Molecular Mechanisms of Cutaneous Aging: Connective Tissue Alterations in the Dermis

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Cutaneous aging is a complex biological phenomenon consisting of two distinct components, (a) the intrinsic, genetically determined degenerative aging processes and (b) extrinsic aging due to exposure to the environment, also known as "photoaging". These two processes are superimposed in the sun-exposed areas of skin, with profound effects on the biology of cellular and structural elements of the skin. This overview summarizes our current understanding of the mechanisms of innate versus extrinsic aging with emphasis on connective tissue alterations, primarily collagen and the elastic fiber network. We also introduce a novel transgenic mouse model, expressing a human elastin promoter-reporter gene construct, suitable for studies on biology and preventive pharmacology of the cutaneous aging. ***Key words: photoaging/solar elastosis/ UV-irradiation/ elastic fibers/collagen. Journal of Investigative Dermatology Symposium Proceedings 3:41–44, 1998***

One of the fastest growing segments of the patient populace seen in dermatology practice today are individuals seeking help for cutaneous aging, i.e., sagging and wrinkling of the skin, the tell-tale signs of youth spent on the beach, middle years on the golf course, or just a visible reminder of the inescapable aging process (Uitto, 1997). The patients' complaints range from minor cosmetic problems to major disfigurement with profound psycho-social consequences. Thus, cutaneous aging represents a spectrum of medical conditions with severity ranging from mild wrinkling to the development of premalignant and malignant lesions.

Cutaneous aging is a complex biologic process affecting various layers of the skin, but the major changes are seen in the dermis. Contributing to the complexity of cutaneous aging is the fact that there are two independent, clinically and biologically distinct, processes affecting the skin simultaneously. The first is innate or intrinsic aging, "the biologic clock," which affects skin in a manner similar to how it probably affects a variety of internal organs, i.e., by slow, irreversible tissue degeneration. The second process is extrinsic aging, or "photoaging," the result of exposure to outdoor elements, primarily ultraviolet (UV) radiation (Gilchrest, 1995). The consequences of innate aging can be observed all over the skin, including sun-protected areas, whereas in sun-exposed areas, particularly on the face and backs of hands, photodamage is superimposed on the background of the ongoing innate aging process. Consequently, the most noticeable changes on facial and neck skin, the prime focus of patient complaints, result from a combination of intrinsic and extrinsic aging processes; however, it has been suggested that as much as 80% of facial aging is attributable to sun exposure (Gilchrest, 1989).

Normal human dermis consists primarily of an extracellular matrix of connective tissue, and three major extracellular components have been recognized to contribute to physiologic properties of the skin. Specifically, fibers consisting of collagen, an abundant extracellular matrix protein that accounts for about 80% of the dry weight of the skin, provides tensile properties to the dermis, so as to allow skin to serve as a protective organ against external trauma (Uitto, 1990). The elastic fibers, which account for 2%–4% of the extracellular matrix in sun-protected skin, form an interconnecting network that provides elasticity and resilience to normal skin (Uitto, 1979). Finally, glycosaminoglycan/proteoglycan macromolecules, even though a minor component accounting for as little as 0.1%–0.3% of the dry weight of tissue, play a role in providing hydration to the skin, largely through the water binding capacity of hyaluronic acid (Davidson, 1965). Thus, processes that alter the relative proportions of these components, or degradative pathways that render these molecules nonfunctional, can result in clinical manifestations recognized as part of the cutaneous aging process.

MECHANISMS OF INNATE AGING

A variety of theories have been proposed to explain aging phenomena in general, and some of them may be applicable to innate cutaneous aging as well. One of these theories relates to the Hayflick phenomenon that postulates that diploid cells, such as dermal fibroblasts, have a finite life-span in culture (Hayflick, 1979). This observation, when extrapolated to the tissue level, coupled with depletion of the stem cell population from the dermis, could be expected to result in cellular senescence and degenerative changes in the dermis.

Another theory revolves around free radicals, suggesting that oxidative stress may damage not only lipid bilayers in cell membranes but also connective tissue components of the dermis, particularly collagen (Dalle Carbonare and Pathak, 1994). Other changes in innate aging process have been attributed to nonenzymatic glycosylation of proteins, such as collagen. This theory has been put forward on the basis of observations in patients with poorly controlled hyperglycemia in diabetes mellitus, who present with premature aging signs, such as early cataracts and premature arteriosclerosis, due to nonenzymatic glucosylation of lens proteins and blood vessel collagens, respectively (Bucala and Cerami, 1992).

Finally, cutaneous aging may be attributed to differential gene expression. This theory implies that with advanced aging and senescence, skin cells, such as dermal fibroblasts, alter their biosynthetic

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repertoire through differential gene expression. In relation to the extracellular matrix of connective tissue, it has been clearly demonstrated that the rate of collagen biosynthesis is markedly lower in the skin of elderly than in fetal tissues or during the early postnatal years (Uitto *et al*, 1989). The low level of collagen synthesis in the elderly individuals may explain compromised wound healing. Furthermore, reduced repair capacity that will not be able to replenish collagen fibers removed by ongoing degradative processes, may lead to atrophy of the dermis. Similarly, the rate of elastin gene expression, as measured by mRNA steady-state levels in fibroblast cultures established from the skin of elderly individuals, is markedly reduced after the fourth or fifth decade of life (Uitto *et al*, 1989). Again, this results in a situation where there is very little repair capacity to re-synthesize elastic fibers that are continually broken down by slow, yet steady, degradative processes.

Collectively, the observations on dermal connective tissue components in innate aging suggest an imbalance between the biosynthesis and degradation, with less repair capacity on the face of ongoing degradation. This imbalance will eventually lead to loss of collagen and elastic fibers, manifesting clinically as atrophy and loss of recoil.

EFFECTS OF PHOTODAMAGE ON DERMAL CONNECTIVE TISSUE

The histopathologic hallmark of photoaging is the massive accumulation of so-called "elastotic" material in the upper and mid-dermis. This phenomenon, known as solar elastosis, has been attributed to changes in elastin, the major component of elastic fibers, primarily because the accumulated material stains positively with elastin-specific histologic stains, such as Verhoeff-van Gieson stain (Chen *et al*, 1986; Mera *et al*, 1987; Bernstein *et al*, 1994). It has been clearly demonstrated, however, that in addition to elastin, this accumulated material is composed of a variety of extracellular matrix components present in the skin. Like normal elastic fibers, solar elastotic material is composed not only of elastin but also of fibrillin, a microfibrillar protein (Dahlbäck *et al*, 1990; Bernstein *et al*, 1994). In addition, normal as well as abnormal solar elastotic material contains versican, a large proteoglycan as well as hyaluronic acid associated with versican, and thus all the principal components of the elastic fibers (Zimmermann *et al*, 1994; Bernstein *et al*, 1995a). Despite containing the normal constituents of elastic fibers, the overall supramolecular organization of solar elastotic material and its functionality are severely perturbed.

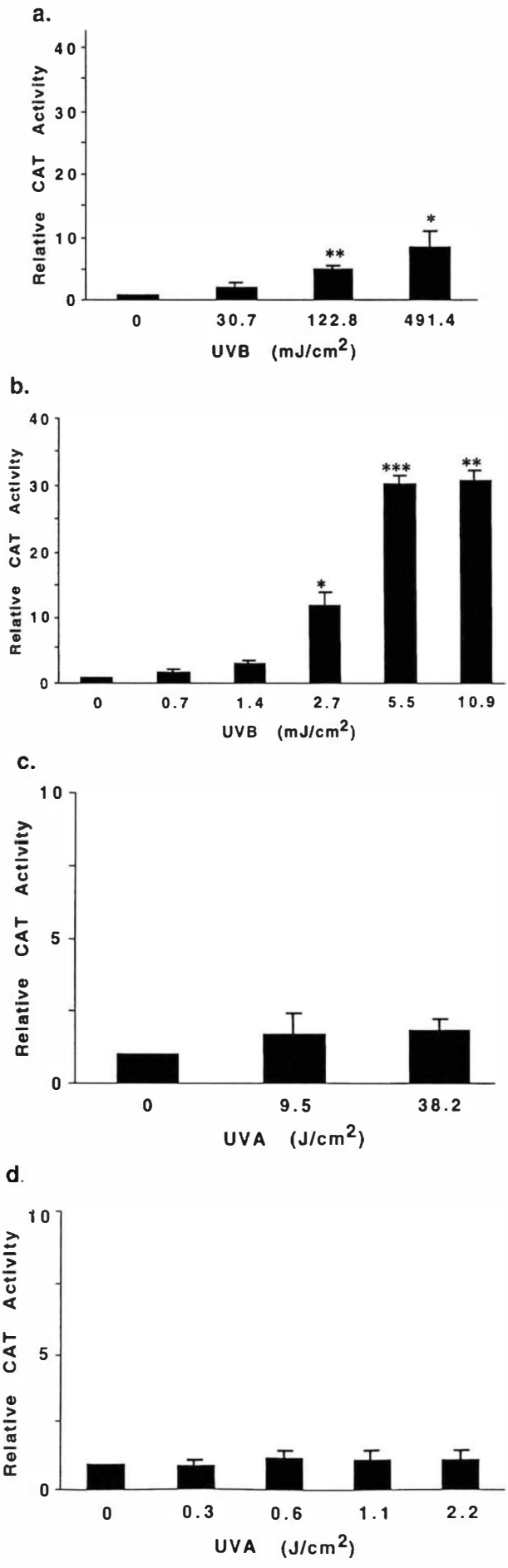
Because of the massive accumulation of elastotic material in sun-exposed areas of skin, photoaging research has in the past focused primarily on the biology and pathology of elastin and the associated microfibrillar components of elastic fibers. Such studies have demonstrated that elastin gene expression is markedly activated in cells within the sun-damaged dermis, as determined by northern hybridizations (Bernstein *et al*, 1994). Elastin gene induction has also recently been demonstrated in response to free radicals, known to play a role in UV-induced photoaging, generated by a xanthine/xanthine oxidase system (Kawaguchi *et al*, 1997). Also, evidence from transient transfections using elastin promoter-chloramphenicol acetyl transferase (CAT) reporter gene constructs have provided evidence for transcriptional activation of the elastin gene as a mechanism for enhanced gene expression (Bernstein *et al*, 1994).

More recently, changes in collagen metabolism have been brought into focus as a major factor leading to photoaging. Specifically, it has been demonstrated that accumulation of elastotic material is accompanied by concomitant degeneration of the surrounding collagenous meshwork, and evidence implicating matrix metalloproteinases (MMPs) as mediators of collagen damage in photoaging has been presented (Griffiths *et al*, 1993; Bernstein *et al*, 1996a; Fisher *et al*, 1997). MMPs comprise a family of degradative enzymes consisting of at least 14 different members with rather broad substrate specificity (Matrisian, 1992). Many of these proteases can degrade native collagen fibers, denatured collagen, elastic fibers, various proteoglycans, and fibronectin, among other components of the dermis. It has previously been demonstrated *in vitro* that UV irradiation of fibroblasts in culture enhances the expression of these proteolytic enzymes (Stein *et al*, 1989; Peterson *et al*, 1992). Subsequently, it was shown that even a single exposure to UV irradiation of human skin is able to increase the activities of MMP, and elevated enzyme activities were shown to be associated with significant degradation of collagen fibers consisting primarily of type I collagen (Fisher *et al*, 1997). At the same time, UV exposure was also shown to induce a specific tissue inhibitor of MMP (TIMP) (Fisher *et al*, 1997). Thus, there appears to be a delicate balance between the induction of the degradative enzymes and their inhibitors in photodamaged skin. Nevertheless, UV exposure appears to result in a more degradative environment that results in loss of cutaneous collagen. Loss of collagen with concomitant accumulation of poorly functional elastotic material can result in a leathery, inelastic, and yellowish appearance of skin, so-called cutis rhomboidalis nuchae, as graphically illustrated in the neck area of an individual spending significant unprotected time outdoors.

A TRANSGENIC MOUSE MODEL FOR PHOTOAGING RESEARCH

As indicated above, solar elastosis is the major alteration in photoaged skin with massive accumulation of elastotic material in the dermis. A number of *in vivo* models of cutaneous photoaging have been developed to measure the amount of newly deposited elastic tissues after repeated UV irradiation exposures. These models require administration of UV radiation for relatively long periods of time, ranging from 3 mo to over 1 y (Kligman *et al*, 1982, 1985; Bissett *et al*, 1987, 1989; Kligman and Sayre, 1991; Kochevar *et al*, 1994). As with humans, animals require a significant portion of their lifetime to acquire a significant amount of solar elastotic material; however, the murine response to UV is somewhat less dramatic than alterations occurring in human dermis. To develop a rapid and sensitive means of studying mechanisms of photoaging and to potentially test compounds that may protect against sun damage *in vivo*, we have adopted a transgenic mouse line containing human elastin promoter linked to CAT reporter gene (Hsu-Wong *et al*, 1994). Specifically, these mice contain 5.2 kb of human elastin promoter stably integrated into the mouse genome. It should be noted that these mice are phenotypical normal, indistinguishable from mice of the same strain not containing the transgene; however, human elastin promoter activation can be measured by assay of the activity of CAT that is not normally expressed in mouse skin but is driven in the transgenic mice by the human elastin promoter. The use of the human promoter is critical to this model, because relative to mice, humans generate significantly larger amounts of elastotic material in response to chronic UV radiation. In addition, dissimilarities between the mouse and human elastin promoters may result in differential response of these promoters to UV irradiation and in secondary pathways stimulated by UV.

The utility of this mouse model for exploration of the photoaging mechanisms has been demonstrated by the fact that UV irradiation of newborn transgenic mice can alter human elastin promoter activity. Specifically, irradiation of newborn transgenic mice with UV resulted in a concentration-dependent enhancement of CAT activity in irradiated skin, the maximum enhancement of ~8-fold being noted with UVB, whereas small, but reproducible, enhancement, up to ~2-fold, of CAT expression was obtained with UVA (**Fig 1*a*, *c***) (Bernstein *et al*, 1995b). Pretreatment of mice with 8-methoxypsoralen, however, results in increased promoter activation in response to UVA (Bernstein *et al*, 1996b). Further increases in CAT activity are measured *in vitro* in response to UVB. Increases up to 30-fold over those of unirradiated controls are achievable using fibroblast cultures derived from the skin of transgenic mice using UVB. No increase in CAT activity is measured *in vitro* after UVA exposure (**Fig 1*b*, *d***); however, the addition of 8-methoxypsoralen to this system results in large increases in CAT activity, up to ~20-fold over that of irradiated controls in response to UVA (Bernstein *et al*, 1996b). Elastin promoter activation has been demonstrated *in vivo* using various lengths of the human elastin promoter in a number of mouse lines. In addition, *in vitro* promoter induction has been measured not only using mouse fibroblasts containing various lengths of the human elastin promoter, but also in rat vascular endothelial cells containing the human elastin promoter (unpublished data). These results confirm the role of UV-induced



elastin promoter activation in photoaging, and they identify UVA as a contributing factor *in vivo*.

This transgenic mouse model serves as a rapid and sensitive system to study cutaneous photoaging in further detail, and for testing of compounds that may protect against photodamage to the skin. We demonstrated the utility of this system for evaluation of agents that protect against cutaneous photodamage by measuring the protection afforded by sunscreens having various sun protection factor (SPF) ratings. Sunscreens previously shown to have SPF ratings of 2, 4, 8, and 15, respectively, were applied to the backs of mice prior to the administration of solar simulating radiation (SSR). Irradiated control mice received the lotion vehicle used for all sunscreen preparations, whereas some mice received no lotion or irradiation. Sunscreens or the lotion vehicle alone produced no alterations in CAT activity. Sunscreens were shown to protect against SSR, with a linear increase in protection with increasing SPF of the sunscreen (**Fig 2**) (Bernstein

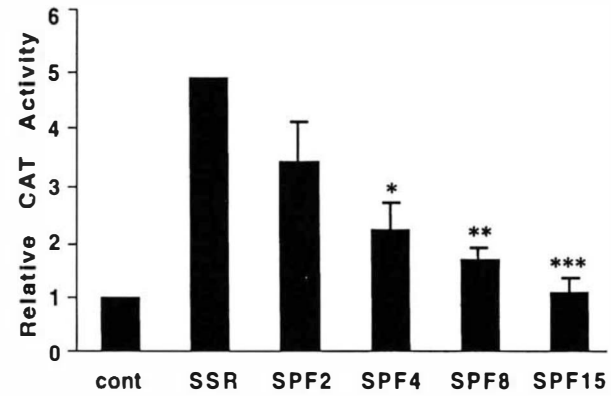


Figure 2. Sunscreens protect against photoaging as measured by elastin promoter activation in transgenic mice. Mice were pretreated topically with either a vehicle alone prior to exposure to SSR, or a sunscreen with an SPF of 2, 4, 8, or 15 (for details of the sunscreen preparations, see Bernstein *et al*, 1997), followed by 30 human MED of SSR. Untreated control mice (cont) received no topical treatment or SSR. SSR resulted in a 4.9-fold increase in CAT activity ($p = 0.001$) in mice treated with vehicle lotion alone (SSR), as compared with mice receiving no SSR (cont.). Application of sun-screens in mice receiving SSR reduced elastin promoter activity by 30%, 55%, 69%, and 78%, for sunscreens with SPF of 2, 4, 8, and 15, respectively. This reduction was statistically significant when compared with mice receiving the lotion vehicle alone followed by SSR (SSR), with the exception of the SPF2 sunscreen, which approached statistical significance ($p = 0.08$). *Bars*: mean ± SEM. Statistical significance is indicated above the bars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Adopted from Bernstein *et al* (1997).

◀ **Figure 1. Transgenic mouse model of cutaneous photoaging.** (*a*) CAT activity responds in a concentration-dependent fashion to UVB in mice. Single doses of 30.7, 122.8, and 491.4 mJ UVB per cm^2 administered over 3.75, 15, and 60 min, respectively, resulted in increasing CAT activities up to 8.5-fold over that of unirradiated control mice (0). The lowest dose given corresponds to ≈1.5 human MED of UVB. (*b*) Cutaneous fibroblasts derived from transgenic mice are exquisitely sensitive to UVB, with maximal promoter activation being about 30-fold higher than in unirradiated controls (0) after a single dose of only 5.5 mJ per cm^2. (*c*) CAT activity increases slightly in response to UVA *in vivo*. Single doses of 9.5 and 38.2 J UVA per cm^2 administered over 1.3 and 5.2 h, respectively, increased CAT activity less than 2-fold over unirradiated controls (0). This difference approached statistical significance ($p = 0.07$). The smallest dose given represents approximately one-fourth of a human MED for UVA. (*d*) UVA irradiation fails to increase CAT activity *in vitro*. Although substantial doses of UVA were administered over as long as 18.4 min (2.2 J per cm^2), CAT activity of fibroblast cultures was virtually unchanged, as compared with unirradiated controls (0). The failure of UVA to increase promoter activity *in vitro*, despite achieving almost 2-fold increases *in vivo*, suggests that the primary target for UVA-induced elastin promoter activation is a cell other than the dermal fibroblast. Thus, the mechanism for elastin promoter activation by UVA differs qualitatively from that of UVB. *Bars*: mean ± SD. Statistical significance is indicated above the bars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Adopted from Uitto *et al* (1997).

et al, 1997). In addition, the use of 8-methoxypsoralen plus UVA permits evaluation of agents that protect against UVA. To test the sensitivity of this system, sunscreens affording increasing amounts of UVA protection were evaluated *in vivo*. Sunscreens with increasing amounts of UVA protection afforded greater protection against elastin promoter activation *in vivo* (Tsunemichi *et al*, 1998). Increased sensitivity is afforded by using fibroblast cultures established from the skin of transgenic mice. The *in vitro* system serves as a way to rapidly screen agents thought to afford protection against UV radiation, and quantitating their relative abilities to prevent UV-induced promoter activation. In addition, the cell culture system can differentiate between the sunscreening ability of agents and the other means they may protect against UV, such as by functioning as a free radical scavenger. Through the use of systems such as this mouse model to rapidly screen agents thought to protect against UV radiation, better strategies to protect skin against photoaging will emerge, hopefully allowing us to have fun in the sun without untoward side-effects.

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REFERENCES

Bernstein EF, Chen YQ, Tamai K, *et al*: Enhanced elastin and fibrillin gene expression in chronically photodamaged skin. *J Invest Dermatol* 103:182–186, 1994

Bernstein EF, Fisher LW, Li K, LeBaron RG, Tan EML, Uitto J: Differential expression of the versican and decorin genes in photoaged and sun-protected skin: Comparison by immunohistochemical and Northern analyses. *Lab Invest* 72:662–669, 1995a

Bernstein EF, Brown DB, Urbach F, *et al*: Ultraviolet radiation activates the human elastin promoter in transgenic mice: A novel *in vivo* and *in vitro* model of cutaneous photoaging. *J Invest Dermatol* 105:269–273, 1995b

Bernstein EF, Chen YQ, Kopp JB, *et al*: Long-term sun exposure alters the collagen of the papillary dermis: Comparison of sun-protected and photoaged skin by Northern analysis, immunohistochemical staining and confocal laser scanning microscopy. *J Am Acad Dermatol* 34:209–218, 1996a

Bernstein EF, Gasparro FP, Brown DB, Takeuchi T, Kong SK, Uitto J: 8-Methoxypsoralen and ultraviolet A radiation activate the human elastin promoter in transgenic mice: *In vivo* and *In vitro* evidence for gene induction. *Photochem Photobiol* 64:369–374, 1996b

Bernstein E, Brown DB, Takeuchi T, Kong SK, Uitto J: Evaluation of sunscreens with various sun protection factors in a new transgenic mouse model of cutaneous photoaging that measures elastin promoter activation. *J Am Acad Dermatol* 37:725–729, 1997

Bissett DL, Hannon DP, Orr TV: An animal model of solar-aged skin: histological, physical and visible changes in UV-irradiated hairless mouse skin. *Photochem Photobiol* 46:367–376, 1987

Bissett DL, Hannon DP, Orr TV: Wavelength dependence of histological, physical and visible changes in chronically UV-irradiated hairless mouse skin. *Photochem Photobiol* 50:763–769, 1989

Bucala R, Cerami A: Advanced glycosylation: chemistry, biology, and implications for diabetes and aging. *Adv Pharmacol* 23:1–34, 1992

Chen VL, Fleischmajer R, Schwartz E, Palia M, Timpl R: Immunochemistry of elastotic material in sun-damaged skin. *J Invest Dermatol* 87:334–337, 1986

Dahlbäck K, Ljungquist A, Lofberg H, Dahlbäck B, Engvall E, Sakai L: Fibrillin immunoreactive fibers constitute a unique network in the human dermis: Immunohistochemical comparison of the distributions of fibrillin, vitronectin, amyloid P component and orcein stainable structures in normal skin and elastosis. *J Invest Dermatol* 94:284–291, 1990

Dalle Carbonare M, Pathak MA: Skin photosensitizing agents and the role of reactive oxygen species in photoaging. *J Photochem Photobiol B* 14:105–124, 1994

Davidson EA: Polysaccharide structure and metabolism. In: Montagna W (ed.). *Aging: Biology of Skin*, Vol. VI. Oxford: Pergamon Press, 1965, pp. 255–270

Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ: Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337:1419–1428, 1997

Gilchrest BA: Skin aging and photoaging: An overview. *J Am Acad Dermatol* 21:510–513, 1989

Gilchrest BA: *Photodamage*. Cambridge, MA: Blackwell Science, 1995, pp. 1–285

Griffiths CEM, Russman AN, Majmudar G, Singer RS, Hamilton TA, Voorhees JJ: Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). *N Engl J Med* 329:530–535, 1993

Hayflick L: The cell biology of aging. *J Invest Dermatol* 73:8–14, 1979

Hsu-Wong S, Katchman S, Ledo I, *et al*: Tissue-specific and developmentally regulated expression of human elastin promoter activity in transgenic mice. *J Biol Chem* 269:18072–18075, 1994

Kawaguchi Y, Tanaka H, Okada T, Konishi H, Takahashi M, Ito M, Asai J: Effect of reactive oxygen species on the elastin mRNA expression in cultured human dermal fibroblasts. *Free Rad Biol Med* 23:162–165, 1997

Kligman LH, Sayre RM: An action spectrum for ultraviolet induced elastosis in hairless mice: Quantification of elastosis by image analysis. *Photochem Photobiol* 53:237–242, 1991

Kligman LH, Akin FJ, Kligman AM: Prevention of ultraviolet damage to the dermis of hairless mice by sunscreens. *J Invest Dermatol* 78:181–189, 1982

Kligman LH, Akin FJ, Kligman AM: The contributions of UVA and UVB to connective tissue damage in hairless mice. *J Invest Dermatol* 84:272–276, 1985

Kochevar IE, Moran M, Granstein RD: Experimental photoaging in C3H/HeN, C3H/HeJ, and Balb/c mice: Comparison of changes in extracellular matrix components and mast cell numbers. *J Invest Dermatol* 103:797–800, 1994

Matrisian LM: The matrix-degrading metalloproteinases. *Bioessays* 14:455–463, 1992

Mera SL, Lovell CR, Jones RR, Davies JD: Elastic fibrils in normal and sun-damaged skin: An immunohistochemical study. *Br J Dermatol* 117:21–27, 1987

Peterson MJ, Hansen C, Craig S: Ultraviolet A irradiation stimulates collagenase production in cultured human fibroblasts. *J Invest Dermatol* 99:440–444, 1992

Stein B, Rahmsdorf HJ, Steffen A, Litfin M, Herrlich P: UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-*fos*, and metallothionein. *Mol Cell Biol* 9:5169–5181, 1989

Tsunemichi T, Uitto U, Bernstein EFA: Novel *in vivo* model for evaluating agents which protect against ultraviolet A-induced photoaging. *J Invest Dermatol* 110:343–347, 1998

Uitto J: Biochemistry of the elastic fibers in normal connective tissues and its alterations in disease. *J Invest Dermatol* 72:1–10, 1979

Uitto J: Molecular pathology of collagen in cutaneous diseases. In: *Advances in Dermatology*. St. Louis: Mosby Year-Book, 1990, pp. 313–328

Uitto J: Understanding of the cutaneous aging. *New Engl J Med* 337:1463–1465, 1997

Uitto J, Fazio MJ, Olsen DR: Molecular mechanisms of cutaneous aging: Age associated connective tissue alterations in the dermis. *J Am Acad Dermatol* 21:614–622, 1989

Uitto J, Brown DB, Gasparro F, Bernstein EF: Molecular aspects of photoaging. *Eur J Dermatol* 7:210–214, 1997

Zimmermann DR, Dours-Zimmermann MT, Schubert M, Bruckner-Tuderman L: Versican is expressed in the proliferating zone in the epidermis and in association with the elastic network of the dermis. *J Cell Biol* 124:817–825, 1994