Nutrition for Healthy Skin

Strategies for Clinical and Cosmetic Practice

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Skin Aging

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Core Messages

- Skin aging is caused by
  (i) UV radiation,
  (ii) Infrared radiation,
  (iii) tobacco smoke and
  (iv) traffic related particulate matter
- Damage to macromolecules such as mtDNA and proteins in dermal fibroblasts drives chronic skin aging

2.1 Introduction

For decades it has been appreciated that aging is the consequence of both genetic and environmental influences. Genetic factors are evident, e.g., in the >100-fold variation among species in the rate of aging; and recent studies of fruit flies, worms, and even mice have identified specific longevity genes whose modification can greatly alter lifespan [22]. Conversely, a role for environmental factors can be deduced both from epidemiologic and laboratory-based experimental data. Such influences include ionizing radiation, severe physical and psychological stress, overeating versus caloric restriction, and in the case of skin ultraviolet irradiation.

In this regard, skin is no exception as skin aging results from intrinsic (genetic, endocrinologic) and extrinsic (environmental) factors. In this chapter I will focus on extrinsic skin aging for the following reasons: (a) The overall topic of this chapter is functional food for skin or, in other words, manipulation of skin aging by nutrition-based strategies; (b) It has already been shown for topical approaches (sunscreens, cosmeceuticals, etc.) that extrinsic skin aging can be effectively manipulated. (iii) And thus, nutrition-based anti-skin-aging strategies will be most effective if they are directed against extrinsic skin aging.

Extrinsic and intrinsic skin aging can be clearly distinguished at a clinical, histological, and molecular level. The two most prominent clinical signs of extrinsic skin aging are the formation of coarse wrinkles and an increase in the number of pigment spots (Fig. 2.1). Interestingly, ethnic differences exist, because, e.g., Caucasian women develop earlier and more severe skin wrinkling whereas Japanese women show more lentigines at a younger age. Among all environmental factors, solar ultraviolet (UV) radiation is most important for extrinsic skin aging, a process accordingly also termed photoaging.

Within recent years substantial progress has been made in elucidating the underlying molecular mechanisms. From these studies it is now clear that both UVB (290–320 nm) and UVA (320–400 nm) radiation contribute to photoaging. UV-induced alterations at the level of the dermis are best studied and appear to

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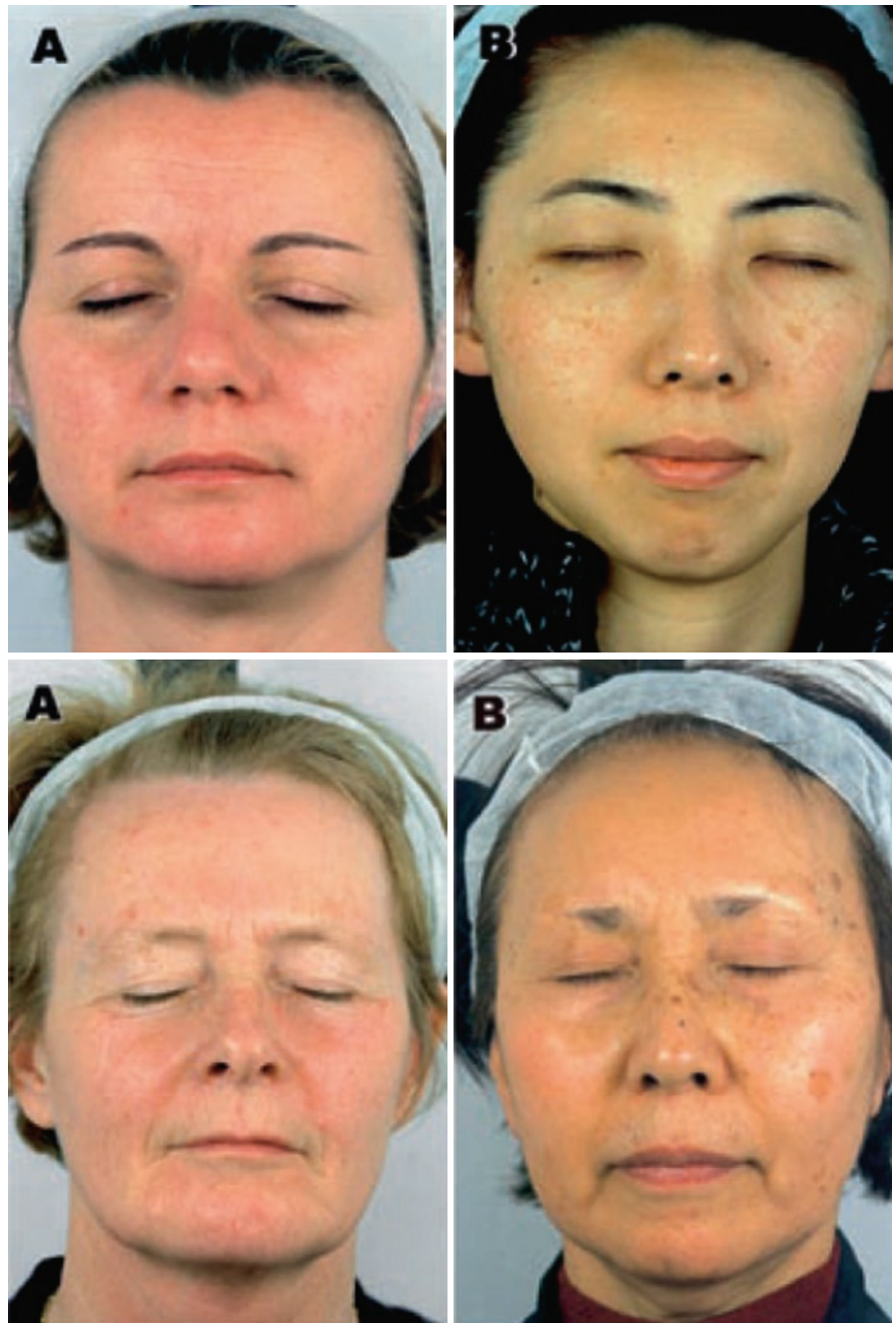

Fig. 2.1 Coarse wrinkle (**a**) and pigment spot (**b**) formation in extrinsic skin aging

be largely responsible for the phenotype of photoaged skin. It is also generally agreed that UVB acts preferentially on the epidermis where it not only damages DNA in keratinocytes and melanocytes but also causes the production of soluble factors including proteolytic enzymes which then in a second step affect the dermis; in contrast UVA radiation penetrates far more deeply on average and hence exerts direct effects on both the epidermal and the dermal compartments (Fig. 2.1). UVA is also 10–100 times more abundant in sunlight than UVB, depending on the season and time of day. It has therefore been proposed that, although UVA photons are individually far less biologically active than UVB photons, UVA radiation

may be at least as important as UVB radiation for the pathogenesis of photoaging [4].

It should be noted that extrinsic skin aging is not exclusively due to solar UV irradiation. Accordingly, also other wavelengths within the solar spectrum, most notably near infrared radiation (IRA; 770–1,400 nm), have been shown to contribute to skin aging, in particular to the formation of coarse wrinkles [25]. The relative contribution of IRA to photoaging is currently not known, but likely to be very relevant. Accordingly, IRA radiation constitutes one-third of the energy that is being emitted by the sun and that reaches the earth surface and thus human skin. Also, IRA radiation deeply penetrates into the human skin with 50% of the energy reaching the dermis, and at a molecular level, the magnitude of IRA radiation-induced collagen breakdown appears to be similar to that caused by UVA radiation.

It is also important to realize that at least two other environmental factors contribute to extrinsic skin aging independent of solar radiation [33]. Accordingly, exposure to tobacco smoke is well known to cause wrinkle formation, elastosis, and teleangiectasia, whereas exposure to traffic-related, airborne particulate matter significantly increases the number of pigment spots (= lentigines) [40].

In the past, the pathogenesis of extrinsic skin aging has been a major research focus and most work has been done with UV radiation. Despite all these efforts the exact mechanisms by which UV radiation causes premature skin aging is not completely clear. In these studies a number of molecular pathways have been described to explain one or more of the key features of photoaged skin. Some of these models are based on irradiation protocols which use single or few UV exposures, whereas others take into account the fact that photoaging results from chronic UV damage and as a consequence employ chronic repetitive irradiation protocols. Still others rely on largely theoretic constructs rather than on experimental observations.

2.2 Mechanisms of UV-Induced Photoaging

Of interest is the fact that most if not all age-accelerating environmental factors damage DNA either directly or indirectly, often through oxidative damage [28]. In addition, rate of aging among species correlates inversely with rate and fidelity of DNA repair [20] and most progeroid syndromes for which the genetic lesion has been identified have impaired DNA replication and/or DNA damage responses [28]. In combination with the fact that cumulative DNA damage accompanies chronologic aging [41], these observations suggest that both the indisputable heritable component and the environmental component of aging result in large part from changing DNA status during the individual's life. The next section of this chapter summarizes the current evidence that damage to mtDNA is of major importance in photoaging and in fact might drive and promote photoaging in a chronic fashion, i.e., over decades [5, 25]. The subsequent sections provide detailed information now available with regard to specific aging targets and signaling pathways responsible for photoaging-associated morphologic and functional changes in skin. These include UV-induced alterations of connective tissue components, vascularization patterns, inflammatory cells, protein oxidation and IRA radiation-induced retrograde signaling cascades. At the end I will present a unifying concept that reconciles the most recent findings in an attempt to provide a novel and comprehensive model to explain photoaging and a framework for the development of nutrition-based strategies to prevent, delay, or reverse skin aging.

2.3 Mitochondrial DNA Mutations and Photoaging

Mitochondria are organelles whose main function is to generate energy for the cell. This is achieved by a multi-step process called oxidative phosphorylation or electron transport chain. Located at the inner mitochondrial membrane are five multi-protein complexes that generate an electrochemical proton gradient used in the last step of the process to turn ADP and organophosphate into ATP. This process is not completely error free and ultimately this leads to the generation of reactive oxygen species (ROS), making the mitochondrion the site of the highest ROS turnover in the cell. In close proximity to this site lies the mitochondrions' own genomic material, the mitochondrial (mt)DNA. The human mtDNA is a 16,559-bp-long, circular, and double-stranded molecule of which four

to ten copies exist per cell. Mitochondria do not contain any repair mechanism to remove bulky DNA lesions; although they do contain base excision repair mechanism and repair mechanisms against oxidative damage, the mutation frequency of mtDNA is approximately 50-fold higher than that of nuclear DNA. Mutations of mtDNA have been found to play a causative role in degenerative diseases such as Alzheimer's disease, chronic progressive external ophthalmoplegia, and Kearns-Sayre syndrome [14]. In addition to degenerative diseases, mutations of mtDNA may play a causative role in the normal aging process with an accumulation of mtDNA mutations accompanied by a decline of mitochondrial functions [42]. Recent evidence indicates that mtDNA mutations are also involved in the process of photoaging [4, 25].

Photoaged skin is characterized by increased mutations of the mitochondrial genome [1, 7, 44]. Intraindividual comparison studies have revealed that the so-called common deletion, a 4,977 base pair deletion of mtDNA, is increased up to tenfold in photoaged skin, as compared with sun-protected skin of the same individual. The amount of the common deletion in human skin does not correlate with chronological aging [24], and it has therefore been proposed that mtDNA mutations such as the common deletion represent molecular markers for photoaging. In support of this concept, it was shown that repetitive, sublethal exposure to UVA radiation at doses acquired during a regular summer holiday induces mutations of mtDNA in cultured primary human dermal fibroblasts in a singlet oxygen-dependent fashion [3]. Even more important, in vivo studies have revealed that repetitive three-times daily exposure of previously unirradiated buttock skin for a total of 2 weeks to physiological doses of UVA radiation leads to an approximately 40% increase in the levels of the common deletion in the dermal, but not epidermal compartment of irradiated skin [5]. Furthermore, it was shown that, once induced, these mutations persist for at least 16 months in UV-exposed skin. Interestingly, in a number of individuals, the levels of the common deletion in irradiated skin continued to increase with a magnitude up to 32-fold. It has been postulated for the normal aging process as well as for photoaging that the induction of ROS generates mtDNA mutations, in turn leading to a defective respiratory chain and, in a vicious cycle, inducing even more ROS and subsequently allowing mtDNA mutagenesis independent of the inducing agent [21]. It is the characteristic of vicious cycles that they evolve at ever-increasing speeds. Thus, the increase of the common deletion up to levels of 32-fold, independent of UV exposure, may represent the first in vivo evidence for the presence of such a vicious cycle in general and in human skin in particular (Fig. 2.3).

The mechanisms by which generation of mtDNA mutations by UVA exposure translates into the morphologic alterations observed in photoaging of human skin are currently being unraveled. In general, a cause–effect relationship between premature aging and mtDNA mutagenesis is strongly suggested by studies employing homozygous knock-in mice that express a proof-reading-deficient version of PolgA, the nucleus-encoded subunit of mtDNA polymerase [38]. As expected, these mice developed a mtDNA mutator phenoytpye with increased amounts of deleted mtDNA. This increase in somatic mtDNA mutations was found to be associated with reduced lifespan and premature onset of aging-related phenoytypes such as weight loss, reduced subcutaneous fat, alopecia, kyphosis, osteoporosis, anemia, reduced fertility, and heart enlargement.

In addition, recent studies demonstrate that UVA radiation-induced mtDNA mutagenesis is of functional relevance in primary human dermal fibroblasts and apparently has molecular consequences suggestive of a causative role of mtDNA mutations in photoaging of human skin as well [2]. Accordingly, induction of the common deletion in human skin fibroblasts is paralleled by a measurable decrease of oxygen consumption, mitochondrial membrane potential, and ATP content, as well as an increase of MMP-1, while TIMP remains unaltered, an imbalance that is known to be involved in photoaging of human skin (see below). These observations suggest a link not only between mutations of mtDNA and cellular energy metabolism, but also between mtDNA mutagenesis, energy metabolism, and a fibroblast gene expression profile that would functionally correlate with increased matrix degradation and thus premature skin aging. In order to provide further evidence for the role of the energy metabolism in mtDNA mutagenesis and the development of this "photoaging phenotype," the effect of creatine was studied in these cells. This applied the hypothesis that generation of phosphocreatine, and consequently ATP, is facilitated if creatine is abundant in cells. This would

allow easier binding of existing energy-rich phosphates to the energy precursor creatine. Indeed, experimental supplementation of normal human fibroblasts with creatine normalized mitochondrial mutagenesis as well as the functional parameters, oxygen consumption and MMP-1, while an inhibitor of creatine uptake abrogated this effect [2].

The studies discussed above always required the UV radiation-induced formation of mtDNA mutations prior to functional analysis and thus it was not possible to differentiate between functional consequences resulting from mtDNA mutagenesis and those which were UV-induced but occurred independent of damage to mtDNA. In recent studies this problem has been addressed by employing unirradiated dermal fibroblasts. Mitochondrial DNA was partially depleted from these cells in order to generate phenocopies of large-scale deletion bearing fibroblasts [35]. Subsequent analysis of their gene expression pattern showed striking similarities to that expressed by dermal fibroblasts in photoaged skin, indicating that the presence of mtDNA deletions in skin fibroblasts resulted in functional alterations which were of pathogenic relevance for photoaging. This assumption was further corroborated and extended by recent studies in which primary human skin fibroblasts from patients with the mitochondriopathy Kearns-Sayre Syndrome (KSS) were used [29]. These cells constitutively carry large amounts of UV-inducible large-scale mutations of mtDNA such as the common deletion. They were used to generate three-dimensional dermal equivalents by seeding them into collagen gels. Interestingly, within 6 weeks after contraction of gels, KSS, in comparison to normal dermal equivalents, showed many features reminiscent of photoaging. These include an overexpression, both at the mRNA and protein level, as well as an increased activity of matrix metalloproteinase-1 (see next paragraph), a rarefication of collagen fibers, an increased amount of fragmented collagen fibers, an increase in oxidized proteins, signs of neovascularization, and an overexpression of lysyl oxidase-1 [29]. Taken together these studies strongly indicate that the presence of large-scale deletions of mtDNA in human dermal fibroblasts is causally related to photoaging because it leads to an altered gene expression pattern in these cells and subsequently to structural and functional alterations of the human dermis which are characteristic for photoaged human skin [25].

2.4 Connective-Tissue Alterations in Photoaging: The Role of Matrix Metalloproteinases and Collagen Synthesis

Photoaged skin is characterized by alterations of the dermal connective tissue. The extracellular matrix in the dermis mainly consists of type I and type III collagen, elastin, proteoglycans, and fibronectin. In particular, collagen fibrils are important for the strength and resiliency of skin, and alterations in their number and structure are thought to be responsible for wrinkle formation.

In photoaged skin, collagen fibrils are disorganized and abnormal elastin-containing material accumulates [36]. Biochemical studies have revealed that in photoaged skin levels of types I and III collagen precursors and cross-links are reduced, whereas elastin levels are increased [9, 37].

How does UV radiation cause these alterations? In principle it is conceivable to assume that UV radiation leads to an enhanced and accelerated degradation and/or a decreased synthesis of collagen fibers and our current knowledge indicates that both mechanisms may be involved.

A large number of studies unambigously demonstrate that the induction of matrix metalloproteinases (MMPs) play a major role in the pathogenesis of photoaging. As indicated by their name, these zinc-dependent endopeptidases show proteolytic activity to degrade matrix proteins such as collagen and elastin. Each MMP degrades different dermal matrix proteins, e.g., MMP-1 cleaves collagen type I, II, III, whereas MMP-9, which is also called gelatinase, degrades collagen type IV, V, and gelatin. Under basal conditions, MMPs are part of a coordinate network and are precisely regulated by their endogenous inhibitors, i.e., tissue-specific inhibitors of MMPs (TIMPs), which specifically inactivate certain MMPs. An imbalance between activation of MMPs and their respective TIMPs could lead to excessive proteolysis.

It is now very well established that UV radiation induces MMPs without affecting the expression or activity of TIMPs [17, 31]. These MMPs can be induced by both UVB and UVA radiation, but the underlying photobiological and molecular mechanisms differ depending on the type of irradiation. In a very simplified scheme, UVA radiation would mostly act

indirectly through the generation of reactive oxygen species, in particular singlet oxygen, which subsequently can exert a multitude of effects such as lipid peroxidation, activation of transcription factors and generation of DNA-strand breaks [31]. While UVB radiation-induced MMP induction has been shown to involve the generation of ROS as well [43], the main mechanism of action of UVB is the direct interaction with DNA via the induction of DNA damage. Recent studies have indeed provided evidence that enhanced repair of UVB-induced cyclobutane pyrimidine dimers in the DNA of epidermal keratinocytes through topical application of liposomally-encapsulated DNA repair enzymes on UVB-irradiated human skin prevents UVB radiation-induced epidermal MMP expression [15].

The activity of MMPs is tightly regulated by transcriptional regulation and elegant in vivo studies by Fisher et al. have demonstrated that exposure of human skin to UVB radiation leads to the activation of the respective transcription factors [16]. Accordingly, UV exposure of human skin not only leads to the induction of MMPs within hours after irradiation, but already within minutes, transcription factors AP-1 and NFkB, which are known stimulatory factors of MMP genes, are induced. These effects can be observed at low UVB dose levels, because transcription factor activation and MMP-1 induction could be achieved by exposing human skin to one-tenth of the dose necessary for skin reddening (0.1 minimal erythema dose). Subsequent work by the same group clarified the major components of the molecular pathway by which UVB exposure leads to the degradation of matrix proteins in human skin Low-dose UVB irradiation induced a signaling cascade which involves upregulation of epidermal growth factor receptors (EGFR), the GTP-binding regulatory protein p21Ras, extracellular signal-regulated kinase (ERK), c-jun amino terminal kinase (JNK), and p38. Elevated c-jun together with constitutively expressed c-fos increased activation of AP-1. Identification of this UVB-induced signaling pathway does not only unravel the complexity of the molecular basis which underlies UVB radiation-induced gene expression in human skin, but also provides a rationale for the efficacy of tretinoin (all-trans-retinoic acid) in the treatment of photoaged skin. Accordingly, topical pretreatment with tretinoin inhibited the induction and activity of MMPs in UVB-irradiated skin through prevention of AP-1 activation.

In addition to the destruction of existing collagen through activation of MMPs, failure to replace damaged collagen is thought to contribute to photoaging as well. Accordingly, in chronically photodamaged skin, collagen synthesis is downregulated as compared to sun-protected skin [18]. The mechanism by which UV radiation interferes with collagen synthesis is not yet known but in a recent study evidence has been provided that fibroblasts in severely (photo)damaged skin have less interaction with intact collagen and are thus exposed to less mechanical tension, and it has been proposed that this situation might lead to decreased collagen synthesis [39].

2.5 UV-Induced Modulation of Vascularization

There is increasing evidence that cutaneous blood vessels may play a role in the pathogenesis of photoaging. Photoaged skin shows vascular damage which is absent from intrinsically aged skin. In mildly photodamaged skin, there is venular wall thickening, while in severely damaged skin the vessel walls are thinned and supporting perivascular veil cells are reduced in number [10]. The number of vascular cross-sections is reduced [23] and there are local dilations, corresponding to clinical teleangiectases. Overall, there is a marked change in the horizontal vascularization pattern with dilatated and distorted vessels. Studies in humans as well as in the hairless skh-1 mouse model for skin aging have demonstrated that acute and chronic UVB irradiation greatly increases skin vascularization [6, 45].

The formation of blood vessels from preexisting vessels is tightly controlled by a number of angiogenic factors as well as factors which inhibit angiogenesis. These growth factors include basic fibroblast growth factor, interleukin-8, tumor growth factor-beta, platelet-derived growth factor, and vascular endothelial growth factor (VEGF). VEGF appears to be involved in chronic UVB damage because UVB radiation-induced dermal angiogenesis in Skh-1 mice is associated with increased VEGF expression in the hyperplastic epidermis of these animals [45]. Even more important, targeted overexpression of the angiogenesis inhibitor Thrombospondin-1 does not only prevent UVB radiation-induced skin vascularization and endothelial cell proliferation, but significantly reduces dermal photodamage and wrinkle formation. These studies suggest that UVB radiation-induced angiogenesis plays a direct biological role in photoaging.

2.6 Photoaging as a Chronic Inflammatory Process

In contrast to intrinsically aged skin, which shows an overall reduction in cell numbers, photoaged skin is characterized by an increase in the number of dermal fibroblasts, which appear hyperplastic, but also by increased numbers of mast cells, histiocytes, and mononuclear cells. The presence of such a dermal infiltrate indicates the possibility that a chronic inflammatory process takes place in photoaged skin and in order to describe this situation the terms heliodermatitis and dermatoheliosis have been coined [26]. More recent studies have shown that increased numbers of CD4+ T-cells are present in the dermis whereas intraepidermally, infiltrates of indeterminate cells and a concomitant reduction in the number of epidermal Langerhans cells have been described [13, 19]. It is currently not known whether the presence of inflammatory cells represents an epiphenomenon or whether these cells play a causative role in the pathogenesis of photoaging, e.g., through the production of soluble mediators which could affect the production and/or degradation of extracellular matrix proteins.

2.7 Protein Oxidation and Photoaging

The aging process is accompanied by enhanced oxidative damage. All cellular components including proteins are affected by oxidation [27]. Protein carbonyls may be formed either by oxidative cleavage of proteins or by direct oxidiation of lysine, arginine, proline, and threonine residues. In addition, carbonyl groups may be introduced into proteins by reactions with aldehydes produced during lipid peroxidation or with reactive carbonyl derivatives generated as a consequence of the reaction with reducing sugars or their oxidation products with lysine residues of proteins.

Within the cell, the proteasome is responsible for the degradation of oxidized proteins. During the aging process this function of the proteasome is diminished and oxidized proteins accumulate. In addition, lipofuscin, a highly cross-linked and modified protein aggregate is formed. This aggregate accumulates within cells and is able to inhibit the proteasome. These alterations mainly occur within the cytoplasm and lipofuscin does not accumulate in the nucleus.

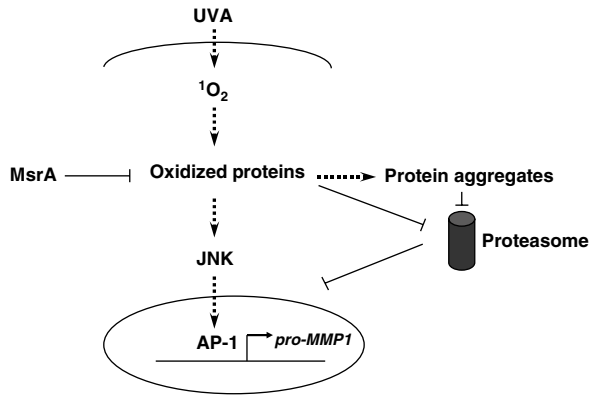

Fig. 2.2 Role of protein oxidation in photoaging of human skin. Ultraviolet radiation generates oxidized proteins through a singlet oxygen-dependent mechanism. Oxidized proteins as well as resulting protein aggregates inhibit proteasomal function. This leads to an accumulation of transcription factors such as AP-1. The resulting increase in MMP-expression eventually leads to collagen degradation and wrinkle formation

In biopsies from individuals with histologically confirmed solar elastosis, an accumulation of oxidatively modified proteins was found specifically within the upper dermis [30]. Protein oxidation in photoaged skin was most likely due to UV irradiation, because repetitive exposure of human buttock skin on 10 days to increasing UV doses as well as in vitro irradiation of cultured dermal fibroblasts to UVB or UVA radiation caused protein oxidation. The functional relevance of increased protein oxidation in UV-irradiated dermal fibroblasts, in particular with regard to the pathogenesis of photoaging, has recently been unraveled. In these studies, it was observed that increased protein oxidation that may result from exposure of human fibroblasts to UVA radiation inhibits proteasomal functions and thereby affects intracellular signaling pathways which are involved in MMP-1 expression (Fig. 2.2).

2.8 Infrared a Radiation-Induced Retrograde Signaling

Similar to UVB or UVA, IRA radiation is a potent regulator of gene expression in human dermal fibroblasts [12]. In particular, there is no more doubt that IRA radiation causes an imbalance between MMP-1 versus TIMP-1 expression in favor of MMP-1 [32] and at the same time decreases COL 1A1 and COL 1A2

expression [11] and thereby leads to a rarefication of collagen fibers and eventually to wrinkle formation. Importantly, the signaling mechanisms involved in IRA radiation-induced gene regulation differ completely from those induced by UVB or UVA radiation [34]. Accordingly, IRA radiation is primarily absorbed by cupper atoms in complex IV of the mitochondrial respiratory chain. The first detectable signaling event is the subsequent intramitochondrial generation of ROS. This intramitochondrial signal is then transmitted to the cytoplasm where it causes an increase in calcium levels, followed by an activation of MAPKs and the subsequent intranuclear transcriptional activation of IRA-responsive genes (Fig. 2.3). The importance of intramitochondrial ROS production for the elicitation of this retrograde signaling response is emphasized by the fact that mitochondrially targeted antioxidants are highly effective in blocking this signaling cascade in vitro and in preventing IRA-radiation-induced MMP-1 upregulation in vivo in human skin [35].

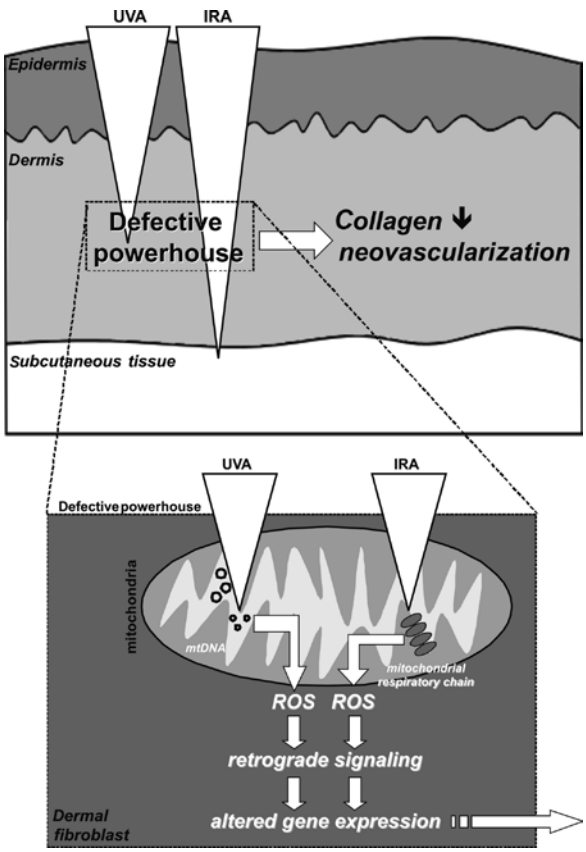

Fig. 2.3 Defective powerhouse model of cutaneous ageing. UVA and IRA via different mechanisms lead to the disruption of the mitochondrial function ("defective powerhouse") which results in changes in the dermal compartment of the skin and leads to photoageing. *Insert /lower panel*: Repetitive UVA irradiation results in the increased formation of large-scale deletions of mtDNA (*left*). Also, even a single dose of IRA leads to a disruption of the mitochondrial electron transport chain (*right*). Both events cause an increased production of ROS and thereby initiate retrograde signalling responses

2.9 Concluding Remarks: The Defective Powerhouse Model of Photoaging of Human Skin

From the above it is evident that major progress has been made recently in identifying molecular mechanisms involved in photoaging. In this regard, skin has proven to serve as an excellent model organ to understand basic mechanisms relevant for extrinsic aging.

Despite all this progress, however, a general, unifying concept linking the different mechanisms and molecular targets described in the previous paragraphs is still missing. In other words, the critical question to answer is: How do mitochondrial DNA mutagenesis, neovascularization, protein oxidation, downregulation of collagen synthesis, and increased expression of matrixmetalloproteinases together cause photoaging of human skin? Which of these mechanisms are of primary importance and responsible for inducing others? Are some or all of the above-mentioned characteristics of photoaged skin merely epiphenomena and, if so, to what extent causally related to premature skin aging?

The current state of knowledge does not allow to answer these questions in a definitive manner. Nevertheless I have proposed a hypothesis which tries to reconcile most of the research discussed above in one model [25].

I envision photoaging of human skin to be initiated and driven by UV radiation-induced mitochondrial DNA mutagenesis in the dermis of human skin. I believe that the persistence of UV radiation-induced mitochondrial DNA mutations and the resulting vicious cycle with further increases in mitochondrial DNA mutations leads to a situation which can best be described as a "defective powerhouse" where inadequate energy production leads to chronic oxidative stress (Fig. 2.3). In the dermis, functional consequences of direct DNA damage and aberrant ROS production in human dermal fibroblasts could be (a) an altered gene

expression pattern which would affect neovascularization and collagen metabolism and possibly also the generation of an inflammatory infiltrate and (b) the oxidation of intracellular proteins, inhibition of the proteasome, and again an altered gene expression pattern with detrimental consequences for collagen metabolism. Evidence supporting this model has recently been generated in human-skin-equivalent models employing dermal fibroblasts which constitutively carry large amounts of UV-inducible mtDNA deletions [29]. Ongoing studies will answer the question whether dermal mtDNA mutagenesis is also of importance for epidermal photoaging (= inside – outside mechanism), or whether epidermal changes are due to direct UV-induced effects, e.g., DNA damage in combination with indirect ROS-induced damage, which would be expected to cause the well-documented UV signature mutations in p53 [8] leading to poorly regulated growth and differentiation of epidermal cells associated with discrete premalignant actinic keratosis and diffuse photoaging (outside–inside mechanism).

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