Photoaging of the skin

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Solar radiation at the surface of the earth includes ultraviolet radiation (UV : 290-400nm), visible light (400-760nm) and infrared radiation (760nm-1mm) (**Fig. 1**).

Extrinsic skin aging is superimposed on intrinsic skin aging process and is due primarily to UVR (solar ultraviolet radiation) and partly by other factors, such as infrared light, smoking and air pollutants. UVR has been divided into ultraviolet B (UVB: 290-320nm) which principally generates pyrimidine dimer type DNA damage through direct absorption and ultraviolet A (UVA: 320-400nm), which indirectly produces base oxidation via UV-induced ROS. Recently, UVA radiation at high dose is reported to produce cyclobutane pyrimidine dimmers.

Intrinsic aging of the skin, on the other hand, is characterized by the decline of biological function, a decrease in adaptation to stress, and structural damage due to reactive oxygen species (ROS) from cellular metabolism.

Recent advances in understanding mechanisms of aging and photoaging have enhanced our ability to develop strategies to prevent, slow, and rejuvenate the altered structure and function of photoaged skin.

In this review, we discuss the mechanisms of photoaging of the skin with relevance to acute and chronic skin reactions to solar UVB, UVA and infrared radiation, and summarize briefly the clinical approaches for prevention and the treatment of photoaging with topical and systemic use of anti-aging materials. Finally, a range of therapeutic modalities available to reverse or retard the visible signs of photoaged skin will be discussed briefly.
1. Solar ultraviolet light and its acute effects on human skin

The acute effects of UVR on human skin have been well characterized. Upregulation of TNF-α is a key early response to ultraviolet B (UVB) by keratinocytes (KCs), and represents an important component of the inflammatory cascade in skin. UVB irradiation induces TNF-alpha expression in both KCs and dermal fibroblasts, with TNF-alpha mRNA induction seen as early as 1.5 h after UVB.\(^1\) The immediate reaction also includes epidermal keratinocyte release of pro-inflammatory cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6).\(^2,3\) This release is believed to be due to DNA lesions characteristic to UVR, cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts.\(^4-6\) UVB radiation induces not only IL-1 and IL-6, and TNF-alpha, but IL-10 and IL-12, UVA radiation, however, induces only IL-10, mainly produced by dermal CD11b + macrophages and neutrophils that infiltrate epidermis after intense UV. IL-10 is shown to be responsible for suppressing T cell-mediated immune response and can induce immune tolerance to neoantigens, whereas IL-12 can reverse UV-induced immune suppression and break immune tolerance.\(^7-9\) Interestingly, Reeve and Tyrrell reported that UVA induces heme oxygenase which upregulates IL-12 and counteracts UVB-induced immunosuppression.\(^10\)

UVB and UVA exposure also depletes cellular antioxidants and results in the production of reactive oxygen species such as hydrogen peroxide, superoxide anion, singlet oxygen, hydroxyl radicals and nitric oxide (NO).\(^11,12\) Free radical-induced peroxidation of membrane lipids play a role in producing proinflammatory prostaglandins via activated phospholipase A2.\(^13\)

Nuclear factor kappa B (NFκB) and activator protein-1 (AP-1) are transcription factors regulated by cellular redox states, and involved in regulation of gene expression.\(^14\) These two transcription factors are responsible for the regulation of a wide range of extracellular signaling molecules involved in inflammation, cell proliferation, apoptosis, tumorigenesis, and tissue repair.\(^15,16\) Because these transcription factors seem to be very important in the UVR-induced degenerative processes associated with aging and photaging\(^17-19\), such as induction of the matrix metalloproteinases, they are frequent targets of anti-aging preventive therapies.

DNA photolesions and DNA fragments resulting from repair are responsible for acute cutaneous responses to UV radiation, including erythema (sunburn), pigmentation (suntan, melanogenesis), and immune suppression.\(^20-25\) In order to maintain genomic integrity most DNA lesions are repaired efficiently by nucleotide excision repair mechanisms constitutively expressed in all live cells in the body.\(^26,27\) However, there are several DNA products that are highly characteristic of specific UVR wavelengths.

Ultraviolet C (UVC : 200-290nm) radiation is efficiently absorbed by cellular and mitochondrial DNA and produces pyrimidine dimers, such as cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts (6-4PP) (Fig 2). UVC radiation, however, does not reach the surface of the earth, since since virtually all is absorbed by stratospheric ozone. UVC radiation emitted from artificial light sources can cause DNA damage (CPD and 6-4PP) in cells to the level of spinous layers, but it does not penetrate to the basal layer.\(^28\) One concern that has developed over the last few decades is the decreasing thickness of the ozone layer over the southern hemisphere, declining by 10 to 40 percent during the winter and spring months. As a rule a 10% reduction in the ozone layer causes about a 20% increase in UVB-radiation and a 40% increase in skin cancers. Thus relatively minor changes in the ozone layer may have a marked impact on health.\(^29\)

UVB is only partially blocked by ozone layer and comprises approximately 1 to 10% of the UVR reaching the earth.\(^30\) UVB is absorbed by nuclear and mitochondrial DNA but does not penetrate very efficiently past the stratum corneum. The UVB that does reach the living layers of the epidermis and dermis, however,

![Fig. 2. DNA damage caused by solar radiation](image)
yields CPD and 6-4PP that have been observed after realistic exposures in human subjects. Further, UVB radiation has been shown to produce 8-hydroxy-deoxyguanosine (8-OHdG), the most common ROS-induced DNA damage, and one that is highly mutagenic if left unrepaired.

The UVA dose required to produce CPD in cultured cells is reported to be about 5J/cm² and therefore produces CPD and 6-4PP about 1/1,000 less effectively than UVB, but UVA can penetrate significantly farther into the living epidermis and dermis. In fact, the UVA-induced mutations of greatest concern occur in the basal layer of the epidermis where they may transform stem cells.

UV exposure most characteristically results in ROS and base oxidation of DNA, including 8-OHdG (Fig 3) and thymine glycol. Thus, the primary difference in damage between UVB and UVA radiation is that UVB yields primarily CPD (TT, CC, CT and TC) and (6-4)PP with some 8-OHdG, and UVA was reported to yield primarily oxidative (DNA) damage in the form of 8-OHdG with minor amounts of CPD (most commonly TT dimers), but the yields of CPDs by UVA radiation was reported to be ~3 fold higher than that of 8-OHdG upon UVB exposure. Visible and infra-red radiations have not been shown to produce CPD, 6-4PP or 8-OHdG.

UVB radiation can induce erythema in skin at a single dose at or above 20-40mJ/cm². The minimal dose of UVB radiation required to produce visible erythema with sharp margin 24h after radiation is defined as the minimal erythema dose (MED). 20 minutes exposure to the sun around noon on a sunny mid-summer day is approximately equal to one MED for Japanese skin type I (Fitzpatrick’s skin phototype II ~ III). UVA radiation can also produce erythema, but requires a dose about 1,000 times higher than UVB.

Suntan (an increase in pigmentation and skin color caused by sun exposure) is attributable primarily to UVB radiation and usually develops by the 3rd day, limited to the area exposed to UVR. UVB-induced pigmentation results from stimulation of both epidermal keratinocytes (KC) and melanocytes (MC). KC exposed to UVB produce and release neuropeptides (α-MSH : α-melanocytes stimulating hormone, ACTH : adrenocorticotropic hormone) and cytokines (ET-1 : endothelin-1, SCF : stem cell factor, ADF : adult T-cell leukemia cell derived factor) which stimulate MC in a paracrine manner to increase their melanin synthesis and transfer of melanosomes to KC.

UVB induces immediate tanning and persistent pigment darkening through oxidation of pre-existing melanin or melanogenic precursors, while UVB induces tanning, which takes a few days or longer to observe and requires activation of melanocytes. Immediate pigment darkening and persistent pigment darkening induced by UVA radiation may occur through epidermal melanin photooxidation which polymerizes the colorless melanogenic precursors 5,6-dihydroxy indole carboxylic acid (DHICA) and 6-hydroxy-5-methoxy indole carboxylic acid (6H5MICA) to irreversible brownish black pigments. This UVA-induced basal and suprabasal pigmentation takes place outside of melanocytes and does not involve enzymatic melanin synthesis.

In tanning, new melanin in melanosomes is transferred from MC to the neighboring KC, possibly by phagocytic function of KC. The melanosomes concentrate in KC above the nucleus in a formation known as the as the nuclear cap. This protective structure blocks UVB and UVA radiation to a level 1/3-1/5 lower than that of pre-irradiated skin. An abundance of evidence connects DNA photolesion repair to UVR-induced erythema. One example is that xeroderma pigmentosum patients (XP) with low nucleotide excision repair (NER) capacity exhibit severe sunburn reactions after a few minutes of sun exposure.

XP group A patients with less than 5% of normal NER levels develop erythema reactions at 1/5 MED of healthy subjects. Cockayne’s syndrome patients (CS) who have a DNA repair defect in transcription coupled repair (TCR) also show hypersensitivity to UVR in their erythema reaction. The increase of UV dose induces more marked erythema and increase of CPD, and protection of human DNA photolesion repair has 3/11/2009 been shown to produce 8-hydroxy-deoxyguanosine (8-OHdG), the most common ROS-induced DNA damage, and one that is highly mutagenic if left unrepaired.
Role of DNA repair

DNA damage in human skin is mostly caused by environmental UVR. Photodamages are known to play a role in the induction of cell death including apoptosis, mutation, and tumorigenesis, in addition to photoaging. To avoid cancer, the entire genome must be accurately transmitted through repetitive cell divisions, as the skin is one of the organs continuously self-renewing. DNA repair mechanisms, particularly nucleotide excision repair (NER) and base excision repair (BER) are present to maintain genomic integrity, but beyond a certain level of DNA damage, mistakes slip past. Among CPDs, thymine-cytosine (C-T) and cytosine-cytosine (C-C) are known to be the most mutagenic, possibly due to the rule of “A” which takes place at photolesions remained in the strand under DNA synthesis. These misleading DNA damages which increase mutation frequency are indeed found at higher rate in p53 gene of UV-induced skin pre-cancer and cancer cells, such as basal cell carcinoma and squamous cell carcinoma. 6-4PPs are repaired more efficiently than CPDs, usually within 6 hs after UV exposure of the cells, whereas, only a half of CPDs are repaired within 24 hs. NER effectively eradicates CPD and (6-4)PP produced in epidermal keratinocytes (KC), Langerhans cells and melanocytes and dermal fibroblasts. Among these cells, KCs have been shown to repair CPD most efficiently than MCs and fibroblasts. Nearly 30 factors are involved in NER. There are two repair systems in NER, global genome repair (GER) and transcription coupled repair (TCR). Photolesions in DNA strands actively transcribed by DNA polymerase II are repaired more rapidly than those in the non-transcribed strand of active genes, and those in global genome.

Further, DNA damage binding proteins (DDB 1 and 2) take part in recognizing DNA damage in TCR and GGR, respectively. Further, CSA and CSB proteins, and XPC-hHR23B-centrin play a recognition step of DNA damage in TCR and GGR, respectively. Further, DNA damage binding proteins (DDB 1 and 2) take part in recognizing DNA damage in TCR and GGR, respectively. Further, CSA and CSB proteins, and XPC-hHR23B-centrin play a recognition step of DNA damage in TCR and GGR, respectively.

Skin by sunscreen reduces both erythema and CPD formation in vivo. Further, Ley suggested a role of CPD in sunburn, using animal (Monodelphis domestica). In addition, we showed higher yield of DNA damage in Japanese photo-skintype I who burn easily and tan slightly. Thymidine dinucleotides (pTT) and the telomere 3-prime overhang sequence (T-oligos) are shown to increase melanogenesis by increased melanogenic proteins leading to 3- to 5-fold UV protection multiple with distinct nuclear capping in many keratinocytes in vivo. Studies using oligonucleotides are also shown to be effective in inducing protective responses in human skin, such as increase of DNA repair capacity and prolonging cell cycle arrest. pTTs are reported to be effective as much as 33% of the TTGAAA sequence in stimulating these UV-induced SOS-like protective responses.

Sunburn is an erythema reaction, and has been shown to be induced by increased prostaglandin E2 (PGE2) and nitric oxide (NO) in circulating blood produced by UV irradiation on skin. UV stimulates epidermal keratinocytes to upregulate mRNA of cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS), which produce PGE2 and NO, respectively. These two chemical mediators are known to increase melanogenesis of melanocytes in vitro cultured system. In XPA cells, COX-2 mRNA level and PGE2 are shown to increase after a low dose of UVB irradiation in vitro.

These results strongly suggest that UV radiation-induced DNA damage enhances the expression of COX-2 and iNOS mRNA, and increased PGE2 and NO produce sunburn and suntan, although the detailed mechanisms of the stimulation of mRNA level of COX-2 and NO by DNA photodamages still remains to be elucidated. Further, direct stimulation of membrane components of epidermal keratinocytes by UV radiation is also a possible mechanism to enhance the expression of mRNA levels of COX-2 and iNOS.

**Fig. 4.** Sunburn induction by solar UVB radiation, by DNA damage remained and signal transduction via cellular membrane. DNA damages produced by UVB and remained, particularly at transcribing strands are responsible for the production of prostaglandins and nitric oxides which increase vascular circulation, leading to sunburn and suntan. UVB also stimulates cell membrane and increase the production of these chemical mediators.
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Transcription factor II H (TFIIH, XPB, XPD, TTDA), XPG protein, XPA protein and recognition protein (RPA) are also involved in this recognition step of both TCR and GGR.

After recognition of the damaged lesion, endonucleases XPF-ERCC1 and XPG make incisions at the 5’ and 3’ sites of the lesion, respectively, resulting in the excision of a photodamaged site, leaving a gap of ~30 DNA nucleotides. The gap is filled by DNA polymerase δ/ε, PCNA (proliferating cell nuclear antigen) and RFC (replication factor C), and finally sealed by DNA ligase 1.

Oxidized bases, such as 8-OHdG, are repaired by the BER system. The oxidized base is excised by DNA glycosylase and remaining AP site is excised by AP endonuclease. The remaining gap lesion is synthesized by DNA polymerase β, XRCC1 and DNA ligase III (Fig 6).

**Fig. 5.** A rule of “A”

Among cyclobutane pyrimidine dimmers, C-T and C-C are shown to be the most mutagenic damages, since a rule of “A” does play a role in repair process to make a mis-pairing on the opposite strand. In DNA synthesis of the strand free from DNA damage, a guanine base is incorporated into the opposite site of cytosine (C) on the opposite strand. Adenine (A), however, is wrongly incorporated at around 50% rate into the opposite site of cytosine at DNA damage of C-T and C-C. This mis-uptake of adenine instead of guanine is called as “A rule of A”. Keratinocytes in epidermis are most frequently exposed to solar UV and are known to frequently develop to basal and squamous cell carcinoma, particularly in white skin populations. Since skin cells of children divide more frequently than adults and elderly, there is more chance for “A rule of A” takes place in epidermal cells, leading to a mutation in the important genes relevant to skin cancer and solar lentigines.

**Fig. 6.** DNA repair of different epidermal and dermal cells

Keratinocytes are reported to repair CPD most efficiently than other cells, MCs and fibroblasts at the low dose of UV irradiation, when measured by ELISA technique using monoclonal antibody against CPD.
3. Photoaging of the skin caused by chronic sun exposure

3-(1) Characteristics of photoaged skin

Photoaged skin is characterized by coarse wrinkles, loss of elasticity, pigmented spots, dryness, verrucous papules, and telangiectasia. The age at onset and expression of these photoaged characteristics appear to differ between racial phenotypes or pigmentary groups 79). It is commonly held that lighter skinned people tend to manifest photoaging by wrinkles, whereas Asian ethnicities exhibit pigmented spots (solar lentigines) rather than wrinkles. The severity of photoaging in any case depends on cumulative sun exposure, and is usually most determined by occupation and life-style 80). Histopathologically, aged skin undergoes progressive disorientation of dermal collagen and elastic fiber bundles 77,78). In photoaged skin, there can be a significant increase in space between fiber bundles, thinning of fibers and increased disorganization of fiber proteins.

Intrinsic and photoaged skin shows an age-dependent reduction of cutaneous microvasculature, leading to decreased skin temperature and decreased nutritional supply which possibly may cause thinning of nail plates and skin 79). It is reported that there are age-dependent decreases in the cutaneous vascularity of both sun-exposed facial and sun-protected buttock skin, but the alteration is more prominent in photoaged skin 80) (Fig 7). In intrinsically aged skin, there is no significant difference in the vascular density, although there is a decrease in vessel size between aged and young skin 81). However, the papillary dermis of photoaged facial skin of elder donors showed apparent decreases in vessel size, vascular number, compared with that of younger skin.

Acutely, UV exposure stimulates angiogenesis through vascular endothelial growth factor (VEGF) upregulation via MEK-ERK activation and thrombospondin-1 (TSP-1) downregulation via PI3K-Akt activation in human epidermis, although with chronic exposure blood vessels may be decreased in UV-damaged skin 82).

Among non-fibrous components in dermis and epidermis, hyaluronan (HA) plays an essential role in supporting tissue architecture, and is also involved in cell migration and differentiation during inflammation 83). Further, HA is known as one of the important factors to protect skin from dryness by its capacity to bind water. An age-dependent decrease in hyaluronan content has been reported 84). In addition, the rate of UVB-induced HA synthesis 24 h after UVB irradiation is also decreased in aged human skin compared to young skin. 85)

HA metabolism in human skin is rapidly and differentially regulated by acute UVB irradiation. HA content in epidermis and dermis decreases 3h after a single UVB exposure due to increased degradation and decreased synthesis of HA. In epidermis, new HA increases by 24 h after UV irradiation, but remains lower in the dermis. In dermis, HA degradation products increase for 24 h-post irradiation, suggesting that balance between HA synthesis and degradation determines HA recovery in tissue after UV irradiation 86).

3-(2) Mechanisms of solar lentigine development

Solar lentigines, small brown pigmented spots sharply demarcated in sun-exposed skin, may be induced by mutations of KC and MC genes which play a role in pigment formation and transfer. Stem cell factor (SCF) mutations in KC have been suggested to be responsible for the development of solar lentigine, although details of the mechanism has not yet been elucidated. Other KC and MC genes which control melanin formation in MC may also be responsible for pigment spot formation. It seems reasonable to suggest that gene mutations would be responsible for the induction of lentigos, since XP patients who have abnormally low ability to repair DNA damage have numerous pigmented spots as early as a few-months of age, limited to the sun-exposed face, after minor exposures to sunlight 52). Through its ability to induce mutations in cutaneous cells, UVB radiation is thought to be the extrinsic factor most responsible for pigment spot development.

Fig. 7. Nucleotide excision repair (NER) mechanisms of CPD

There are two distinctively different repair system in NER, global genome repair (GGR) and transcription coupled repair (TCR). UV-induced CPD and 6-4PP in DNA strand which are actively transcribed by DNA polymerase II are repaired more rapidly than those in the non-transcribing strand of active genes and those in global genome.
3-(3) Mechanisms of wrinkle formation

Transcription factor AP-1 is a critical mediator of acute photodamage that is involved in both overexpression of matrix metalloproteinases (MMPs) and reduction of type I procollagen. Increased MMP activity would be expected, over time, to degrade dermal connective tissue. In human skin UVR-induced ROS activate signaling kinases in epidermal KCs and dermal fibroblasts. AP-1 (activation protein-1) is activated through MAPK signaling pathway, and controls the transcription of matrix metalloproteinases MMPs [87]. Another transcription factor NF-kB is also activated by UV radiation, and stimulates the transcription of inflammatory cytokines which attract neutrophils which express neutrophil collagenase-2 (MMP-8).

MMPs up-regulation occurs after a low dose UV exposure, less than one minimal erythema dose [88]. Therefore, daily exposures to a low dose solar UV radiation below sunburn are thought to be sufficient to induce MMPs up-regulation and to degrade skin collagen and elastic fiber, leading to wrinkle formation.

Keratinocytes exposed to UBV radiation produce and secrete cytokines IL-1α, IL-6, and TNFα, which stimulate epidermal KC and dermal fibroblasts, in autocrine and paracrine manners, respectively, and upregulate the levels of mRNA and MMPs 1, 2 (gelatinase A), 9 (gelatinase B) and 12 which degrade dermal collagen and elastic fibers, leading to the development of wrinkle formation (Fig 8) [89-91].

Human skin constitutively expresses three distinct collagenases, 1 and 2, and 3, also referred to as matrix metalloproteinases (MMP) -1, -8 and -13 respectively. UBV radiation induces MMP-1, -3, and -9 in normal human epidermis. UVA radiation also induces mRNA expression MMP-1, -2, and -3 in fibroblasts [92], MMP-1 and MMP-8 cleave fibrillar collagen type I and III in the dermis, then which are further degraded by MMP-2 and -9. Elastic fibers and collagen type V and VII are also cleaved by other MMPs, such as MMP-12 derived from macrophages [93], and serine protease derived from inflammatory infiltrating cells, and skin fibroblast elastase. UBV radiation may contribute to wrinkle formation by inducing fibroblast elastase via cytokines released by UVB-exposed keratinocyte or directly by UV radiation [94].

Collagen synthesis is also stimulated by UBV and UVA radiation, but degradation exceeds the production of collagen and elastic fibers, resulting in the reduction of dermal fiber components. UVR upregulates the mRNA level of MMPs via reactive oxygen species, suggesting that anti-oxidants might have a protective effect on UVR-induced wrinkle formation (Fig 9) [95]. Alteration of basement membrane is also reported to play a role in wrinkle formation [96]. Type VII collagen is reduced in photoaged skin at the dermo-epidermal junction, although a role for this in wrinkle formation has not yet been fully elucidated. At present, wrinkles are understood to result from several related factors, including the decrease of collagen and elastic fibers in dermis [97], the degradation of basement membrane at the dermal-epidermal junction, and an decrease in the three dimensional organization of the extracellular matrix.

The histopathological changes seen in intrinsically aged skin are characterized by the general atrophy of the extracellular matrix with decreased elastin and disintegration of elastic fibers. Photoaged skin is characterized by a loss of mature collagen, and basophilic degeneration of connective tissue, evidenced by denatured elastin fiber and collagen fibers. Elafin, a molecule found in actinically damaged skin, has been shown to be induced by UVA in fibroblasts in vitro. It inhibits the binding of elastase to elastin by forming an elafin-elastin complex which is increased in photoaged upper to middle dermal connective tissue [98]. The complex prevents elastic fibers from elastolytic degradation, leading to the accumulation of elastic fibers, and thus, elafin is now understood to be a molecule integral to actinic elastosis. Accompanying the changes in collagen and elastin, there is an increase in the deposition of glycosaminoglycans.

In summary, solar UV radiation has been implicated in wrinkle formation through its exacerbation of the decline in tensile strength and elasticity and its ability to cause the degradation of the supporting structural components of the dermal extracellular matrix. Infrared radiation is another factor in sun exposure that is now believed to play a role in the development of solar elastosis and premature skin aging [99,100]. Experimentally, heat increases...
Mechanisms of UVB-induced wrinkle formation

Keratinocytes exposed to UVB radiation produce and secrete cytokines, such as IL-1α, IL-6 and TNFα which stimulate keratinocytes and dermal fibroblasts to produce matrix metalloproteinases (MMPs), leading to destruction of collagen and elastic fibers, and formation of wrinkles in sun-exposed skin.

collagenase and stromelysin mRNA in dermal fibroblasts. The expression of MMP-1 and MMP-3 mRNA and protein levels is increased by heat in a dose-dependent manner, possibly mediated by the activation of ERK and JNK. Further, heat increases the expression of IL-6 mRNA in cultured dermal fibroblasts, which upregulates the expression of MMP-1 and MMP-3, leading to the degradation of extracellular matrix proteins and induction of wrinkles. A major regulator of collagen types I, II and III synthesis, TGF-β3, has been shown to be downregulated by heat treatment in cultured fibroblasts and also in human skin in vivo. The dose used experimentally to induce altered expression of MMPs was extremely high compared to the dose we are exposed to the sunlight in usual life. Therefore, it remained to be elucidated in the future how much chronic solar infrared radiation may contribute to the enhanced photoaging of the skin.

4. Preventions and treatment of photoaging

Chronically sun-exposed skin is characterized by pigmented spots (lentigos), deep wrinkles (so-called leathery skin) and verrucous papules, superimposed on chronologically degenerated skin. This structural and functional change is termed photoaging. There are essentially three strategies to prevent photoaging: (1) prevention of UV penetration into skin, (2) inhibition of inflammation by antioxidants and anti-inflammatory molecules, (3) medically based rejuvenation treatments of photoaged skin.

4-(1) Prevention of UV penetration by sunscreens

The primary approach to preventing photoaging is by sun avoidance and by the proper use of sunscreen, appropriate clothing, and hats. Adopting a healthy attitude about sun exposure can prevent the unwelcome signs of aging such as wrinkles and lentigos as well as non-melanoma skin cancer. When out-of-doors, this would imply that shade seeking behavior would prevail. Proper daily use of protective materials against solar ultraviolet radiation (UVR) should prevent both acute and chronic damage of the skin.

Sunscreen use is generally accepted to reduce the level of DNA damage and protect sun-exposed skin from erythema, suggesting a protective role against UVR-induced photoaging and skin carcinogenesis. Our epidemiological study of the effect of annual UVR on skin pigmented freckles in women, conducted in the northern and southern Japan, Akita prefecture and Kagoshima prefecture, respectively, showed that the number of small pigmented spots at age 40 of women living in Kagoshima (exposed to a higher UVR flux) was almost equal to those of women at around 60 years old at Akita, indicating that chronic UVR efficiently promotes pigmented lesions. In another epidemiological study on skin cancer incidence in Japan, subjects who live in Okinawa, where annual ambient UVR is about 2 times higher than subjects who live in Kasai-city, Hyogo prefecture had a 4-5 times higher incidence of precancerous actinic keratoses (Table 1).
Further, it is shown that the yearly exposure dose of school children not using any specific photoprotection in USA and Japan is roughly 150 MEDs to 300 MEDs (122). Exposure to UVA is of particular concern because the UVA energy reaching the earth’s surface 90-99% of the total, UVA passes through glass, and only decreases by 50% in winter. Further, UVA radiation causes DNA damage, such as CPD and 8-OHdG and induces photoaging as mentioned earlier. There are two types of sunscreens, chemicals which absorb UV photons and physical agents which reflect or scatter UV light. Sunscreens have long been known to protect skin from UV-induced erythema, as reflected by SPF values. In the 1980’s and early 1990’s sunscreen was expected to protect skin from UV-induced carcinogenesis (111-114). In the past few years, however, the importance of broad-spectrum protection covering both UVB and UVA radiation has been recognized (116-119). Taken together, it is recommended to use daily broad-spectrum sunscreen that blocks both UVB and UVA radiation (20,21). At present, a safe level of daily UVR exposure to the public has not been established, however, it can recommended that the public reduce their life-time UVR exposure to a level as low as possible. Many commercial sunscreens on the market are formulated to be broad-spectrum, and new technology has increased their photostability. Modern sunscreens such as Parsol 1789 and Mexoryl SX and XL cover at least part of the UVA spectrum, and together with efficient UVB absorbers and reflective micro-sized titanium dioxide are highly effective broad-spectrum filters, often used to protect patients with UVA sensitivity (121). Consumers can be advised to adjust their sunscreen to the environmental and activity related conditions: a broad-spectrum sunscreen with SPF 50 and PA +++ for outdoor activities on sunny days in summer, and a sunscreen with SPF 10-20, PA ++ for daily, incidental exposure. Currently, there is controversy over the link between low serum levels of vitamin D and the risk of cancers originated in several organs (123,124). Suberythermal doses of UVB (several minutes to a quarter of hour exposure at noon in summer) produce vitamin D3 (Vit D3) for daily calcium and bone metabolism (125). However, repeated suberythermal UVR exposures on human skin have been shown to induce significant DNA damage in epidermal cells and even sunburn erythema after consecutive exposures (107). At this time, the American Academy of Dermatology recommends that the sun protection measures outlined above be followed and that vitamin D levels be maintained by dietary and supplemental vitamin D.

### Table 1 Incidence of skin pre-cancer in Kasai city and ie-son in Japan

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<th>Place</th>
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<th>No. of subjects</th>
<th>No. of patients a</th>
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a: Number of actinic keratoses patients diagnosed in each year
b: No. of patients corrected at age and sex based on the Japanese population in 1990

4-(2) Prevention of UV-induced ROS and inflammation

Enzymes which convert ROS to harmless water and molecular oxygen protect skin from ROS-induced damages. The levels of these major endogenous anti-oxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase are shown to decrease after a single and repeated exposure to UVB radiation in mice and pig (127,128) and also in aged and photoaged skin of human (129). The level of catalase in epidermis is much higher than in the dermis, and decreases after a single UVB and UVA exposure, recovering 3-4 weeks after exposure. Topical and oral administration of biologically relevant antioxidants, such as vitamin E, vitamin C, coenzyme Q, polyphenols and carotenoids have minimal evidence that they provide photoprotection and reduce acute photodamage in human skin (129,134). Recently, we found that CoQ10 suppresses UV-induced MMP1 production of fibroblasts by inhibiting the production of cytokines in KC. We speculate that anti-oxidative activity of CoQ10 may inhibit the production of inflammatory cytokines in UV irradiated KCs (135). Several polyphenolic antioxidants of plant, such as green tea, grape seed, pomegranate and others, have been shown to be effective in vitro for prevention of cellular photodamage, and green tea extract has been shown to reduce photoaging and skin cancer (136-142). Resveratrol derived from grape skin is a novel agent for anti-aging and anti-photoaging treatment of the skin, possibly through its antioxidant properties and through regulation of energy metabolism in mitochondria and epidermal cell differentiation (143,144).

Topical retinoids have been demonstrated to inhibit UV-induced inflammation mediated by AP-1 and NFκB transcription factors (145,146). All-trans-retinoic acid (ATRA) prevents UV-induced accumulation of c-Jun protein, resulting the suppression of AP-1 binding to MMPs gene. Further, ATRA is shown to stimulate the breakdown of jun protein through ubiquitin-proteasome degradation. In conclusion, we recommend the use of sunscreen from childhood to prevent acute severe sunburn and to reduce the level of accumulated DNA damage caused by daily repeated exposures, and to both retard the onset of visible photoaging, and reduce the risk for melanoma and non-melanoma skin cancer.

4-(3) Treatment and rejuvenation of photoaged skin

Retinoids are one of the most commonly used topical agents to reverse the signs of photoaging. Topical use of ATRA for several months proved to reduce wrinkle numbers, length and depth by increasing fiber components in dermis and make epidermis thicker (147,148). A new retinoic acid agonist, N-retinoyl-D-glucosamine has been shown to be effective on photoaged skin without the irritation commonly seen in ATRA treatment (149). Further ATRA and its derivatives are shown to reduce melanin pigment such as mottled hyperpigmentation, freckles and solar lentigines by topical use possibly by increased turnover rate of epidermis (150). To treat acute inflammation, topical immune suppressant, tacrolimus, non-steroidal anti inflammatory drugs (NSAIDs) and corticosteroid hormone are extremely effective. Recent advances in non-ablative laser and light therapy of pigmented skin and wrinkles have made it possible possible to treat photo-damaged skin effectively and safely.

Noninvasive cosmetic procedures are now popular worldwide, since they are effective, and relatively painless and safe compared to deep chemical peels more common decades ago (151,152). Chemical peeling by salicylic acid in polyethylene glycol can be used to
treat photodamaged skin and has been shown to suppress skin tumor development in irradiated hairless mice (153).

Pigmented lesions are commonly treated by laser, IPL (intense pulsed light), superficial chemical peel and topical application of whitening agents, cosmetics and drugs. Further, a highly effective drug delivery system, electroporation, is also available for whitening and wrinkle care. In many cases, patients are treated by combined use of these modalities, depending on the disease and skin conditions.

Chemical peels are effective for both superficial wrinkle amelioration and for whitening of pigmented spots. Glycolic acid (GA) and salicylic acid (macrogole) are popular in Japan. Dainichi et al. showed that chemical peeling by macrogole suppresses p53 expression and normalizes keratinocyte differentiation, leading to the reduction of UV-induced skin cancer development in mice (153). These acid formulations essentially dissolve the upper layer of skin, whereas trichloroacetic acid (TCA) can be used for lower dermal layer (medium depth peel).

Laser resurfacing is a technique used where the molecular bonds of the skin cells are dissolved by a laser. It is used for the treatment of wrinkles, solar lentigines, sun damage, scars, actinic keratoses and telangiectasias or “spider veins”. Laser treatment is based on the theory that selective removal of skin tissue triggers a wound-healing response, remodeling of collagen fibers, dermal matrix, and rebuilds epidermal components. For discoloration, Q-switch ruby laser is commonly used, particularly useful for the treatment of melanin pigment located in dermis. IPL is also very effective to reduce and often erase epidermal pigmentation in solar lentigines and freckles by killing keratinocytes containing melanin (154). Complete resurfacing was first done with a CO2 laser. More commonly now, laser resurfacing is done with a fractional laser. The term “fractional” pertains to the method in which the laser light is transferred. Tiny pinpoints of laser light are used to deliver the laser to the surface of the skin in only a fraction of the area. Several hundred or thousands of pinpoints may be used per square inch, leaving healthy skin in between the ablated areas. This is intended to allow more rapid healing and less risk.

Radiofrequency devices delivers energy by waves in the range of radio signals and aims to destroy the upper and some of the lower skin layers, leading to contraction and tightening of the skin. This has been associated with a high degree of pain and inflammation.

Topically applied botanical extracts and synthetic molecules have been in widespread use for centuries in the case of the former and more recently in the case of the latter to rejuvenate photoaged skin. Table 2 lists some of the most commonly used chemicals (cosmetics and drugs) for skin rejuvenation (Table 2).

<table>
<thead>
<tr>
<th>Table 2 Whitening and anti-wrinkle agents, and their mechanisms</th>
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<tbody>
<tr>
<td>1 Arbutin</td>
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<td>3 Vitamin C derivatives</td>
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<td>4 Canzo extracts</td>
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<td>5 Retinoids</td>
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<td>6 Kamitsure extract</td>
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<td>7 Linoleic acid</td>
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<td>8 Ellagic acid</td>
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<td>9 Racinol</td>
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<td>10 a-hydroic acid</td>
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<td>11 Hydroquinone</td>
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<td>12 Vitamin E</td>
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<td>13 Tranexamic acid</td>
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<td>15 Glutathione</td>
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<td>16 Retinoic acid</td>
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<td>17 Vitamin C</td>
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<tr>
<td>18 Emilin(tetrapeptides)</td>
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<td>19 Astaxanthin</td>
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</tbody>
</table>
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