Aging and Anti-Aging Strategies

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Contents

Abstract

Ageing of human skin may result from both the passage of time (intrinsic ageing) and from cumulative exposure to external influences (extrinsic ageing) such as ultraviolet radiation (UVR) which promotes wrinkle formation and loss of tissue elasticity. Whilst both ageing processes are associated with phenotypic changes in cutaneous cells, we summarize, in this chapter, related mechanisms involved, discuss on potentential treatment until now disposable, and suggest preventive measures.

Introduction

During the last century, life expectancy at birth rose by a remarkable 30 years in Western countries and in Japan, initially because of reductions in infant, child, and maternal mortality and then because of declining mortality in middle and old age. So, during the past century, humans have gained more years of average life expectancy than in the last 10,000 years: we are now living in a rapidly ageing world [1]. Accordingly, the extraordinary increase of the elderly in developed countries underscore the importance of studies on ageing and longevity and the need for the prompt spread of knowledge about ageing in order to satisfactorily decrease the medical, economic and social problems associated to advancing years for the increase of the subjects which are not autonomous and are affected by invalidating pathologies [2].

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Ageing is a complex process, induced by an intricate interaction of genetic, epigenetic, stochastic and environmental factors. They determine the lost of molecular fidelity followed by an increased entropy [3, 4]. As result, loss complexity and random accumulation of damages (i.e., particularly damages to nuclear and mitochondrial DNA) at cellular, tissue, organ levels and/or of whole body arise, compatibly with the disposable soma theory of ageing [5]. Thus, it establishes a condition, which modifies both architecture and functioning of physiological processes and regulatory systems. This determines a deterioration of the homeostasis. Accordingly, it becomes more easily vulnerable to internal and external stressors, with frailty, disability and disease. On the other hand, the loss of DNA integrity, the principal random damages able in modifying cellular fidelity and inducing cellular and whole body senescence, determines the decline of the functionality of stress resistance and survival pathways (i.e., autophagic uptake mechanisms, chaperone systems, DNA repair mechanisms, apoptotic process, immune/inflammatory response), involved in cellular and organism defense to environmental stress and maintaining homeostasis.

As above mentioned, it is generally accepted that ageing has two principal determinants: the intrinsic disposition (genetic makeup, somatic capacity and composition) delineating what is maximally possible, and extrinsic factors (life style, nutrition, environmental influences) determining how the pre-set frame of opportunity is exploited in the course of the individual ageing trajectory. Extrinsic ageing is thus closely related to the quality, with which life-supportive tasks are adjusted to the environmental condition, and inseparably linked to mechanisms of stress response and adaptation. Insufficient adaptation and/or collateral maladaptation due to trade-offs with other probiotic or species-protective processes (e.g., fertility or tumour suppression) are thought to be major principles of extrinsic ageing [6].

As a result, it generates an imbalance between inflammatory and anti-inflammatory networks, which results in the low chronic grade of ageing pro-inflammatory status, "*inflamm-ageing*." Such chronic inflammatory response could build up with time and gradually causes tissue damage. It is, indeed, considered as one of the driving forces for age-related diseases such as diabetes, atherosclerosis, Alzheimer's disease and cancer (i.e., skin cancer) [7].

Augment of age-related body fat and consequent increase of visceral adiposity, age-related decline of sex hormones, oxidative and genotoxic stress, cellular and tissue damage, nutrition, alterations of physical condition of gut microbiota, other organs (brain, liver) and *immune and endocrine* systems have been associated with inflamm-ageing [8–10]. In addition, factors linking to physiological stress, such a long-term smoking and depression, seem also to contribute to inflammageing [8–10]. However, the most important factor for age related inflammation is the long-life pathogen burden [11]. Some recent studies have, indeed, evidenced associations between past infections and levels of chronic inflammation and increased risk of heart attack, stroke, and cancer [8, 10].

In line with these observations, it is possible to underline that a low grade of systemic inflammation characterizes ageing and inflammatory markers are significant predictors of mortality in old humans [12]. One the other hand, it has been recently proposed that during evolution the host defense and the ageing process have become linked together [4]. Host defense and ageing mechanisms seem to be overlapping. In particular, host defences seem to be involved in ageing process, to active inflammatory network and also to evocate the release of so-called *senescence associated secretory phenotype* (SASP), represented by a myriad of factors, such as the pro-inflammatory cytokines, chemokines, adhesion molecules, eicosanoids, growth factors, metallo-proteinases, nitric oxide, etc. [13, 14]. A large range of defense factors and mechanisms are involved in inducing of inflammatory network and related release of SASP, and they are all (or the major number) linked to the NF-κB pathway, an ancient signaling pathway specialized to the host defense [15, 16]. Namely, its induction is linked to several recognition pathway, i.e., Toll-

like receptors (TLRs) and inflammasome, as well as through different upstream kinase cascades via canonical or non-canonical pathways [9]. SASP occurs in several cells, such as fibroblasts and epithelial cells, and it participates, together the phenomenon of inflammageing, in the low chronic inflammation, improving both entropic ageing process and onset risk for age-related degenerative diseases, i.e., skin cancer. Several pathways and factors in the different cellular types induce the release of SASP [13]. On the other hand, ageing of different cell types, tissues and organs is associated with distinct patterns of altered gene expression and tissue function, whereas isolated genetic defects in ageing-relevant pathways give rise to segmental, tissue-selective ageing phenotypes. For most tissues it remains, however, unclear, which age-related alterations play a leading and causative role in the ageing process, and which ones are just epiphenomena. In this chapter, particular emphasis is given in describing skin ageing, related mechanisms and factors involved and potential anti-ageing strategies.

Skin Ageing

The ageing process is noticeable within all organs of the body, and manifests itself visibly in the skin. So, skin ageing is particularly important because of its social impact, and also represents an ideal model organ for investigating the ageing process. In skin, as well as in all organs, ageing is caused by a combination of factors. Metabolic processes and mitochondria cause increases in the levels of reactive oxygen species (ROS), which cause damage to all cellular macromolecules, including lipids, proteins and nucleic acids [17]. In addition, environmental factors such as smoke, pollution and ultraviolet (UV) radiation exposure (photo-ageing) make important contributions to skin ageing [18]. Aged human skin shows peculiar features that mirror the physiological decrement of its functions with time. With age, the skin becomes thinner, more transparent, flattened and fragile. Fine wrinkles appear, desquamation and wound healing are delayed, and skin appendages and their functions are reduced; thus, the skinis dry, and hair loss is common. Skin pigmentation produces typical age spots; occasionally, seborrhoeic keratosis, a benign neoplastic condition, appears [19].

In order to elucidate the related mechanisms, we briefly report the structure of skin and its function. As well recognized, skin is the physical barrier between the body's internal organs and the environment, and its failure causes loss of body temperature control; percutaneous loss of fluid, electrolytes and proteins; inability to prevent penetration of infective agents and dangerous substances; and inability to respond to tissue in curie [20, 21]. Three stratified regions compose normal skin: the hypodermis, which is the inner subcutaneous tissue and consists mostly of adipose cells supporting the upper connective tissue; the dermis; and the external and more complex layer, the epidermis. The dermis consists of fibroblasts that are able to synthesise and secrete several matrix proteins (different types of collagen, fibronectin, elastin, and glycans) into the extracellular space, conferring elasticity and resilience as well as resistance and strength to skin. The epidermis, which is the real protective barrier, continuously self-renews. It is rich in stratified cells called keratinocytes that proliferate in the basal layer and are committed to terminal differentiation through the upper spinous, granular and corneous layers. Epidermal stem cells, located in the basal layer and in the hair follicle bulge, are responsible for the continuous renewal of this organ, giving rise to transient amplifying cells that are able to undergo a specific number of divisions (clonal expansion) before ascending through the upper layers and terminally differentiating over a period of 2–3 weeks [20, 21]. Other cellular types are present in the epidermis such as pigment-producing melanocytes, antigen-presenting Langerhans cells and sensorial cells. All of these cells are subject to age-related changes and contribute to the acquisition of an aged skin phenotype. Precisely, epidermal stem cells are maintained at normal levels throughout life. Consequently, it supposes that skin ageing is rather due to their impaired mobilization or reduced capacity to respond to proliferative signals, likely

limited to very low number of stem cells with advancing age. In the skin, existence of several distinct stem cell populations has been reported. The self-renewal and multi-lineage differentiation of skin stem cells make these cells attractive for ageing process studies, but also for regenerative medicine, tissue repair, gene therapy, and cell-based therapy with autologous adult stem cells not only in dermatology. In addition, they provide in vitro models to study epidermal lineage selection and its role in the ageing process [19]. However, cutaneous ageing consists of distinct processes due to either intrinsic or extrinsic factors [20, 21]. Ageing of non-exposed skin areas is mainly due to intrinsic genetic or metabolic factors, while exposed areas of the body, such as the face and hands, are also influenced by extrinsic factors, particularly sunlight [20–22].

Intrinsic skin ageing is a physiological process that occurs as a result of chronological changes in tissue. A 10–50 % thinning of the epidermis between ages 30 and 80 years is the result of gradual tissue atrophy. In tissues with high turnover, such as the epidermis, stem cells and their transient amplifying progeny undergo an additional process, replicative senescence, which is a progressive reduction in their proliferative potential accompanied by the accumulation of senescent cells and a decline in tissue regenerative capability. All diploid cells undergo a finite number of successive divisions (Hayflick limit). The phenomenon of telomere shortening provides an explanation for this limit: the repetitive DNA sequences at the end of linear telomeric DNA shorten by approximately 50–200 bp per cell division; when they reach a minimal length, further cell divisions are prohibited. This phenomenon is probably not the only cause of cellular senescence, as demonstrated by the fact that non-dividing tissues, such as the brain, also age [22]. The balance between damage and repair ability appears to be the most accredited principle associated with cellular senescence theories. The metabolic rate is directly correlated with an increase in oxidative DNA damage and damage to other macromolecules, a cumulative effect that, together with an impaired cellular response and a specific gene expression signature, produces a senescent phenotype under these stress conditions [22]. Senescent cultured keratinocytes appear enlarged, flattened and vacuolised; they are arrested in the G1 phase of the cell cycle, positive for senescence-associated (SA) – β-gal staining and express senescence markers such as $p16^{INK4A}$ and hypo-phosphorylated retinoblastoma protein (Rb) [22]. In older subjects, a reduced cell number is also evident in dermal fibroblasts, melanocytes, other skin cell types and populations of hypodermic adipocytes. These events, together with a decreased cutaneous microvasculature, also lead to dermal atrophy, which is characterised by disaggregation and disintegration of collagen and elastic fibres.

The extrinsic ageing, also known as photo ageing, is clinically, biologically, and molecularly distinct from intrinsic ageing. Photoageing is typically characterized by prominent alterations of the cellular components and the extracellular matrix of the connective tissue. Photoageing primarily depends on the degree of sun exposure and skin pigment. Individuals who have outdoor lifestyles, live in sunny climates, and are lightly pigmented will experience the greatest degree of photoageing [22]. Solar UV radiations hurt epidermal and connective tissues, activating complex molecular cascades able to accelerate physiological ageing [22]. Photoageing is characterized by specific and peculiar clinical and histopathological features. The former include deep wrinkles, roughness and dryness, laxity, atrophy, yellowish complexion, hyper-chromic areas (solar lentigo, flat seborrheic keratoses, and freckles) and hypochromic areas, telangectasie, purpura, cutaneous fragility and pseudostellate scars, finally resulting in preneoplastic and neoplastic lesion development on chronically photo-exposed areas. UV damages can be linked mostly to the photochemical overproduction of ROS and reactive nitrogen species (RNS). ROS and RNS UV-generated can directly alter cellular components (DNA, proteins, lipids), and also affect regulation of gene expression of signalling molecules/cascades such as mitogen activated protein kinases (MAPKs) and interrelated inflammatory cytokines as well as NF-kB and activator protein-1 (AP-1) [22]. It is also well documented that photoexposure induces

the activation of the enzymatic systems, e.g., lipoxygenase (LOX) and cyclooxygenase (COX), which are responsible for the production of inflammatory mediators. Of particular interest is gene regulation and oxidative activation of matrix metalloproteinase (MMP), a family of Zn-dependent endopeptidases which are produced by different cell types and taken together are capable of degrading all the components of the intercellular matrix of the connective tissue. MMPs take part in the development of the alterations, typical of photoageing. Even limited exposure to solar light may induce MMP synthesis beyond the control of specific inhibitors [22]. The role played by ROS in controlling MMP activity has been largely documented, and a critical role is due to the activation of the transcription factor AP-1 [22].

The adverse acute and long-term effects of solar exposure are well established, and in general, are related to skin type. It is widely assumed that sensitivity to UV is directly related to pigmentation or tanning ability and this assumption is primarily based on epidemiological evidence that shows that skin cancer and photoageing are much less common in people who tan well or who have high levels of constitutive pigmentation [22]. Furthermore, studies comparing dark-skinned peoples with related albinos [22] show the latter to have a higher incidence of photoageing. Pigmentation, whether constitutive (i. e., base skin colour) or induced by UV, depends on the balance between two classes of melanins: the eumelanins that are insoluble black or brown nitrogenous pigments and phaeomelanins that are alkali-soluble yellow to reddish-brown pigments that usually contain sulphur as well as nitrogen [22]. Unlike eumelanins, which are mainly protective, phaeomelanins are considerably photolabile, and may produce highly cytotoxic and mutagenic free radical species on photoexcitation, which would account for the greater proclivity of red-haired Celtic-type population to photoageing, skin cancer, and sunburn [22].

In addition, individual ability to counteract noxious molecular events induced by UV, depends by the activation of a complex defence system against oxidative stress. Among the numerous defensive genes expressed during cellular stress response to UV exposure, a critical role seems to be played by a heterogeneous family of proteins, the heat shock proteins (HSPs) [22]. This family includes the HSP70, an inducible protein able to refold damaged proteins, and Heme Oxygenase 1 (HO-1), a redox sensitive enzyme with strong cytoprotective effects in several tissues, including skin [22]. Cellular ability to maintain adequate expression levels of protective genes such as HSP70 and HO-1, in response to a stressful insult, such as UV exposure, seems to be essential to preserve cellular homeostasis, and to delay ageing related degenerative processes [22]. Individual variability in the efficacy to activate these defensive genes is due to mechanisms not completely understood that include genetic makeup, responsible also for different phototypes and age. At molecular level post trascriptional regulation might represent a putative mechanism to modulate individual efficiency in the activation of cellular stress response. Post transcriptional regulation is fundamental to modify the half-life of some messenger RNAs [22]. Both HSP70 and HO-1 have been shown to be post transcriptionally regulated in various cell lines [22], and this process is thought to be altered during ageing. This evidence has been proposed as a possible cause for the impaired efficacy of defensive genes such as HSPs [22]. In recent years, natural derived polyphenols have attracted considerable attention because of their skin photoprotection effects [22]. Many of these substances have been shown to activate specifically the expression of some HSPs and in general genes involved in cellular stress response [22]. Having a better understanding of the protective role played by HSPs in photoageing processes and mechanisms, regulating their activation, will allow identifying the novel pharmaceutical strategies to prevent photoageing.

The immune system may either have a protective role against sunburn and skin cancer, or conversely, promote solar damage. The skin is poised to react to infections and injury, such as sunburn, with rapidly acting mechanisms (innate immunity) that precede the development of acquired immunity and serve as an immediate defense

system. Some of these mechanisms, including activation of defensins and complement, modify subsequent acquired immunity. An array of induced immune-regulatory and pro-inflammatory mediators is evident, at the gene expression level, from the microarray analysis of both intrinsically aged and photoaged skin. Thus, inflammatory mechanisms may accentuate the effect of UV radiation to amplify direct damageing effects on molecules and cells, including DNA, proteins, and lipids, which cause immunosuppression, cancer, and photoageing. A greater understanding of the cutaneous immune system's response to photo-skin interactions is essential to comprehensively protect the skin from adverse solar effects. Sunscreen product protection, measured only as reduction in redness (current "sun" protection factor) may no longer be sufficient, as it is becoming clear that protection against UV-induced immune changes is of equal if not of greater importance [23].

However, both intrinsic and extrinsic mechanisms, in a vicious circle, through ROS production and telomere shortening, are responsible of a skin pro inflammatory status, hence worsening skin ageing.

MicroRNA in Skin Ageing

MicroRNAs (miRNAs) are small noncoding RNAs that take part in post-transcriptional regulation either by arresting the translation or by cleavage (degradation) of mRNA targets. MiRNA regulation is performed by pairing the miRNA to sites in the messenger RNA of protein coding genes. miRNAs have been thought to be involved in many pathobiological processes (i.e., cell proliferation, death, differentiation, tissue degeneration, cancer, age-related diseases) and are believed to regulate the expression of approximately one-third of all human genes. Mature miRNAs bind to their target mRNAs by complete or incomplete complementation of their 50-end nucleotides 1–8 (seed sequences) with a binding site in the 30- or 50-untranslated regions of target transcripts or in the coding sequences. This process results in direct cleavage of the targeted mRNAs or inhibition of translation. Currently, nearly 1700 human miRNAs have been identified [24].

Recent findings demonstrate that microRNAs play key roles in regulating the balance between a cell's proliferative capacity and replicative senescence. Here, we focus on the molecular mechanisms regulated by senescence-associated microRNAs and their validated targets in both keratinocytes and dermal fibroblasts. In particular, we aim to highlight the contribution of miRNAs as modulators and regulators of cellular replicative senescence, focusing on keratinocytes and dermal fibroblasts [25].

The first study to identify senescence-associated miRNAs (SA-miRNAs) in human epidermal keratinocytes revealed a set of regulated miRNAs. In particular, miR-137 and miR-668 are upregulated during replicative cellular senescence and organismal ageing and they are able to induce senescence in proliferating human keratinocytes with a concomitant induction of the senescence markers p53 and $p16^{INK4A}$ [26]. However, the direct targets that are important for the described phenotype have not yet been identified. Rivetti di Val Cervo and colleagues determined that miR-138, miR-181a, miR-181b and miR-130b were upregulated during replicative senescence in keratinocytes [27]. These authors found that upregulation of these miRNAs (also singularly) in proliferating cells is sufficient per se to induce SA-β-galactosidase activity, suggesting that they interfere with important pathways involved in cell proliferation and maintenance. It is interesting that three of the miRNAs identified in this study, miR-138, miR-181a and miR-181b, target *SIRT1* mRNA, suggesting that Sirt1 activity is crucial for keratinocyte replicative senescence. In fact, *SIRT1* knockdown in proliferating keratinocytes induces cellular senescence [27]. Sirt1 is a member of the NAD^+-dependent deacetylase family. By the deacetylation of proteins, which regulates cellular stress responses, replicative senescence, immune responses and metabolism, Sirt1 protects cells and organisms against age-related diseases [28].

On the other hand, miR-130a targets *p63*mRNA. p63, which is a transcription factor and member of the p53 family, is known as the master gene of epithelia development [25]. P63 is

strongly involved in counteracting ageing/senescence both in vitro and in vivo. Indeed, p63- and p63 isoform-specific depletion induces premature ageing in different mouse models [25]. In human keratinocytes, *p63* knockdown is sufficient to induce cellular senescence, again suggesting an important role for p63 in counteracting cellular senescence and ageing in general. This potential role is strongly supported by the finding that p63 directly inhibits the expression of the senescence-inducing miRNAs miRNA-138, miRNA-181a, miR-181b and miR-130b [27]. The importance of miR-181a, miR181, miR138 and miR-130a, as well as their targets *p63* and *SIRT1*, has also been examined in a study of human skin ageing in vivo that was performed on a cohort of healthy young (<10 years) and older (>60 years) subjects. In this study, the SA-miRNAs were significantly upregulated in aged skin, in parallel with a significant downregulation of their targets *p63* and *SIRT1* [27]. The studies described thus far clearly demonstrate that modulation of SA-miRNA expression affects the execution of many gene expression programmes in human keratinocytes, resulting in the promotion or counteraction of cellular senescence. Many of the target genes identified (e.g., *p63*, *SIRT1*) are also strongly associated with organismal ageing.

In aged skin, human dermal fibroblasts (HDFs) lose the ability to remodel and organise the extracellular matrix (ECM), and evidence has shown that these features could also be mediated by miRNAs. In particular, this effect is due to decreased expression of transmembrane receptors, such as integrins, and components of the ECM, such as collagens, in senescent dermal fibroblasts. In a recent study, miR-152 and miR-181a were shown to induce senescence in proliferating human dermal fibroblasts. Interestingly, miR-152 has a specific role in ECM remodelling; in fact, its direct target is integrin alpha 5 (*ITGA5*). Integrin-α5 promotes cell adhesion and migration on fibronectin through activation of focal adhesion kinases; thus, it plays an important role in enhancing cell adhesion and migration. Through the downregulation of *ITGA5*, miR-152 is able to significantly reduce dermal fibroblast adhesion, suggesting an important role in the aged dermis. In addition, in senescent fibroblasts, the expression of collagen XVI (*COL16A1*) is downregulated, and it has been demonstrated that *COL16A1*mRNA is a direct target of miR-181a, which in turn is upregulated. Collagen XVI is a minor component of the skin ECM; it is expressed in the dermal-epidermal junction zone of the papillary dermis and connects ECM proteins to cells, ensuring mechanical anchoring. These findings suggest that miR-152 and miR-181a may have a complex role in the dermal ECM remodelling that is typical of aged skin [25].

The importance of miRNAs in inducing the replicative senescence of fibroblasts was highlighted by another study, in which it was shown that the expression of miR-29a and miR-30 increases during fibroblast senescence. miR-29a and miR-30 induce senescence by directly repressing the expression of *BMYB*. B-Myb is a transcription factor, and it regulates cellular senescence by controlling the expression of a variety of genes involved in cell proliferation; therefore, inhibition of *BMYB* expression by siRNA or exogenous overexpression of miR-29a and miR-30 results in senescence [25].

By contrast, the miR-17-92 cluster and miR-106 were found to be downregulated during dermal fibroblast senescence [25]. Many studies indicate that the miR-17-92 cluster contributes to the transcriptional regulation of genes involved in cell cycle control and tumorigenesis, such as *BCL2L11* (*BIM*), *p63*, *p57*, *p27* and *p21*, thus suggesting that these miRNAs counteract senescence by promoting proliferation [25]. Transcription of the miR-17-92 cluster is activated by the transcription factors e2f1 and e2f3 and repressed by p53 [25]. These transcriptional regulators account for the decreased expression of the miR-17-92 cluster in senescent cells. Fewer e2f family members have been observed in senescent cells, whereas p53, which is a decisive switch in ageing and tumorigenesis, is increasingly active in senescence [25]. Thus, miR-17-92 is actively, although it remains unclear how and why miR-17-92 is downregulated during ageing and senescence.

Microarray studies have also identified miRNAs involved in premature cellular

senescence after exposing cells to UVB irradiation and examining expression changes [25]. MiR-34c-5p is upregulated in irradiated cells and targets the 3′-UTR of *E2F3*. E2f3 plays an essential role in cell cycle progression, proliferation and development and protects dermal fibroblasts from UVB-induced premature senescence via the regulation of the senescence-related genes *p53* and *p21WAF-1* [25]. MiR-101 is also upregulated upon UVB irradiation and targets the 3′-UTR of *EZH2*. There is evidence that the functional interaction of miR-101 and *EZH2* is implicated in UVB-induced senescence of human dermal fibroblasts; however, the upregulation of miR-101 and the concomitant downregulation of Ezh2 are not sufficient to block the UVB-induced senescence phenotype, thus suggesting redundancy in this system [25].

All the evidence obtained by studying UV-induced senescence in vitro strongly suggests that miRNAs also have an important role in extrinsic skin ageing.

Finally, several studies on keratinocyte and fibroblast senescence have identified many miRNAs that target components of the conserved signalling pathways involved in ageing. In particular, senescence-associated miRNAs affect cell cycle regulators, chromatin modifiers, cell metabolism and cell adhesion. Additional studies on the skin senescence-associated miRNAs, including the identification of additional targets and their functions, will provide insight into how ageing mechanisms are regulated at the cell, tissue and organismal levels. Furthermore, it will be crucial to understand the upstream factors that control the differential expression of miRNAs during skin senescence and ageing.

Potentetial Anti-Ageing Interventions

The clinical treatment of choice, or "gold standard," to benefit both intrinsically aged and photoaged skin is the topical application of a class of molecules, the retinoids, which are derivatives of vitamin A [29]. Their positive effects on UV-damaged, photoaged skin are well characterised and influence both the collagenous and elastic dermal matrices. Clinically, the skin appears "rejuvenated," with significant reductions in the appearance of fine lines and wrinkles [30]. This is in part explained by the induction and deposition of newly synthesised collagens I and III [30] coupled with a significant increase in the number of anchoring fibrils [30]. More recent work has identified that retinoids can also induce deposition of fibrillin-rich microfibrils in the upmost papillary dermis, adjacent to the the dermal–epidermal junction [30], so potentially re-establishing a physical link between superficial skin layers and mature elastic fibres in the deep dermis. In an in vivo system, it has been shown that induction of fibrillin-1 expression occurs prior to that of the collagens, so making this elastic fibre component a useful biomarker of skin repair [30]. This system has now been used to assess the potential for repair of over-the-counter cosmetic products [30]. However, whilst the deposition of fibrillin in the dermal matrix following the application of both retionoids and over-the-counter cosmetic products is promising, it is still unclear whether the resultant newly formed fibrillin-rich microfibrils are structurally and functionally analogous to those thatthey seek to replenish. Finally, the effects of systemic treatments, such as the TGF antagonist losartan [30], on the structure and function of the ageing elastic fibre system remain to be determined.

Several anti-oxidants are also incorporated into topical skin care products, including vitamins C and E, co-enzyme Q10, ferulic acid, green tea, idebenone, pycnogenol and silymarin. Resurfacing procedures have been shown to sometimes spur the formation of new collagen with a normal staining pattern, as opposed to the basophilic elastotic masses of collagen characteristic of photo-aged skin [30]. It is possible that the potential of growth factors, cytokines and telomerase will eventually be harnessed via technological advancement and innovation in the burgeoning fields of tissue engineering and gene therapy [30].

Although there are several treatments available for aged skin, prevention of extrinsic ageing remains the best approach and should be encouraged to all patients. Of course, this entails

avoiding exposure to the sun, using sun-screen when sun avoidance is impossible, avoiding cigarette smoke and pollution, eating a diet high in fruits and vegetables, and taking oral anti-oxidant supplements or topical anti-oxidant formulations. The regular use of prescription retinoids can also help prevent or treat wrinkles.

Prevention

The formation of rhytides is considered the most conspicuous and common manifestation, and nearly a *sine qua non* feature, of skin ageing. Wrinkles appear as a result of changes in the lower, dermal layers of the skin. It might come as a surprise to many consumers, given the ubiquity of advertising that touts the newest topical formulations to eliminate wrinkles and the related expenditure of millions of dollars by consumers on these "anti-ageing" products, that few skin care product ingredients have the capacity to penetrate far enough into the dermis to ameliorate deep wrinkles. Prevention of wrinkle development, therefore, has assumed a fundamental status in anti-ageing skin care [30]. To prevent the formation of wrinkles, it is necessary to halt the degradation of the skin's three primary structural constituents, collagen, elastin and hyaluronic acid (HA), since all three components are known to decline with age. Consequently, most anti-ageing procedures and products are designed or formulated with the intention of salvaging at least one of these basic cutaneous substances. Because the technology required to suitably deliver these compounds into the skin has not yet been developed, topical products containing collagen, elastin or HA are unable to serve as adequate replacements for what is lost from the skin through ageing. Although no products replenish these key skin components, some products do promote the natural synthesis of these substances. For example, collagen production has been shown to be stimulated by the use of retinoids, vitamin C and copper peptide [30]. Collagen synthesis may also be brought about through the use of oral vitamin C. In animal models, retinoids have been shown to increase production of HA and elastin. HA levels are also thought to be augmented with glucosamine supplementation. There are no products yet approved for increasing the production of, or enhancing, elastin [30].

Because inflammation is a known contributor to the degradation of collagen, elastin and HA, reducing inflammation is another integral approach to preventing wrinkle formation. Anti-oxidants, all of which display various distinguishing characteristics and activities, are believed to be an important focus in this endeavour, as these free radical scavengers protect the skin via several mechanisms that are just beginning to be elucidated [30].

In terms of preventing the effects of photo-ageing, it is not yet known which anti-oxidants are the most effective. Using topical and oral anti-oxidants in combination will likely be the favoured recommendation in the near future. Anti-oxidants should also be used in combination with sunscreens and retinoids to enhance their protective effects. Indeed, it is worth remembering that not all sunscreens have an anti-oxidant effect and not all anti-oxidants have a sunscreen effect. However, a recent study has demonstrated that vitamins C and E combined with ferulic acid impart both a sunscreen effect and an anti-oxidant effect [30].

Conclusion

Skin ageing is a dynamic, multifactorial process, best characterized and understood in dichotomous expressions: intrinsic or natural ageing is cellularly determined, is inevitable and results in cutaneous alterations; extrinsic ageing, which also manifests in cutaneous changes, originates from exogenous sources and is avoidable. In other words, intrinsic ageing is a natural result of the passage of time, and not subject to the realm or whims of human control or behaviour. Extrinsic ageing results from various factors, but exposure to the sun is the primary source. Therefore, photo-ageing is roughly synonymous with, although technically a subset of, extrinsic ageing.

The American Academy of Dermatology, practicing dermatologists and other clinicians

have been preaching the mantra that "there is no such thing as a healthy tan," with some portion of the populace absorbing this message. Citing the attendant wrinkling and pigmentary changes associated with photo-ageing and the potentially more serious consequences of chronic sun exposure can be effective approaches for doctors, as this method appeals to an individual's strong concern about appearance. The clinical appearance of photoageing is characterized by rough, dry skin, mottled pigmentation and wrinkling. Such cutaneous manifestations, particularly when extensive or severe, can be harbingers of skin cancer. It is important for physicians to impress upon patients that photodamage represents the cutaneous signs of premature ageing. A summary of the role of telomeres in cellular ageing and cancer and/or a brief discussion of the differences between intrinsic and extrinsic ageing might prove useful in altering the behaviour of patients and stemming the tide of photodamage, photo-ageing, and photo-induced skin cancers.

The only known defences against photo-ageing beyond sun avoidance are using sunscreens to block or reduce the amount of UV reaching the skin, using retinoids to inhibit collagenase synthesis and to promote collagen production, and using anti-oxidants, particularly in combination, to reduce and neutralize free radicals.

Cross-References

- ▶ Cosmetic Anti-Aging Ingredients
- ▶ Cosmetics and Aging Skin
- ▶ Topical Growth Factors for Skin Rejuvenation
- ▶ Topical Peptides and Proteins for Aging Skin

References

1. Caruso C, Passarino G, Puca A, Scapagnini G. "Positive biology": the centenarian lesson. Immun Ageing. 2012;9:5.
2. Christensen K, McGue M, Petersen I, Jeune B, Vaupel JW. Exceptional longevity does not result in excessive levels of disability. Proc Natl Acad Sci U S A. 2008;105:13274–9.
3. Hayflick L. Entropy explains aging, genetic determinism explains longevity, and undefined terminology explains misunderstanding both. PLoS Genet. 2007;3:e220.
4. Salminen A, Kaarniranta K. Genetics vs. entropy: longevity factors suppress the NF-kappaB-driven entropic aging process. Ageing Res Rev. 2010;9:298–314.
5. Kirkwood TB, Holliday R. The evolution of ageing and longevity. Proc R Soc Lond B Biol Sci. 1979;205:531–46.
6. Kirkwood TB, Melov S. On the programmed/non-programmed nature of ageing within the life history. Curr Biol. 2011;21:R701–7.
7. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci. 2014;69 Suppl 1:S4–9.
8. Licastro F, Candore G, Lio D, Porcellini E, Colonna-Romano G, Franceschi C, Caruso C. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. Immun Ageing. 2005;2:8.
9. Balistreri CR, Caruso C, Candore G. The role of adipose tissue and adipokines in obesity-related inflammatory diseases. Mediators Inflamm. 2010;2010:1–19.
10. Balistreri CR, Colonna-Romano G, Lio D, Candore G, Caruso C. TLR4 polymorphisms and ageing: implications for the pathophysiology of age-related diseases. J Clin Immunol. 2009;29:406–15.
11. Candore G, Caruso C, Colonna-Romano G. Inflammation, genetic background and longevity. Biogerontology. 2010;11:565–73.
12. Balistreri CR, Candore G, Accardi G, Colonna-Romano G, Lio D. NF-κB pathway activators as potential ageing biomarkers: targets for new therapeutic strategies. Immun Ageing. 2013;10:24.
13. Salminen A, Kauppinen A, Kaarniranta K. Emerging role of NF-κB signaling in the induction of senescence-associated secretory phenotype (SASP). Cell Signal. 2012;24:835–45.
14. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest. 2013;123:966–72.
15. Gilmore TD, Wolenski FS. NF-κB: where did it come from and why? Immunol Rev. 2012;246:14–35.
16. Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol. 2012;4:1–19.
17. Rattan SI. Theories of biological aging: genes, proteins, and free radicals. Free Radic Res. 2006;40:1230–8.
18. Yaar M, Gilchrest BA. Photoageing: mechanism, prevention and therapy. Br J Dermatol. 2007;157:874–87.
19. Zouboulis CC, Makrantonaki E. Clinical aspects and molecular diagnostics of skin aging. Clin Dermatol. 2011;29:3–14.
20. Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol. 2008;17:1063–72.
21. Farage MA, Miller KW, Elsner P, Maibach HI. Characteristics of the Aging Skin. Adv Wound Care (New Rochelle). 2013;2(1):5–10.

22. Naylor EC, Watson RE, Sherratt MJ. Molecular aspects of skin ageing. Maturitas. 2011;69:249–56.
23. Zhuang Y, Lyga J. Inflammaging in skin and other tissues – the roles of complement system and macrophage. Inflamm Allergy Drug Targets. 2014;13:153–61.
24. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
25. Mancini M, Lena AM, Saintigny G, Mahé C, Di Daniele N, Melino G, Candi E. MicroRNAs in human skin ageing. Ageing Res Rev. 2014;17:9–15.
26. Shin KH, Pucar A, Kim RH, Bae SD, Chen W, Kang MK, Park NH. Identification of senescence-inducing microRNAs in normal human keratinocytes. Int J Oncol. 2011;39:1205–11.
27. Rivetti di Val Cervo P, Lena AM, Nicoloso M, Rossi S, Mancini M, Zhou H, Saintigny G, Dellambra E, Odorisio T, Mahé C, Calin GA, Candi E, Melino G. p63-microRNA feedback in keratinocyte senescence. Proc Natl Acad Sci U S A. 2012;109:1133–8.
28. Donmez G, Guarente L. Aging and disease: connections to sirtuins. Aging Cell. 2010;9:285–90.
29. Kafi R, Kwak HSR, Schumacher WE, et al. Improvement of naturally aged skin with vitamin A (retinol). Arch Dermatol. 2007;143:606–12.
30. Baumann L. Skin ageing and its treatment. J Pathol. 2007;211:241–51.