvı dengesinin korunmasında önemli rol oynadığı bilinmektedir. Ayrıca, bir duyu organı olarak deri, çeşitli yapısal protein, glikan, lipid sentezi, işlenmesi ve/veya metabolizması için çalışan bir biyo-fabrikadır ve klasik bir endokrin organının gerekliliklerini yerine getirmektedir. Gerçekten de, insan derisi hücreleri büyüme faktörleri ve seks steroidleri dahil çeşitli hormonlar ve D vitamini gibi vitaminler üretmektedir. Yaşın ilerlemesiyle, deri yapısal ve morfolojik özelliklerini kaybeder ve sonuç olarak yukarıda bahsedilen tüm işlevleri bozulur. Bu bozulma çeşitli çevresel fiziksel, kimyasal ve mekanik saldırılar ile kümülatif olarak artar. Yüz, boyun, ve eller gibi derinin açıkta kalan bölümleri dış faktörlerin etkisiyle en çok zararı görürken bu bölgelerin aşırı maruz kalması erken deri yaşlanmasına yol açabilir. Ultraviyole (UV) radyasyon, kızılötesi (IR) radyasyon ve tütün içiciliği en saldırgan çevresel ajanlardır. Açıkta olmayan alanlarda, yaşlanma genetik yatkınlık ve endokrin ortamındaki değişiklikler gibi içsel faktörlere bağlanmaktadır ve tüm organizmanın degradasyon sürecini yansıtmaktadır. Klinik yaşlanma belirtileri güneşe maruz kalan bölgelerde güneşten korunan bölgelere göre daha fazladır. İçsel ve dışsal olarak yaşlanan deriye tipik histolojik özellikler ve biyokimyasal değişiklikler eşlik eder. Temel mekanizmalar halen çok iyi anlaşılamasa da, yaşlı derinin oluşmasında birden çok yolun etkisinin olduğunu gösteren kanıtlar gittikçe artmaktadır. Hücresel yaşlanma, hücresel DNA onarım kapasitesinde düşüş ve telomerlerin kaybı, ekstranükleer mtDNA'da nokta mutasyonları, oksidatif stres, kromozom anormalliklerinin ve gen mutasyonlarının sıklığının artması dahil çeşitli teoriler önerilmiştir. Ayrıca, hormonların farklı bir rol oynadığı kanıtlanmıştır. Gelecek yıllarda, 80 ve üzeri yaşlardaki kişilerin sayısının artması beklendiğinden, sağlık giderlerinin karşılanması ve hastalık önleme üzerinde durulması gereken önemli konular haline gelecektir.

Anahtar Kelimeler: Deri, dış faktörler, iç faktörler, yaşlanma, hücresel yaşlanma, telomer, DNA onarım kapasitesi, mtDNA, hormonlar, oksidatif stres, progeroid sendromlar

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Like all other organs, skin suffers progressive morphologic and physiologic decrement with increasing age and provides the first obvious evidence of the ageing process. Skin ageing can be classified into light-induced ageing (photoageing, exogenous ageing) and endogenous ageing. The latter occurs in non-exposed areas, which are not in direct contact with environmental factors such as ultraviolet (UV) and infrared (IR) irradiation (e.g. the inner side of the upper arm)[1] and is mainly attributed to genetic factors, and alterations of the endocrine environment. In contrast to photoageing, endogenously aged skin reflects degradation processes of the entire organism. This article addresses the fundamental mechanisms involved in skin ageing.

CELLULAR SENESCENCE

The theory of cellular senescence describes the observed loss of the cells proliferative potential after a limited number of cell divisions.[2] According to this theory, cells possess a 'biological clock', which signals the end of their replicative life span, and as a consequence, they cannot be stimulated to enter the S1 phase by physiological mitogens, arresting at the G1 phase. This process can be partly explained by the selective repression of growth regulatory genes.

Studies on keratinocytes,[3] fibroblasts[4-6] and melanocytes,[7,8] have revealed that they all show an age-associated decrease in cumulative population doublings. Fibroblasts, for instance, taken from a normal human tissue go through only about 25-50 population doublings when cultured in a standard mitogenic medium. Towards the end of this time, proliferation slows down and finally stops and the cells enter a state from which they never recover. The reduction in proliferative capacity of skin derived cells in culture from old donors and patients with premature ageing syndromes and the accumulation in vivo of senescent cells with altered patterns of gene expression 8 also support the theory of cellular senescence.

THE FREE RADICAL THEORY

Oxygen radicals (ROS) are increasingly considered as the major contributors to ageing and the protective mechanism against oxidative stress is observed as an indispensable function.[9] It has been shown that oxygen radicals levels rise and anti-oxidant activity declines with advancing age.[10-12]

Skin possesses many defensive mechanisms in order to reduce the production of ROS from internal sources. For example, the activity of enzymes which indirectly produce oxygen metabolites can be altered (xanthine oxidase modulation). There is a repair system consisting of enzymes and small molecules,[13-16] antioxidant enzymes such as catalase and peroxidase and low molecular weight antioxidants, such as tocopherols ascorbic acid, NADH and carnosine, which can donate an electron and then scavenge ROS.[10]

Excess ROS production leads to accumulation of cellular damage,[17,18] which includes oxidation of DNA resulting in mutations and oxidation of membrane lipids leading to reduced transport efficiency and altered trans membrane signalling, processes which have as consequence the ageing phenotype. A disturbed stress response is also known to be associated with a defect in proteolytic sytems such as lysosomal activity and ubiquitine-proteosome pathway in somatic cells.[19] As a consequence altered proteins cannot be eliminated resulting in accumulation of misfolded and damaged proteins in the cells.

Moreover, accumulative evidence suggest that ROS play a crucial role by participating in multiple MAP kinase pathways, which induce AP-1 and in turn the signal cascade, already mentioned above.

The free radical theory has been also supported by the fact that strategies that reduce metabolism and the production of ROS, such as dietary caloric restriction (DCR) can extend lifespan of experimental animals.[20] Studies conducted in animal models demonstrated that DCR can retard the ageing process by influencing stress response and altering the expression of metabolic and biosynthetic genes.[21] Cancer prevention due to alterations of hormone metabolism, hormone-related cellular signalling, oxidation status, DNA repair and apoptosis has been also associated with DRC.[22,23] In skin tissues of mice with DCR weight control a palette of genes showed a differential expression when compared to mice receiving normal diet.[24] DCR could show profound inhibitory impact on the expression of genes relevant to cancer risks (e.g. neuroblastoma ras oncogene, Neuroblastoma myc-related oncogene 1, Rab40c, Myeloblastosis oncogene-like 2, Lung carcinoma myc-related oncogene 1, Myeloblastosis oncogene , RAB5B, RAP2B, RAB34).

GENES AND MUTATIONS

The mechanisms, which seem to be associated with ageing are complex.[25,26] Recent studies on models such as the yeast Saccharomyces cerevisiae,[27] the nematode Caenorhabditis elegans,[28] the fly Drosophila melanogaster,[29-31] the mouse Mus musculus[32] and humans[33] show

that single gene mutations can contribute to the initiation of ageing and induce premature ageing syndromes. However, there are no special genes that can cause ageing-associated damages. The manifestation of ageing is mostly due to the failure of maintenance and repair mechanisms[34,35]

Studies on human keratinocytes have demonstrated altered expression of growth-regulating molecules with age; there is an increase of the baseline expression of the differentiation-associated genes like SPR2 and interleukin 1 receptor antagonist[36] and EGF binding and receptor phosphorylation is reduced and thought to be the result of age related changes in a critical downstream signalling element.[37]

In senescent fibroblasts, genes like the c-fos proto-oncogene,[38] the helix-loop-helix Id-1 and Id-2 genes[39] and components of the E2F transcription factor[8,40] have been shown to be downregulated, and negative growth regulators are overexpressed including the p21 and p16 inhibitors of cyclin dependent protein kinases.[41] Other changes seen in senescent skin fibroblasts include increased expression of IL-1 and of the EGF-like cytokine heregulin that modulates the growth and differentiation.[42] Moreover, elastin gene expression is markedly reduced after the age of 40–50, as determined by mRNA steady state levels.[43]

Futhermore, recent studies indicate that endogenous and exogenous ageing may share some fundamental pathways, and may have some common mediators. Photoageing is thought to be the superposition of UV-irradiation from the sun on intrinsic ageing.[44]

Some of the similarities are changes in the MAP kinase signalling pathways, like decreases in ERK-dependent MAP kinase activity and increases in stress-activated JNK and p38 kinase,[45] which result in reduced cell proliferation, differentiation, and cell survival 46 and enhanced growth arrest, apoptosis and stress-related responses.[46,47] As a consequence of the stress-activated MAP kinase pathways, the expression of c-jun and c-Jun N terminal kinase – an upstream activator of c-jun, is elevated in aged compared with young skin.[45] As c-jun is a constituent of the transcription factor AP-1, AP-1 is also elevated and subsequently the AP-1 regulated connective tissue degrading enzymes MMP-1 (interstitial collagenase), MMP-3 (stromelysin 1) and MMP-9 (gelatinase B). Parallely, there is an observed reduction in the expression of tissue inhibitors of metalloproteinases.[48-50] Another common feature is the increased insoluble degraded collagen and the reduction of type I and III procollagen synthesis, which may result from the impaired TGFß signalling pathway.[51,52]

Ly et al measured mRNA levels in fibroblasts isolated from young, middle-aged, and elderly patients with progeria and found chromosomal pathologies that lead to misregulation of key structural, signalling, and metabolic genes associated with the ageing phenotype.[53]

In recent studies researchers have been focusing on gene mutations accompanying known progeroid syndromes e.g. Hutchinson-Gilford progeria, Werner's syndrome (WS), Rothmund-Thomson syndrome, Cockayne syndrome, Ataxia teleangiectasia and Down syndrome.[54] The most common skin disorders of these syndromes which are characterised by an acceleration of the ageing phenotype are alopecia, skin atrophy and sclerosis, teleangiectasia, poikiloderma, thinning and graying of hair and several malignancies. Most of these syndromes are inherited in an autosomal recessive way and mostly display defects in DNA replication, recombination, repair, and transcription. Expression gene patterns of skin cells derived from Werner patients,[55] old and young donors showed that 91% of the analyzed genes had similar expression changes in WS and in normal ageing implying transcription alterations common to WS and normal ageing represent general events in the ageing process.

Further studies conducted to investigate changes in gene expression during skin ageing have been performed on naturally aged human foreskin obtained from children and elderly males. Some of the mechanisms proposed to be involved in the induction of ageing comprise disturbed lipid metabolism, altered insulin and STAT3 signalling, upregulation of apoptotic genes partly due to the deregulation of FOXO1, dowregulation of members of the jun and fos family, differential expression of cytoskeletal proteins (e.g., keratin 2A, 6A, and 16A), extracellular matrix components (e.g., PI3, S100A2, A7, A9, SPRR2B), and proteins involved in cell-cycle control (e.g., CDKs, GOS2).[56]

THE MITOCHONDRIAL DNA (MTDNA) THEORY

Genetic damage and instability outside the nuclear genome has been also suggested to contribute to ageing.[57] The mtDNA synthesis takes place near the inner mitochondrial membrane, where the sites of formation of ROS are and the fact that mtDNA lacks excision and recombination repair, has made many investigators to believe that cumulative damage of the mtDNA may play a key role in the pathogenesis of the ageing phenotype.[58,59]

Examination of human fibroblast mtDNA in aged individuals revealed point mutations at specific positions in the control region for replication. Notably, a T414G transversion was found in a significantly higher proportion of persons older than 65 years of age when compared with younger persons. These results lent support to the notion that cumulative damage to mtDNA during life contributes to the ageing process.[53]

THE TELOMERE HYPOTHESIS

The telomere hypothesis of cellular ageing 60 proposes that loss of telomeres due to incomplete DNA replication and absence of telomerase provides a mitotic clock that signals cycle exit, limiting the replicative capacity of the somatic cell 61. Human telomeres consist of repeats of the sequence TTAGGG/CCCTAA at chromosome end, which are not replicated in the same manner as the rest of the genome but instead are synthesised by the enzyme telomerase .[61-63] By mechanisms that remain unclear, telomerase also promotes the formation of protein cap structures that protect the chromosome ends. Telomerase is active in germline cells and in humans, telomeres in these cells are maintained at about 15 kilobase pairs (kbp). In contrast, telomerase is not expressed in most human somatic cells like skin cells.[64,65] As a result, their telomeres become 50-100 nucleotides shorter with every cell division, and their protective protein caps progressively deteriorate. Eventually, after many cell generations, DNA damage occurs at chromosome ends. The damage activates a p53-dependent cell-cycle arrest that resembles the arrest caused by other types of DNA damage.

The lack of telomerase in most somatic cells has been proposed to help protect humans from the potentially damaging effects of runaway cell proliferation, as occurs in cancer. Telomere loss is thought to control entry into senescence.[65-69]

HORMONE DECLINE AND SKIN AGEING

Further factors that may play a predominant role in the initiation of skin ageing is the physiological hormone decline occurring with age. Over time important circulating hormones decline due to a reduced secretion of the pituitary, adrenal glands and the gonads or due to an intercurrent disease. Amongst them, growth factors [i.e. growth hormone (GH) and insulin-like growth factor-I (IGF-I)], and sex steroids (e.g. androgens and estrogens) show significant changes in their blood levels.

By now, in models of animal ageing, such as in organisms as diverse as the nematode Caenorrhabditis elegans, the fly Drosophila melanogaster, and the mouse Mus musculus, the importance of hormonal signals on the ageing phenotype has been already documented. Suppression of hormones such as insulin-like peptides, growth hormone (GH) and sterols[70] or their receptors can increase lifespan and delay age-dependent functional decline. Conboy et al[71] showed that the age-related decline of progenitor cell activity of mice could be reversed by exposure to young serum and that the cells could retain much of their intrinsic proliferative potential even when old, underlining the great importance of the systemic environment. In an in vitro model of human hormonal ageing, human skin cells cultured under hormone-substituted conditions showed altered lipid synthesis and metabolism and affected expression of genes being involved in biological processes, such as DNA repair and stability, mitochondrial function, oxidative stress, cell cycle and apoptosis, ubiquitin-induced proteolysis, and transcriptional regulation indicating that these processes may be hormone-dependent.[72] These studies illustrate the importance of the hormone environment for deterioration of the human organism and the ageing process.

The growth hormone (GH)/ insulin-like growth factor-I (IGF-I) axis is considered to be one of the most important signalling pathways involved in ageing. Serum levels of IGF-I have been reported to increase from birth to puberty, followed by a slow decline through adulthood. This reduction has been correlated with the progressive decline of GH with advancing age.[73] Patients with isolated GH deficiency (IGHD), multiple pituitary hormone deficiency (MPHD) including GH, as well as primary IGF-I deficiency (GH resistance, Laron syndrome) present signs of early skin ageing such as dry, thin and wrinkled skin. Other resulting characteristics of GH/IGF-I deficiency are obesity, hyperglucemia, reduced body lean mass, osteopenia, lowered venous access, hypercholesterolemia, cardiovascular diseases and, subsequently, premature mortality.[74-77] Treatment of normal elderly males with GH resulted in amelioration and reverse of the ageing signs and symptoms.[78-80] However, recent reports of an association of GH substitution and increased risk of prostate, lung, colon, breast cancer as well as a possible decrease of insulin insensitivity all make further investigations necessary regarding safety and efficacy of GH substitution in the ageing population.[81]

On the other hand, menopause, which is characterised by a rapid decline of sex-steroids, has been associated with a worsening of skin structure and functions, which can be at least partially repaired by hormone replacement therapy or local estrogen treatment.[82] Improvement of epidermal skin moisture, elasticity and skin thickness,[83] enhanced production of surface lipids,[84] reduction of wrinkle depth, restoration of collagen fibers[85] and increase of the collagen III/I ratio 20 have all been reported under hormone replacement therapy.

In in vitro studies the effects of GH, IGF-I androgens and estrogens at age-specific levels on human skin cells have been documented. IGF-I showed to play an important role in the regulation of the lipid synthesis in human sebocytes while 17β-estradiol showed no significant effects on the biological activity of the cells. Dermal fibroblasts showed to be more susceptible to 17β-estradiol treatment, while IGF-I could significantly stimulate fibroblast proliferation. Furthermore, an interplay between the 17β-estradiol and IGF-I signalling pathway was documented in both cell types. These results indicate the importance of IGF-I in the reduction of skin surface lipids and thickness with advanced age.

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