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As first published as:
Madalene C.Y. Heng, M.D., FRACP, FACD
"Chapter 19. Signaling Pathways Targeted by Curcumin: Basis for Anti-Photoaging and Anti-Carcinogenic Therapy"; and
"Protocol Chapter 2. Protocols for Work-Up and Anti-Aging Treatment for Photodamaged Skin."
Anti-Aging Therapeutics Volume X, 151-160 and 319-320.
Chicago, IL USA: American Academy of Anti-Aging Medicine, 2008.

Excerpted from A4M's *Anti-Aging Therapeutics Volume X*

Reprint prepared for Madalene C.Y. Heng, M.D., by
A4M and Madalene C.Y. Heng, M.D.

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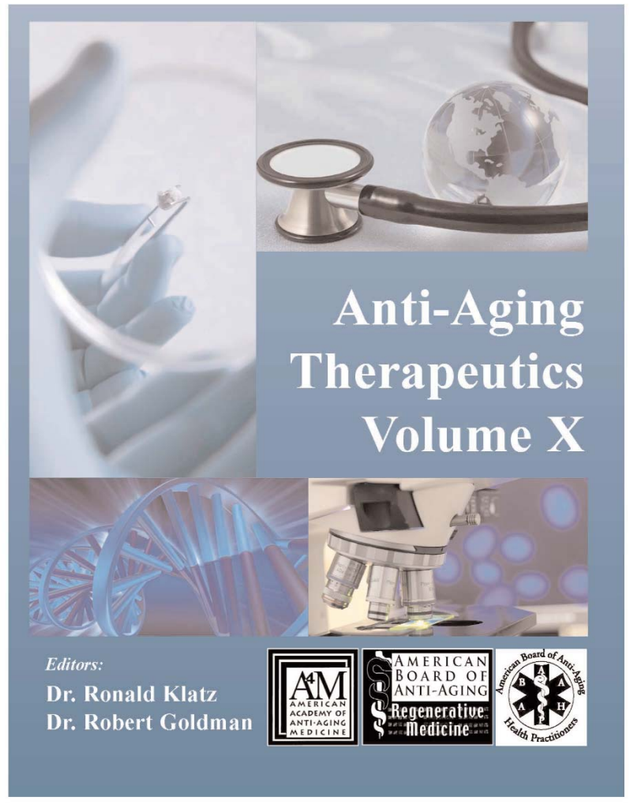

Chapter 19
Signaling Pathways Targeted by Curcumin: Basis for Anti-Photoaging and Anti-Carcinogenic Therapy

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ABSTRACT

Increasing interest has been focused on certain dietary botanicals and their potential use in the treatment of photoaging skin and prevention of photocarcinogenesis. In this review, the biochemical mechanisms and injury pathways involved in photoaging and photocarcinogenesis are summarized, with specific focus on potential targets for the preventive use of curcumin in these conditions.

INTRODUCTION

Photoaging of the skin has assumed increasing importance particularly in the context of an increasingly aging population. Chronic solar exposure not only produces photoaging, characterized by skin fragility, scaling, and pigmentary changes, but is also associated with the development of premalignant and malignant non-melanoma and melanoma skin cancers (Runger 1999; Bachelor 2003).

Photocarcinogenesis is initiated by DNA damage, most commonly induced by UVB (280-320 nm) and UVA (320-400 nm) solar radiation. It has since been observed that although the UVB wavelengths causes burning of the skin, these rays have low penetrating properties, and are not capable of penetrating tissue much below the surface of the epidermis. More recently, the role of UVA (320-400 nm) in photocarcinogenesis has been increasingly recognized (Runger 1999; Bachelor 2004). Unlike UVB, which, because of its limited penetrating properties predominantly affects the epidermal keratinocytes, UVA damages basal cells and melanocytes and easily penetrates into the mid-dermis, where it interacts with dermal fibroblasts, stromal tissue, and blood vessels. It is now believed that the changes of photoaging, including loss of elastic tissue, dryness, scaling, and patchy hyperpigmentation, result mainly from chronic UVA exposure. In comparison with UVB rays, it has been observed that although wavelengths in the UVA spectrum do not produce burning, the UVA rays are capable of penetrating far below the epidermis, and the effects of prolonged UVA exposure have been observed to affect the mid and even the lower dermis. The inadequacy of sunscreens to protect against UVA-induced free radical formation has been recently reported and has important implications both in photoaging and in melanoma and non-melanoma photo-induced tumorigenesis (Haywood 2003; Bachelor 2004).

Epidemiologic studies have implicated sunlight exposure as a risk factor in the development of basal cell and squamous cell carcinomas, although correlation is better for squamous cell carcinomas than for basal cell carcinomas and melanomas. Point mutations and mutagenic bipyrimidine dimers have been observed with combined UVB and UVA exposure. Point mutations of the type seen in UVB exposure have been observed in the p53 gene on chromosome 17p in 40-56% of basal cell carcinomas. However, limited association between basal cell carcinomas and sites of UVB (280-320 nm) exposure suggests that additional factors must be involved It is possible that the mutagenic effects of UVA may add to the genotoxic effects of UVB in initiation of melanomas and non-melanoma skin cancers.

Increasing interest has been focused on certain dietary botanicals and their potential use in the treatment of photoaging skin and prevention of photocarcinogenesis. In this review, the biochemical mechanisms and injury pathways involved in photoaging and photocarcinogenesis are summarized, with specific focus on potential targets for the preventive use of curcumin in these conditions.

STEPS INVOLVED IN PHOTOCARCINOGENESIS

Specifically, photocarcinogenesis involves three steps: (A) tumor initiation with DNA damage induced in a single cell as a result of the genotoxic effects of the mutagenic photoproducts; (B) tumor promotion, with clonal expansion of the clone of DNA-damaged cells, and (C) tumor transformation of the damaged clone by further DNA and stromal changes, leading to disregulated growth and acquisition of metastatic potential.

A. Tumor Initiation (DNA Damage)

It has been shown that although oxidative lesions are the main type of DNA damage involved with UVB exposure, other genotoxic products are generated with solar UVA exposure that may be even more mutagenic. In particular, unlike UVB-generated [6-4]-photoproducts, which are quickly repaired, UVA-generated bipyrimidine photoproducts are poorly repaired and isomerize into Dewar products that are highly mutagenic (Douki 2003). In addition, the induction of singlet oxygen formation by UVA is the basic event leading to signal transcription-factor-mediated gene expression in UVA-damaged skin.

B. Tumor Promotion with Clonal Expansion

1. Induction of Gene Transcription: Activation of Transcription Factors

(a) Nuclear Factor-kappa B (NF-κB) **–** Nuclear factor-kappa B (NF-B) is a family of related protein dimers that bind to a common sequence on the DNA, the κB site. In the quiescent state, the NF-κB dimers are located in the cytoplasm. When activated by free radicals generated by ultraviolet light exposure, as well as by other injurious stimuli such as radiation, endotoxins, carcinogens, tumor promoters, and inflammatory cytokines, the activated NF-κB dimers, a complex made of p50/p65 subunits, are translocated to the nucleus. NF-κB then goes on to induce transcription of over 200 genes involved in cell proliferation, cell transformation, inhibition of apoptosis, and metastases.

Curcumin, the active ingredient in turmeric, is an indirect but potent inhibitor of NF-κB activation. The process of activating NF-κB dimers involves the removal of the inhibitory protein, IκBα, by phosphorylation of its kinase (IκBα kinase, a serine/threonine kinase), which in turn is activated by phosphorylase kinase. Curcumin, a selective phosphorylase kinase inhibitor, blocks NFκB activation by blocking its IκBά kinase Blocking NF-κB activation is an important mechanism for the anti-inflammatory and anticarcinogenic effect of curcumin.

(b) Activator protein-1 (AP-1) – AP-1 is a transcription activator which bears similarity to a DNA-binding protein encoded by the tumor transforming viral oncogene. The complex consists of members of the JUN and FOS family of proteins. The inducers of AP-1 include environmental stresses such as ultraviolet light, various growth factors, and inflammatory cytokines. AP-1 has been implicated in growth regulation and cell transformation by activating the cyclin D1 gene, which promotes the initiation of cells into the G1 phase of the cell cycle. AP-1, by suppressing the p53 tumor suppressor gene, causes uncontrollable growth and cell transformation. Curcumin has been shown to also suppress the activation of AP-1.

2. Cell Proliferation

(a) Mitogen Activated Protein (MAP) Kinases **–** The mitogen activated protein kinase pathway which results in cell proliferation of epidermal cells involves activation of MAP kinase kinase kinase (MAP 3Kinase), which then activates MAP kinase kinase (MAP 2Kinase). These kinases are serine threonine kinases, which when activated go on to activate MAP kinase (MAPK), a growth factor-dependent receptor tyrosine kinase (see below under Growth Factor Signaling Pathways). Activation of the tyrosine kinase at the cell membrane level is responsible for triggering intracellular pathways resulting in cell growth and cell proliferation.

The MAP kinases are responsible for activating NK-κB-induced proliferative pathways, including the extracellular signal regulated protein kinases (ERK), c-jun N-terminal kinases (JUN), stress-activated protein kinases (SAPK), and p38 protein kinases. In skin cancers, stress activated pathways are particularly important, since stress activated promoters, such as ultraviolet light, activate NF-κB through phosphorylation of ERK, JNK, SAPK and p38 kinases. All these kinases are serine/threonine kinases believed to be activated by phosphorylase kinase and blocked by curcumin.

(b) Growth Factor Signaling Pathways **–** Growth factors are proteins that bind to receptors on the cell surface, with resultant activation of cell proliferation and/or differentiation. Growth factors that are implicated in carcinogenesis include: epidermal growth factor (EGF), platelet-derived growth factor, fibroblast growth factors (FGFs), insulin-like growth factor (IGF), transforming growth factors (TGFα and TGFβ) as well as cytokine growth factors such as TNFα (tumor necrosis factor-ά) and IL-1 (interleukin-1). These growth factor-induced signaling pathways are involved in non-malignant proliferation, e.g. psoriasis, as well as in proliferation of transformed cells.

The binding of growth factors to its tyrosine-kinase based receptor results in phosphorylation of the receptor, activation of the receptor, and triggering of serine-threonine based signaling pathways

resulting in cell growth and proliferation. Curcumin has been shown to inhibit the tyrosine-kinase activity of this receptor, and also inhibits serine/threonine-dependent pathways. Furthermore, it is probable that the effect of curcumin may be achieved through its inhibition of phosphorylase kinase. Phosphorylase kinase, which is involved in tyrosine-kinase dependent phosphorylation reactions, is also involved in serine/threonine kinase-dependent phosphorylation (see MAP kinases). In addition, since inhibition of phosphorylase kinase depletes ATP levels, the curcumin treated ATP-depleted cell may also have difficulty maintaining the growth factor receptor in its folded state.

3. Apoptosis-Cell Survival Balance

The balance between cell survival and cell death determines the number of existing cells. In cancer, the balance is tipped towards cell survival of UV-damaged cells. Cell death (apoptosis) helps to remove excess, damaged, or abnormal cells. It has been observed that activation of NF-κB promotes cell survival, and downregulation of NF-κB sensitizes the cells to apoptosis induction. Inhibition of NF-κB by curcumin promotes apoptosis of photodamaged cells and retards photoaging as well as the development of skin malignancies.

(a) Apoptotic Proteins – Apoptotic proteins include the caspase family, in particular caspase 8, caspase 9, and caspase 3, which trigger DNA fragmentation when activated, leading to loss of membrane potential, and leakage of cytochrome c into the cytoplasm. Other apoptotic proteins include PARP and Bax proteins, which are also involved in the apoptotic process. It has been observed that NF-κB-dependent expression of cell survival genes block apoptosis, thus promoting survival of photodamaged cells. On the other hand, curcumin, which inhibit NF-kB activation, sensitizes cells to apoptosis induction (Aggarwal B, 2003), thus killing off photodamaged cells. Curcumin has been observed to cause p53-dependent apoptosis in human basal cell carcinoma cells. (Jee *et al*,1998).

(b) Antiapoptotic proteins – Anti-apoptotic proteins such as Bcl-2 and Bcl-xL inhibit apoptosis and increase cell survival. On the other hand, downregulation of apoptosis suppressor proteins such as Bcl-2 or Bcl-xL by curcumin has been shown to induce apoptosis in cancer cell lines. This leads to activation of nuclear DNA fragmentation through mitochondrial disruption and cytochrome c release through activation of the caspase-dependent apoptotic pathways. NF-κB-dependent expression of cell survival genes, including survivin, TRAF1, and TRAF2, block apoptosis of photodamaged cells. By downregulating anti-apoptotic proteins, curcumin promotes apoptosis of photodamaged cells, thus improving photoaging skin and reduce the development of premalignant and malignant skin lesions.

(c) Cell Survival Kinase (Akt) – The cell survival kinase, Akt, is a serine/threonine protein kinase activated by growth and survival factors. Akt is activated by phosphorylation at the Thr308 and Ser473. Activated Akt promotes cell survival by activating NF-kB signaling pathway, and by inhibiting apoptosis of photodamaged cells. Since the activation of NF-κB is dependent on removal of its inhibitory IκBά protein achieved by activation of the serine threonine IκBά kinase, inhibition of IκBα kinase would result in inhibition NF-κB. Both IκBά kinase (NFκB activator) and Akt (survival kinase) are serine/threonine kinases activated by phosphorylase kinase and inhibited by curcumin. Thus, curcumin promotes apoptosis of photodamaged cells both by promoting NFκB-dependent apoptosis and by inhibiting Akt-dependent cell survival.

C. Cell Transformation and Metastatic Potential

1. Dysregulated Cell Cycle and Tumor Transformation

Proteins that regulate the cell cycle, in particular the timing of cell cycling events are important in tumor transformation since loss of this regulation is the hallmark of the cancerous cell. These proteins are known as the cyclins, which are in turn, regulated by cyclin-dependent kinases.

Cyclin D1, a subunit of cyclin dependent kinase, cdk-4, and cdk-6, is the rate-limiting factor regulating the G1 phase of the cell cycle. Overexpression of cyclin D1, and other cyclin-dependent kinases, causes excessive growth promotion and dysregulation of the cell cycle associated with tumorigenesis, with increased expression related to proliferating cell nuclear antigen expression. Curcumin has been known to block the dysregulated cell cycle in cancers. Cyclin D1 expression is regulated by NF-κB, and suppression of NF-κB by curcumin leads to downregulation of cyclin D1. Curcumin also induces AF-1/p-21-mediated G1 phase arrest of the cell cycle (Aggarwal B, 2003), thus retarding proliferation of premalignant and malignant cells.

2. p53 Transcription Factor and Tumor Transformation

p53 is a transcription factor which functions as a tumor suppressor. It regulates many cellular processes including signal transduction and cell cycle control. It is also responsible for cellular response to DNA damage and subsequent cellular genomic stability. It activates the transcription of genes such as p21WAF1 and Bax to induce apoptosis of DNA damaged cells, resulting in the inhibition of growth of DNA damaged cells, including cancer cells. Mutant p53 loses its ability to bind DNA effectively. Consequently, the p21WAF1 protein is not formed to regulate cell division, with resultant uncontrollable growth and tumor formation. In one study, over 90% of squamous cell carcinomas and more than 50% of basal cell carcinomas were linked to deletion of p53 suppressor gene expression. The antitumorigenic effect of curcumin may lie in its ability to upregulate p53 and p21WAF-1/CIP1, perhaps by apototic removal of damaged cells with mutated p53 and low P21 WAF-1 expression. It has been observed that curcumin selectively induces apoptosis in deregulated cyclin D1-expressed cycling G2 phase tumor cells in a p53-dependent manner.

3. Proteins in Tumor Invasion and Metastases: Cell Adhesion Molecules and Matrix Metalloproteinases

The penetrating properties of UVA into the dermis allow ultraviolet radiation of this wavelength band to affect dermal fibroblasts and mesenchymal tissue, inducing the production of tissue metalloproteinases seen in photoaging skin. Tissue injury and resultant inflammatory response result in generation of cytokines and growth factors, which activate transcription factors, such as AP-1 and NF-kB. These synergize to activate metalloproteinase promoter genes, inducing gene transcription. In the case of UVA exposure, it has been shown that singlet oxygen generated as a result of UVA exposure may mediate transcription factor-induced expression of cell adhesion molecules. The upegulation of matrix metalloproteinases promote invasiveness of the tumor into the dermis and deeper tissues. The expression of cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), which allows tumor anchorage and vascular invasion, is also intimately involved in tumor metastases. Two metalloproteinases, MMP-2 and MMP-9, have been observed to be closely involved in promoting tumor metastases. Specifically, MMP-2 and MMP-9 are responsible for digestion of collagen IV in basement membranes, and collagen V in the subendothelial fibrillary component of epithelial and endothelial cells, thus enabling the tumor cells to invade into the dermis, as well as penetrate blood vessels. Metalloproteinase-2 (MMP-2) expression, in particular, has been shown to correlate with aggressiveness of cutaneous squamous cell carcinomas. Curcumin downregulates the expression of both matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), and may reduce the potential for tumor metastases, which rely on these proteins for tissue invasion.

CURCUMIN: A SELECTIVE INHIBITOR OF PHOSPHORYLASE KINASE

Curcumin (diferuloylmethane) is a dietary phytochemical found in the rhizome of the plant (*Curcuma longa*) from which turmeric is derived. Its anticarcinogenic properties have been extensively reviewed by Aggarwal *et al*. (2003). The pathways targeted by curcumin are summarized in Fig 1. As detailed above, curcumin appears to block carcinogenesis in a multi-targeted fashion, which at first glance appears confusing because its many reported effects are difficult to reconcile from the viewpoint of an underlying fundamental mechanism. We propose a unifying concept which may explain the multifaceted inhibitory effects of curcumin in inflammation, anti-aging, and photocarcinogenesis through its selective inhibitory activity on phosphorylase kinase, a protein kinase with unique properties and multiple specificities.

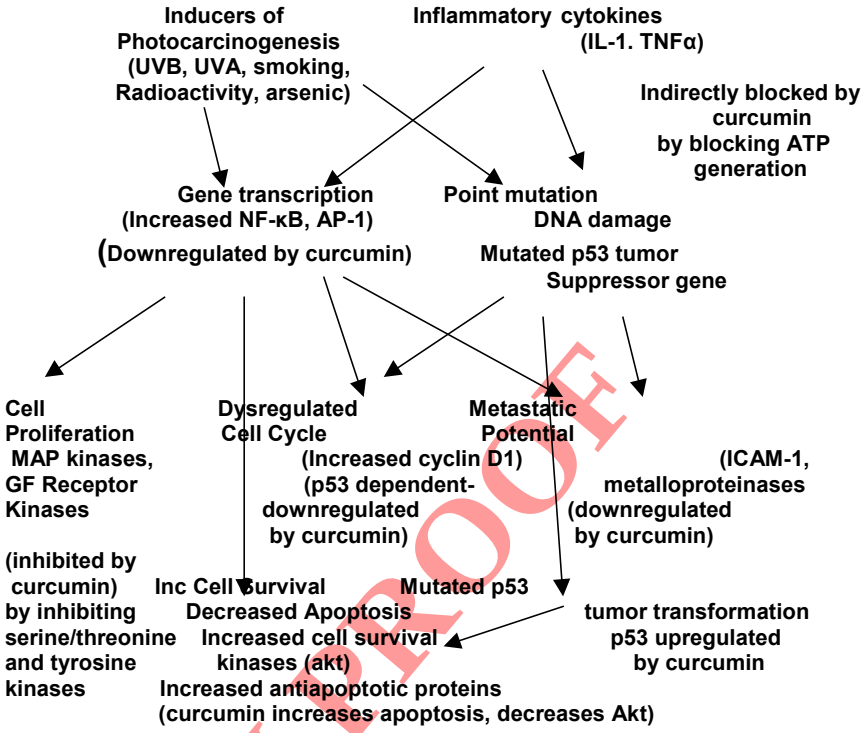


Figure 1. Pathways in photocarcinogenesis: targets for curcumin.

Phosphorylase Kinase: a Protein Kinase with Multiple Specificities

Protein kinases usually catalyze phosphotransfer reactions from ATP to either serine/threonine or tyrosine residues. This is because protein kinases, with the exception of phosphorylase kinase, allow only one configuration at its substrate binding site. In the case of phosphorylase kinase, however, the substrate binding site may be altered by utilizing a hinge joint between the subunits of the phosphorylase kinase molecule, thus altering the size of the substrate binding site. In addition, the substrate binding site can be made to alter its shape by causing it to swivel first in one plane (by binding to magnesium) and in another plane (by binding to manganese). In this way, phosphorylase kinase is able to phosphorylate substrates of multiple specificities, including protein kinases with serine/threonine, tyrosine, phosphatidylinositol, troponin etc… as specific moieties.

In support of the above mode of action of phosphorylase kinase, Graves *et al* (1999) provided evidence that in the phosphorylase kinase molecule, the spatial arrangement of specificity determinants can be manipulated so that phosphorylase kinase can utilize other substrates. It is possible that this flexibility may be the result of both the presence of the hinge joint between the subunits of phosphorylase kinase, a flexibility further enhanced by the ability of the molecule to alter the substrate binding site by metal ion (magnesium or manganese) specificity. This flexibility enables phosphorylase kinase to take part in a multiplicity of phosphorylation reactions.

Moreover, Yuan *et al* (1991) provided evidence of dual specificity depending on ion binding (magnesium, manganese). The ability of phosphorylase kinase subunits to adapt to different enzyme configurations, allow for one enzyme to accept many substrates, including serine/threonine kinases, tyrosine kinase, and phosphatidylinositol kinase

Phosphorylase Kinase: an ATP Generator

In addition, phosphorylase kinase is the only known enzyme which catalyzes the phosphorylation of glycogen phosphorylase b to glycogen phosphorylase a, since no other kinase has yet been observed to be able to duplicate this reaction. In doing so, phosphorylase kinase breaks down glycogen to produce ATP. Also known as ATP-phosphorylase b phosphotransferase, phosphorylase kinase, therefore, integrates multiple calcium-calmodulin-dependent signaling pathways while coupling these reactions to glycogenolysis and ATP-dependent phosphorylation.

Curcumin: a Selective Phosphorylase Kinase Inhibitor

We have previously reported that curcumin gel inhibits phosphorylase kinase activity in the skin (Heng *et al*, 2000). In addition, we have also demonstrated that inhibition of phosphorylase kinase activity by curcumin correlates with apoptosis of cells expressing proliferating cell nuclear antigen (PCNA) as shown by the Ki-67 immunocytochemical marker (Heng *et al*, 2000). Proliferating cell nuclear antigen (PCNA) is expressed in both premalignant (actinic keratoses, solar lentigenes) and malignant (basal cell carcinoma, squamous cell carcinoma and malignant melanoma), as well as in non-malignant cell proliferation (psoriasis, eczema). By inhibiting phosphorylase kinase, curcumin thus benefits photodamaged cells by inhibiting serine/threonine kinases (e.g. IκBά kinase, a kinase responsible for NFκB activation; MAP kinases responsible for cell proliferation; and Akt responsible for increased cell survival of photodamaged cells. Curcumin also inhibits cyclin kinases involved in cell cycling. By its action on tyrosine kinase inhibition, it inhibits growth factor dependent proliferation. By upregulating the p53 suppressor gene, it promotes apoptosis of photodamaged cells, and promotes p53-dependent cell regulation, and inhibits cell transformation.

CLINICAL IMPLICATIONS

Unfortunately, curcumin does not seem to be well absorbed when taken orally, and high doses of curcumin have failed to produce detectable blood levels. Curcumin in a topically gel, however, has been effective in many skin problems, particularly in skin lesions induced by injury. The injury pathway induced by ultraviolet light which results in photoaging and photocarcinogenesis are summarized in Fig 2, which also illustrates the potential role of curcumin gel in the treatment of a variety of skin conditions.

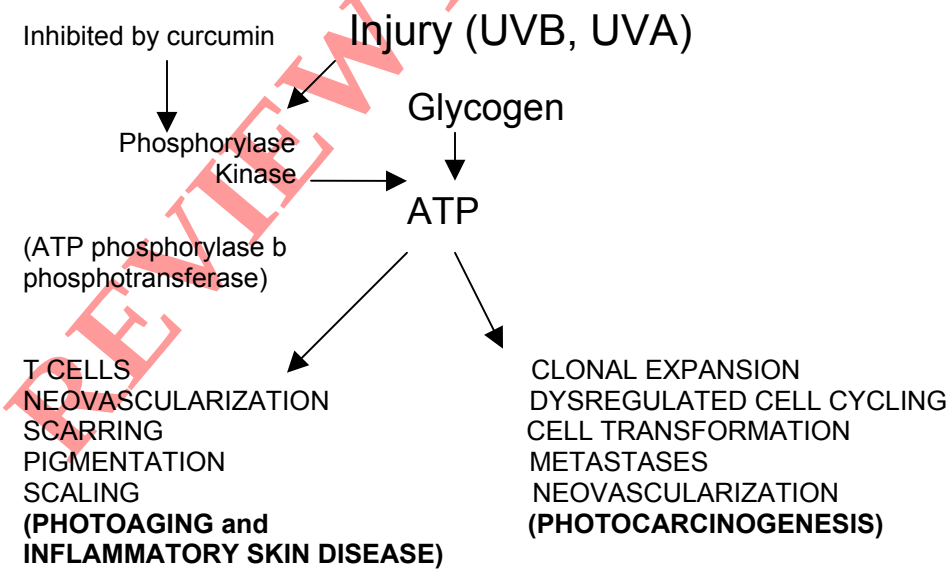


Figure 2. Injury pathway

Inflammatory Skin Disease and Scars

We have shown that inhibition of phosphorylase kinase activity by topical curcumin gel results in resolution of increased T lymphocyte population in inflammatory skin disease (psoriasis, eczema, burns, acne) thus accounting for the anti-inflammatory activity of curcumin gel (Heng *et al*, 2000). Clinically, curcumin in the form of a topical gel has been observed to have anti-inflammatory properties and to decrease redness and inflammation in sun-damaged skin. It has been observed to produce healing of superficial burns with minimal or no scar formation. Our observations also include a decrease in scarring in acne and pseudofolliculitis with curcumin gel. We had previously reported that curcumin gel produced resolution of psoriasis, correlating with suppression of phosphorylase kinase activity (Heng *et al*, 2000).

Photodamaged Skin

In photodamaged skin, topical curcumin in a gel base has been observed to improve the texture of photodamaged skin resulting in decreased appearance of wrinkle formation. It has also been effective in decreasing solar induced hyperpigmentation, and improving solar-induced telangiectasia. In photodamaged skin with actinic keratoses and solar lentigenes, curcumin gel has been observed to reverse these changes. These observations are consistent with reports that curcumin is capable of inducing apoptosis of damaged cells (Aggarwal 2003, Jee 1998), and shown in the following series of photographs.

We present clinical photographs (before and after curcumin gel application) taken from patients with various types of photodamaged skin as clinical evidence of the efficacy of curcumin gel in improving patients with these conditions.

In Figure 3, the patient had confluent actinic keratoses over most of her exposed areas, with scars from multiple surgeries for non-melanoma skin cancers. A large keratotic lesion was observed in the lower presternal chest (Fig. 3a, magnified in Fig. 3c) which partially resolved after 6 months with curcumin gel and sunscreen (Fig. 3b, magnified in Fig. 3d). Resolution was observed after 12 months with curcumin gel (Fig. 3e). Note the residual loss of pigmentation in the presumed apoptotic area after resolution of the keratotic lesion (Fig. 3e) and the general improvement in the remaining skin (Fig. 3e).

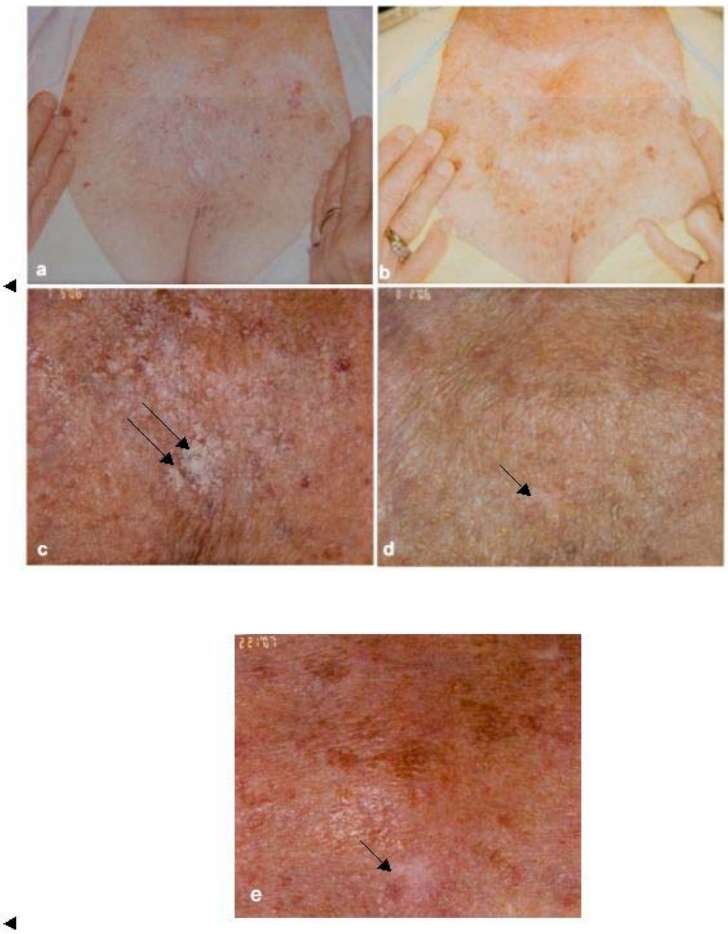
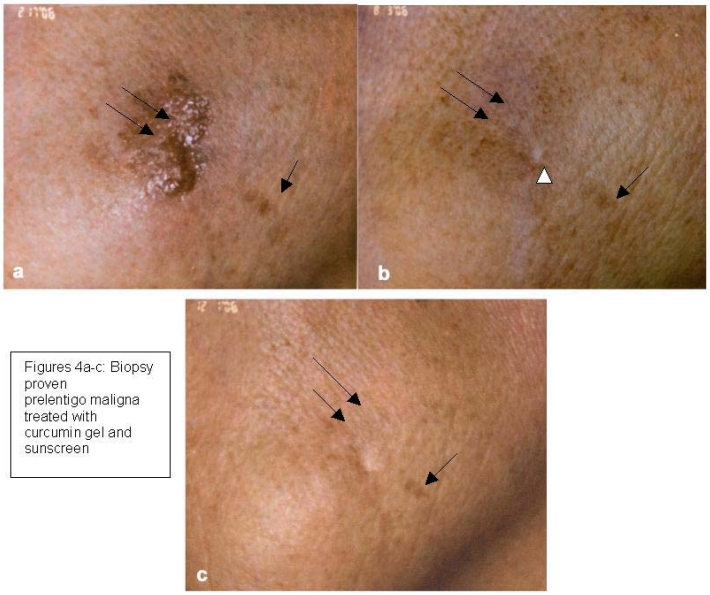


Figure 3: (a and c) severely photodamaged skin anterior chest, with large actinic keratosis before treatment with curcumin gel and sunscreen; (b and d) marked improvement 6 months after curcumin gel and sunscreen therapy; (e) resolution of actinic keratosis with apoptotic changes (unequal arrows) 12 months after curcumin and sunscreen treatment.

We have also observed that the more advanced the premalignant lesion, the more dramatic the resolution. For example, in Fig. 4a-c, the large biopsy proven prelentigo maligna on the left was mostly resolved after 10 months of curcumin gel (with sunscreen on top). However, the less involved smaller pigmented lesion to the right of the prelentigo maligna (Fig. 4a-c) was less improved after 10 months of curcumin gel (also with sunscreen on top) sunscreen).



Figures 4a-c: Biopsy proven prelentigo maligna treated with curcumin gel and sunscreen

Figure 4: (a) Biopsy proven prelentigo maligna on cheek of patient before treatment by curcumin gel and sunscreen; (b) improvement noted after 6 months of treatment with curcumin gel and sunscreen; (c) further improvement (unequal arrows) was noted 10 months after initiation of therapy. Observe marked improvement in the lesion on the left (double arrows), and much less improvement in the lesion on the right (single arrow). Arrowhead indicates the scar resulting from the punch biopsy

Curcumin gel decreases pigmentation induced by solar damage, particularly if used together with a good sunscreen (SPF 45-70). In such patients, wrinkles secondary to photoaging were observed to improve with increasing use. In Fig. 5a-c, there is resolution of pigmentary solar lentigenes over 12 months, with gradual decrease in wrinkles over this period (Figs. 5a-c).

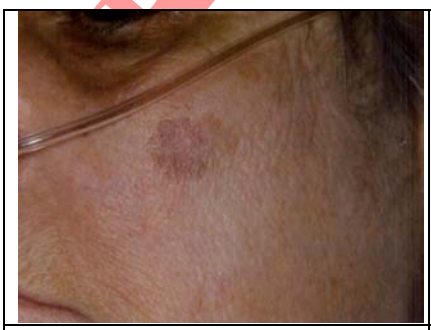
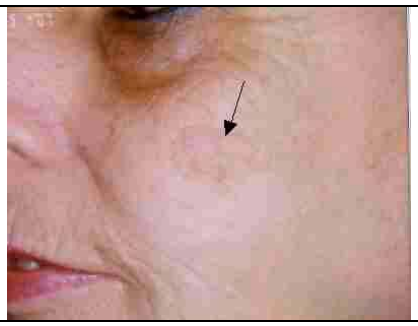
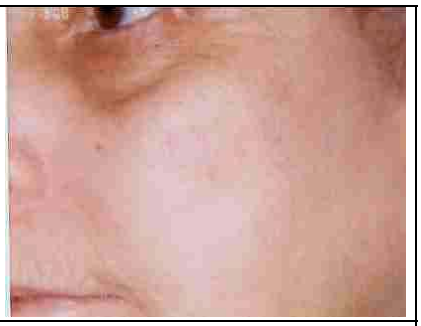
		

Figure 5a: Solar lentigo associated with photodamaged skin showing many wrinkles	*Figure 5b: Decreased pigmentation in solar lentigo (arrow) and wrinkles after 6 months of curcumin gel with sunscreen (more sunscreen applied to dark spot than surrounding skin).*	*Figure 5c: Further improvement in solar lentigo with marked improvement in wrinkles after 12 months of curcumin gel with sunscreen.*

We have also observed improvement in skin quality of photodamaged skin, with decreased atrophy, and improvement in telangiectasia resulting in decreased erythema after 6 months or more of curcumin gel application (Fig. 6a, b).

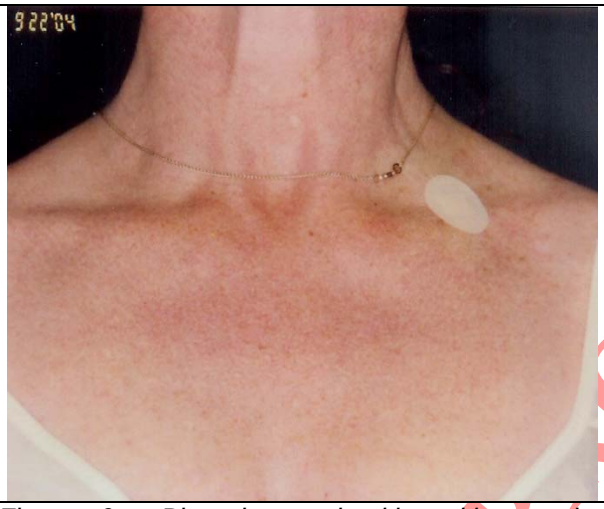


Figure 6a: Photodamaged skin with atrophy, telangiectasia and pigmentary changes treated with sunscreen alone.

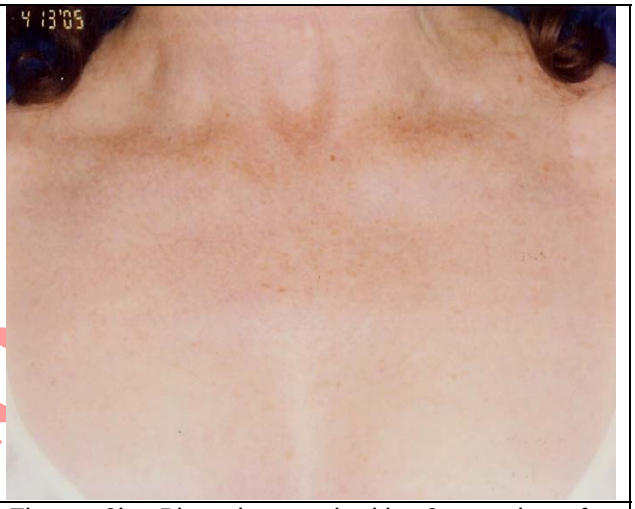


Figure 6b: Photodamaged skin 6 months after curcumin gel applied under sunscreens. Note improvement in skin atrophy, telangiectasia, and pigmentation.

In severely photodamaged skin with increased skin roughness from multiple actinic keratoses, curcumin gel therapy used together with sunscreens result in improvement of the skin quality and smoothness. Many actinic keratoses are seen to resolve with curcumin gel together with sunscreen without surgery (Figs. 7a, b).

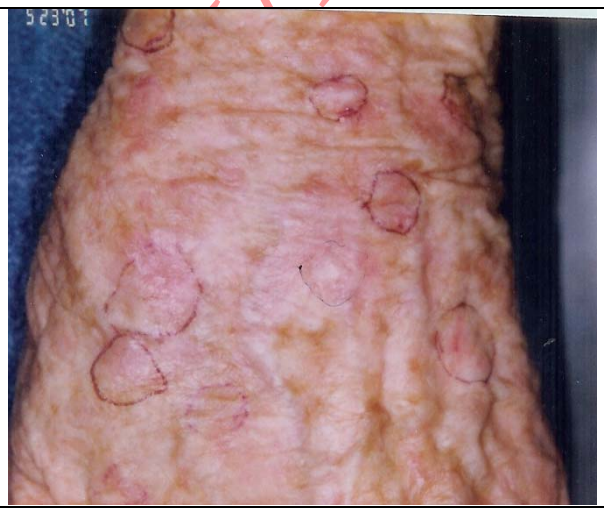


Figure 7a: Photodamaged skin with multiple actinic keratoses over the dorsum of the hand not resolving when treated with sunscreen alone.

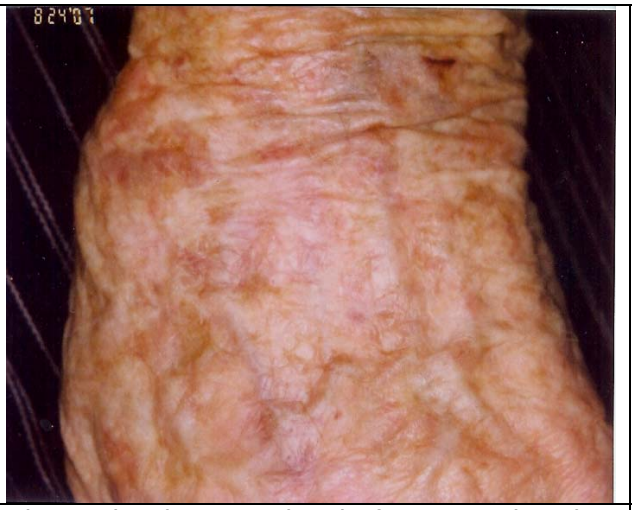


Figure 7b: The same hand after 3 months when curcumin gel was added to sunscreen. The keratotic lesions were pretreated with rubbing alcohol to enhance the penetration of curcumin gel under the keratotic scale. Sunscreen was applied before sun exposure. Note the resolution of many of the keratotic lesions.

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Protocol Chapter 2

Protocols for Work-Up and Anti-Aging Treatment for Photodamaged Skin

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INTRODUCTION

Photoaging skin is characterized by cosmetic changes such as atrophy, pigmentation, and telangiectasia, as well as wrinkling and increased laxity of the skin. More importantly, photodamaged skin is associated with the development of skin cancers, such as basal cell carcinomas, squamous cell carcinomas, and malignant melanomas, which are preceded by premalignant lesions such as actinic keratoses, solar lentigenes, and premalignant lentigo maligna. Both the premalignant and malignant lesions are associated with DNA damage. While the premalignant lesions are associated with DNA damage in the promoter sequence, additional damage to the p53 suppressor gene transforms the cell into the malignant phenotype. DNA damage may occur as point mutations, or by the release of bipyrimidine photoproducts. Point mutations on the DNA are caused by both ultraviolet A (UVA) and ultraviolet B (UVB) exposure. These tend to be more easily repaired. However, bipyrimidine photoproducts from UVA exposure tend to convert to Dewar products, causing damage which is poorly repaired, and therefore highly mutagenic. While UVB is known to produce redness, burning and blistering, these rays (290-320 nm) do not penetrate much below the skin surface, and cause more damage to the keratinocytes than to basal cells and melanocytes. On the other hand, UVA penetrates down to the deep dermis, and is the major wavelength causing basal cell carcinomas, malignant melanomas and dermal changes associated with photoaging such as solar elastoses, telangiectasia, wrinkling, and sagging of the skin. While UVB rays are blocked by clothing and sunscreens, UVA rays are effectively blocked only by bone. For this reason, melanomas and basal cell carcinomas have been observed to develop in areas usually covered by clothing. In view of the lack of adequate protection against UVA exposure by current sunscreens, the use of alternative measures directed at antiphotocarcinogenesis is being explored. More recently, curcumin, the active ingredient in turmeric, has been shown to have anticarcinogenic properties, with the ability to selectively induce apoptosis in photodamaged cells, while leaving normal cells unaffected. We have observed improvement in premalignant lesions such as solar lentigenes, actinic keratoses, as well as erythema, pigmentation, telangiectasia, and wrinkling. However, the improvement, although lasting, is not immediate, but requires at least 6 months to a year or more to effect.

SUGGESTED PROTOCOLS

Initial Work-Up:

This includes a detailed past and family history of non-melanoma skin cancers (basal cell carcinomas, squamous cell carcinomas) and malignant melanomas (type, level, lymph node dissection etc). A positive family history of melanoma suggests that there may be a genetic predisposition, particularly if the patient is young or has multiple melanomas. It is believed that in some cases, there is a genetic defect in the DNA ligase, the enzyme that repairs DNA damage after UV exposure.

The history should include amount of sun exposure (work-related, golf, walking/running, swimming, fishing, surfing, sailing, and gardening), and the time of day of sun-exposure. Smoking is also a predisposing factor, as well as exposure to arsenic and radioactive products.

The patient should have a thorough total body skin check from the scalp to the toes to look for abnormalities such as premalignant lesions (solar lentigenes, dysplastic nevi, actinic keratoses), and malignant lesions (lentigo maligna, malignant melanoma, Bowen's disease (squamous cell carcinoma in situ), squamous cell carcinomas, and basal cell carcinomas.)

Skin Biopsies and Excisions:

The malignant lesions should be biopsied and removed/excised accordingly.

Photography

Premalignant lesions should be photographed for future monitoring. The patient should be followed up every 3 months and the same lesions/locations photographed using the same magnification and same lighting. Should these lesions fail to improve with the curcumin gel, they should be biopsied, and dealt with according to the biopsy results.

Curcumin Gel Therapy

(1) Photodamaged Skin, Pigmentation, Telangiectasia, Wrinkles:

After a morning shower, the skin is "pat-dried" with a towel. The product spreads more easily if the skin is slightly damp (but not wet). A dab of curcumin gel is transferred from the jar with a Q tip, spreading the curcumin gel all over the affected area with the fingers to cover as large an area as the gel will allow until the area feels dry. This ensures that only a thin layer of gel is applied to the skin. More gel may be applied as necessary. Do not double dip to avoid transferring the bacteria from the skin back into the jar. After use, close the lid tightly to avoid drying out of the product. Application of rubbing alcohol before the curcumin gel is not necessary.

Photographs with close-ups of the skin before treatment is started, with sequential photographs every three to six months during therapy, is recommended in order to detect subtle changes in skin wrinkling, telangiectasia, and pigmentation. The photographs should be with Polaroid film, complete with dates of exposure so that the "Before" and "After" photographs can also be assessed by the patient at the time of the visit.

(2) Actinic Keratoses:

Areas with multiple actinic keratoses are treated in the same way as for photodamaged skin by first applying a thin layer all over the affected area (face, forearms, hands etc…). If the keratosis has a thick scale, the thick scale will prevent the curcumin gel from penetrating the skin beneath the scale. To enhance the penetration of curcumin through the scale, pretreat the keratosis with a little alcohol. Then massage curcumin gel into the wet alcohol, which will then "drag" the curcumin gel through and under the scale.

(3) Solar Lentigenes and Premalignant melanomas:

Biopsies may first be performed to make sure that the lesion is not a lentigo maligna or a malignant melanoma. Curcumin gel should be applied over the lesion and the rest of the surrounding skin. After the curcumin gel is dry, apply a layer of sunscreen solely over the pigmented lesion. Photography is essential at 3 monthly intervals. Be prepared to perform surgery if the lesion enlarges or does not improve.

Sunscreens

Select a sunscreen with a higher SPF (sun protection factor) number i.e. 45 to 70. Make sure that the patient is not allergic to the sunscreen. The sunscreen should be applied above the curcumin gel layer if the patient plans to be exposed to sunlight.

For pigmentary lesions such as melasma and solar lentigenes, curcumin gel is first applied all over the face or hands/forearms. When the curcumin gel is dry, which should be almost immediate or shortly after application, sunscreens are applied to the areas of pigmentation, taking care to apply more sunscreens to the darker areas and less to the lighter areas, so that the dark areas will lighten more than the light areas.

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