Review

# Curcumin targeted signaling pathways: basis for anti-photoaging and anti-carcinogenic therapy

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## Abstract

Photocarcinogenesis is caused by DNA damage from solar radiation in the ultraviolet range, resulting in the development of both melanoma and non-melanoma skin cancers. Although the ultraviolet B (UVB) spectrum has previously been considered the more carcinogenic of the two, recent evidence suggests that ultraviolet A (UVA) irradiation may have damaging effects that are not generally appreciated. Furthermore, it is becoming apparent that although sunscreens have been in use for many years, they are relatively ineffective in protecting against UVA-induced photoaging and UVA-induced skin cancers. More recently, attention has been directed on certain dietary phytochemicals, in particular curcumin, in the attempt to repair photodamaged skin as a means of preventing degeneration into solar-induced skin cancers. Curcumin has been shown to protect against the deleterious effects of injury by attenuating oxidative stress and suppressing inflammation. In this review, the curcumin-targeted signaling pathways directed against solar-induced injury are reviewed. The ability of curcumin to block multiple targets on these pathways serve as a basis for the potential use of this phytochemical in photoaging skin and photocarcinogenesis.

## Introduction

Photoaging of the skin, with associated skin fragility and increased risk of both melanoma and nonmelanoma skin cancers[1,2] is the result of chronic damage induced by prolonged exposure to both ultraviolet B (UVB) and ultraviolet A (UVA) in natural sunlight in most individuals. Although the damaging effects of both UVB and UVA components in natural sunlight are believed to be synergistic, there is increasing evidence that the UVA, which makes up 95% of the solar ultraviolet light reaching the earth,[3] may be the more damaging of the two. Thus, the dangers of UVA exposure in tanning salons and Psoralen Ultraviolet A therapy may be underestimated and should not be ignored.

Epidemiologic studies have implicated sunlight exposure as a risk factor in the development of basal cell carcinomas and squamous cell carcinomas, although the correlation for squamous cell carcinomas to sun-exposed skin is better than for basal cell carcinomas and malignant melanomas.[4–6] Point mutations and mutagenic bipyrimidine dimers have been observed with combined UVA and UVB exposure. Although point mutations of the type seen in UVB exposure have been observed in the p53 gene on chromosome 17p, such mutations have only been observed in half (40–56%) of basal cell carcinomas.[7] Ultraviolet B which causes redness, burning and blistering, penetrates only the superficial epidermis to the keratinocyte layer, and is more likely to cause squamous cell carcinomas. On the other hand, the relatively asymptomatic UVA rays, with penetrating properties down to the mid and lower dermis, are probably responsible for DNA damage in basal cell carcinomas and malignant melanomas, as well as for dermal changes in photoaging. It is of interest that p53 mutations, which correlate with mutagenesis, do not correlate with photoaging.[8]

It has been observed that sunscreens alone provide adequate protection neither against photoaging changes nor against the development of photocarcinogenesis.[4–6] The inadequacy of suncreens to protect against UVA-induced free radical formation has been reported, which probably accounts for the failure of sunscreens to prevent photoaging and photocarcinogenesis.[2,6] Like x-radiation, UVA penetrates through clothing and skin, and is only blocked by bony structures, accounting for basal cell carcinomas and melanomas in areas covered by clothing. More recently, increasing interest has been focused on certain botanicals and their potential use in the treatment of photoaging skin and prevention of photocarcinogenesis.[9,10] Curcumin (diferuloylmethane) is a dietary phytochemical found in the rhizome of the plant (Curcuma longa) from which turmeric is derived. In this study, the biochemical mechanisms and signaling pathways of UV-induced photocarcinogenesis are summarized, and potential targets on this signaling pathways blocked by curcumin are detailed. The interruption of these pathways by curcumin serves to minimize the effects of UV-induced



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injury, and enhances the repair of photodamaged and pre-cancerous skin.

## Recent update on the role of UVA in solar-induced injury

Of the two types of ultraviolet solar radiation capable of filtering through the clouds, UVB which causes redness, burning and blistering, used to be thought to be the more mutagenic of the two, while UVA, which is relatively asymptomatic, was thought to be safe. However, recent studies suggest otherwise. In fact, the role of UVA in skin cancers induced by sunlight exposure was suspected by investigators as long as 15 years ago.[11] The well-known genotoxic effects of UVB radiation have been attributed to generation of bipyrimidine photoproducts, in particular cyclobutane pyrimidine dimers (CPDs) and [6-4]-photoproducts. However, it has been observed that UVA-induced photoproducts differ both in type and quantity from those generated by UVB. Using a highly accurate and quantitative assay based on high performance liquid chromatography coupled with tandem mass spectroscopy, Mouret *et al.*[12] determined the type and yield of formation of DNA damage in whole human skin. These investigators found that although CPDs were found with both UVB and UVA radiation, the CPDs were more abundant with UVA radiation, and were qualitatively different, resulting in less rapid removal of the UVA-induced CPD photoproducts. Furthermore, the UVA-induced CPDs were observed by ligation-mediated polymerase chain reaction to form predominantly at thymine-thymine dipyrimidines, correlating with the mutation spectrum.[13] Ultraviolet B radiation, on the other hand, produced more [6-4]-photoproducts which were completely removed 24 h after exposure. Quantitatively, much less conversion to Dewar photoproducts was observed with UVB than UVA-induced injury.[14] The UVA-induced bipyrimidine photoproducts were considered the main type of DNA damage that contributed to the genotoxic effect of solar UVA radiation.[15] These bipyrimidine products generated by UVA radiation tended to convert to Dewar photoproducts that were poorly repaired and, therefore, more mutagenic.[14,15] In addition, it was observed that UVA-induced CPDs form predominantly at thymine-thymine (TT) dipyrimidines compared with thymine-cytosine (TC), cytosine-thymine (CT) or cytosine-cytosine (CC) dipyrimidines found in UVB or simulated sunlight exposure. Moreover, it was reported that there was correlation of the TT dipyrimidines with the mutation spectrum.[13] Recently, it was also observed that UVA generates pyrimidine dimers directly in DNA, and does not require intermediary photosensitizers to transfer the energy from the UVA to DNA to produce CPDs.[3] The TT dipyrimidine predominance in UVA-induced damage may explain the increased susceptibility of UVA radiation to induce DNA toxicity.

Further potential damaging effects of UVA-induced injury is demonstrated by observation of induction of a bystander effect in human cells treated with UVA radiation.[16] The bystander effect is not seen with UVB radiation. The bystander effect is the induction of damage in nonirradiated cells by the presence of irradiated cells, implying involvement of mechanisms for amplification of deleterious effects in areas not exposed to the radiation. The bystander effect induced by UVA radiation as well as the ability of UVA rays to penetrate deep into the dermis, may contribute to the difficulty in blocking UVA-induced damage by sunscreens and protective clothing.

## Steps involved in photocarcinogenesis

Specifically, photocarcinogenesis involves three steps: (A) tumor initiation with DNA damage induced in one or more cells as a result of the genotoxic effects of the mutagenic photoproducts, (B) tumor promotion, with clonal expansion of the clones of DNA-damaged cells, and (C) tumor transformation of the damaged clones by further DNA damage, leading to disregulated growth, and associated stromal and blood vessel changes, resulting in the acquisition of metastatic potential by damaged tissue.

### Inducers of photocarcinogenesis (Tumor Initiation)

It has been shown that although oxidative lesions are the main type of DNA damage involved with UVB exposure,[17] other genotoxic products are generated with solar UVA exposure that are even more mutagenic. Unlike UVB-generated [6-4] photoproducts, which are quickly repaired, UVA exposure-generated bipyrimidine photoproducts are poorly repaired and isomerize into Dewar products that are highly mutagenic.[14,15] In addition, the induction of singlet oxygen formation by UVA is key to signal transcription-factor-mediated gene expression in UVA-damaged skin.[18] The formation of thymine-thymine (TT) type cyclobutane pyrimidine dimers (CPDs) in UVA-induced damage reflects a greater tendency of UVA radiation to induce DNA genotoxicity.[13] Genetic damage in the region of the promoter sequence induces the pre-malignant status, exhibiting proliferative properties leading to clonal expansion.

### Tumor promotion with clonal expansion

#### *Induction of gene transcription: activation of transcription factors*

*Nuclear Factor-kappa B.* Nuclear factor-kappa B (NF-κB) is a family of related protein dimers that bind to a common sequence on the DNA, the κB site.[19] In the unacti-

vated state, the NF-κB dimers are located in the cytoplasm. When activated by free radicals, radiation, endotoxin, carcinogens, ultraviolet light, tumor promoters or inflammatory cytokines, the activated NF-κB dimers, a complex made of two subunits, p50/p65, are translocated to the nucleus[20] then goes on to induce transcription of over 200 genes involved in cell proliferation, cell migration, cell transformation, inhibition of apoptosis, and increased metastatic potential.

Curcumin, the active ingredient in the spice, turmeric, is an indirect, but apparently potent inhibitor of NF-κB activation.[21] The activation of NF-κB requires the p65 subunit to be phosphorylated at serine residues 276, 529 and 536 before it undergoes nuclear translocation.[22] In addition, the process of activating NF-κB dimers involves the removal of the inhibitory protein IκBα by phosphorylation of its kinase (IκBα kinase).[30]

The IkB kinases (alpha and beta) exist as a complex of two catalytic subunits (alpha and beta) with the gamma subunit (chaperone) containing a zinc finger domain required for activation of the IkB kinase. The rapid activation of IkB kinase by tumor necrosis factor alpha (TNFα), a strong-inducer of NF-κB, requires both phosphorylation of serine residues (Ser-171, Ser-181)[23] and receptor-mediated tyrosine residues (Tyr-188 and Tyr-199[24]) on its beta subunit, as well as phosphorylation of the zinc finger domain on the gamma subunit.[25] This results in rapid degradation of the inhibitory protein, IκBα. Similarly, the zinc finger domain of IκB kinase (gamma subunit) is also selectively required for signal activation by UV radiation.[26]. However, as UV light is a slow and weak inducer of NF-κB, phosphorylation occurs on Ser-32 and Ser-36 on IκB (beta subunit), with the phosphoacceptor sites on the activation loop serving as a recognition site for ubiquitin ligase, with resultant degradation of IκB via ubiquitin-dependent proteolysis. In both cases, degradation of the inhibitory IκBα frees the NF-κB to translocate to the nucleus, where it regulates gene transcription.[26,27] In addition, phosphorylation of other serine moieties appear to affect the activity of other subunits of IκB kinase activity. For example, Ser-68 phosphorylation is also involved in the activity of IκBα kinase.[28]

Curcumin, a selective phosphorylase kinase inhibitor of phosphorylase kinase,[29] blocks NF-κB activation as well as the activation of its IκBα kinase.[29] It is believed that the the action of curcumin is mediated through inhibition of phosphorylase kinase.[29,31] Phosphorylase kinase, which is activated 5 min after injury, including UV-induced injury, is believed to be responsible for phosphorylation of both serine/threonine and tyrosine-dependent sites on p65 subunit of NF-κB and IκB (alpha and beta) catalytic subunits. Moreover, phosphorylase kinase, which increases adenosine triphosphate (ATP) supplies through phosphorylation of glycogen phosphorylase, is also responsible for the ATP-dependent IκB gamma chaperone protein, which maintains the zinc finger in the appropriately folded state for activation of IκBα complex. Thus, curcumin, by inhibiting phosphorylase kinase, inhibits NF-κB activation and activation and degradation of IκB by inhibiting phosphorylation of serine residues on NF-κB (p65 subunit), by inhibiting phosphorylation of serine and tyrosine residues on IκB catalytic subunits and gamma subunit, and by interfering with ATP-dependent receptor folding, ubiquitination and degradation of IκB, thereby interfering with freeing of NF-κB for nuclear translocation.

*Activator protein-1.* Activator protein-1 (AP-1) is a transcription activator which bears similarity to a DNA-binding protein encoded by the tumor transforming viral oncogene.[32] 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 cyclin D1 gene which promotes the initiation of cells into the G1 phase of the cell cycle,[33] and by suppressing the p53 tumor suppressor gene, which in turn leads to uncontrollable growth and cell transformation.[32] Exposure to UV light induces activation of AP-1, the activation of which is associated with phosphorylation of both fos and junκ subunits.[34]

Curcumin suppresses the activation of AP-1 by inhibiting serine phosphorylation of the of c-jun N-terminal kinase (JNK), a serine/threonine kinase.[35,36] In *in vitro* studies on human keratinocytes, curcumin has been shown to inhibit UVB-induced gene expression by inhibiting activation of AP-1, with JNK and p38 kinase as upstream synergistic elements.[37]

## *Cell Proliferation*

*Mitogen activated protein kinases.* Signal transduction pathways serve as targets for chemoprevention in skin cancers.[38] In particular, mitogen-activated protein kinase activation has been studied in UV-induced signal transduction,[39] with p38 mitogen activated protein (MAP) kinase detected upstream to AP-1 activation.[37] The MAPK pathway involves activation of MAP kinase kinase kinase (MAP3Kinase, raf-1), which then activates MAP kinase kinase (MAP2Kinase, MEKK), which in turn activates MAP kinase (MAPK).[40] MAP kinases are growth factor-dependent receptor tyrosine kinases while the upstream kinases are serine-threonine kinases. The MAP kinases, which are responsible for activating NF-κB-induced proliferative pathways, include extracellular signal-regulated protein kinases (ERK), JNKs or stress-

 

activated protein kinases (SAPKs), and p38 kinases.[38–43] ERKs are activated by growth-inducing tumor promoters, such as phorbol esters, epidermal growth factors and platelet-derived growth factor/PDGF,[40,41] as well as by ultraviolet light. In skin cancers, stress activated pathways are particularly important, as stress activated promoters, such as ultraviolet light, arsenic and irradiation, which are of particular importance in skin cancer promotion, activate NF-κB through phosphorylation of JNKs, SAPKs (serine/threonine kinases) and p38 kinases (tyrosine kinases). These kinases, including JNK and p38 MAP kinases, have been shown to be modulated by curcumin.[35,37] Curcumin blocks phosphorylation of both serine/threonine kinases and tyrosine kinases through its inhibitory effects on phosphorylase kinase.

*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, platelet-derived growth factor PDGF), fibroblast growth factors, insulin-like growth factor (IGF), transforming growth factors (TGFα and TGFβ) as well as cytokine growth factors such as TNFα and interleukin (IL)-1. These growth factor signaling pathways are involved in nonmalignant proliferation such as psoriasis as well as in proliferation of transformed cells.

Using quantitative real-time polymerase chain reaction to elucidate the effect of UVA and UVB irradiated cells with sham-irradiated cells as controls, Skiba *et al.*[44] observed significant increases in mRNA levels for growth factors such as TNF-α and IL-1β, with TNF-α mRNA detected almost immediately after irradiation with both UVA and UVB, but not in sham-irradiated cells. The inhibition of curcumin-inhibited growth factor gene expression is the result of inhibition of NF-κB activation and ERK signaling.[45]

The binding of growth factors to its tyrosine-kinase based receptor results in phosphorylation of the receptor, activation of the receptor, and triggering of signaling pathways resulting in cell growth and proliferation. Curcumin has been shown to inhibit the tyrosine-kinase activity of this receptor, as well as to deplete the protein itself by interfering with the ATP-dependent chaperone protein which maintains the receptor in the appropriately folded state.[46] It is probable that the effect of curcumin may be achieved through its inhibition of phosphorylase kinase. In addition to its stimulatory effect on serine/threonine kinases, phosphorylase kinase also stimulates tyrosine-kinase dependent phosphorylation, and generates ATP from breakdown of glycogen. As inhibition of phosphorylase kinase by curcumin also depletes ATP levels, the curcumin-treated ATP-depleted cell may also have difficulty in maintaining the growth factor receptor in the appropriately folded state, resulting in inhibition of growth factor-dependent signaling.

*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. Cell death (apoptosis) helps to remove excess, damaged or abnormal cells. It has been observed that activation of NF-κB promotes cell survival, and down-regulation of NF-κB sensitizes the cells to apoptosis induction.[19,29] Inhibition of NF-κB by curcumin promotes apoptosis of photodamaged cells, and retards development of skin malignancies, thus allowing for repair of photodamaged skin.

*Apoptotic proteins.* Apoptotic proteins include the caspase family, in particular capase 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.[47] Other apoptotic proteins include PARP and apoptosis (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.[19] On the other hand, phytochemicals, such as curcumin, which inhibit NF-κB activation, sensitize cells to apoptosis induction.[29,48] Curcumin has been observed to cause p53-dependent apoptosis in human basal cell carcinoma cells,[49] and to induce apoptosis in deregulated cyclin D1-expressed cells at the G2 phase of the cell cycle in a p53-dependent manner[50] through activation of caspase 8, with release of cytochrome c in a mitochondrial-mediated apoptotic pathway.[51,52]

*Anti-apoptotic proteins.* Anti-apoptotic proteins such as Bcl-2 and Bcl-xL inhibit apoptosis and increase cell survival, while down-regulation of apoptosis suppressor proteins such as Bcl-2 or Bcl-xL by curcumin has been shown to induce apoptosis in cancer cell lines.[52] This leads to activation of nuclear DNA fragmentation through mitochondrial disruption and cytochrome c release involving activation of the caspase-dependent apoptotic pathways.[51] NF-κB-dependent expression of cell survival genes, including survivin, TRAF1 and TRAF2, blocks apoptosis of the photodamaged cells.[19] By downregulating anti-apoptotic proteins, curcumin promotes apoptosis of photodamaged cells, thus improving photoaging skin and reduce the survival of cells that may become pre-malignant and malignant skin lesions.

*Cell survival kinase.* The cell survival kinase, Akt, is a serine/threonine protein kinase activated by growth and survival factors. Akt is activated by phosphorylation at the

Thr-308 and Ser-473.[53,54] Activated Akt promotes cell survival by activating NF-κB signaling pathway,[54,55] and by inhibiting apoptosis of photodamaged cells.[55] As the activation of NF-κB signaling is dependent on removal of its inhibitory molecule, IκBα, by IκBα kinase, inhibition of IκBα kinase would result in inhibition NF-κB. Both IκBα kinase (NF-κB 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 NF-κB-dependent apoptosis of photodamaged cells and by inhibiting Akt-dependent cell survival of the UV-induced DNA damaged cells. Suppression of Akt, induced by ERK 1/2 signaling pathways, has been reported in curcumin-induced autophagic removal of damaged cells.[56]

### Cell transformation and metastatic potential

*Dysregulated cell cycle and tumor transformation*

Proteins that regulate the cell cycle, in particular the timing of signaling events, are important in tumor transformation as 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 kinases cdk-4 and cdk-6, is the rate-limiting factor regulating entry into the G1 phase of the cell cycle.[33] Overexpression of cyclin D1 causes excessive growth promotion and dysregulation of the cell cycle associated with tumorigenesis, with increased expression related to proliferating cell nuclear antigen expression and prognosis.[57,58] Curcumin blocks cell proliferation by down-regulation of cyclin D1 expression and phosphorylation events.[59] In head and neck cancers, as well as breast, and prostate cancers, curcumin has been shown to inhibit progression of the cell cycle by downregulating the expression of cyclin D1 both at the transcriptional and post-transcriptional levels.[48,59] Cyclin D1 expression is regulated by NF-κB, and suppression of NF-κB by curcumin leads to downregulation of cyclin D1.[48] Curcumin also induces AP-1/p21-mediated G1 phase arrest of the cell cycle,[60] thus retarding the proliferation of pre-malignant and malignant cells.

*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. Moreover, p53 transcription factor is responsible for cellular response to DNA damage and subsequent cellular genomic stability and activates the transcription of genes such as gene expressing p21WAF1 and Bax to induce apoptosis of DNA damaged cells, resulting in the inhibition of growth of DNA damaged cells, including cancer cells.[61,62] 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 have cancers that were linked to deletion of p53 suppressor gene expression.[63] 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.[7] The antitumorigenic effect of curcumin may lie in its ability to upregulate p53 and p21WAF-1/CIP1.[64] It has been observed that curcumin, selectively induces apoptosis in deregulated cyclin D1-expressed cycling G2 phase tumor cells in a p53-dependent manner.[49] Curcumin also inhibits proteosomal function and induces apoptosis through mitochondrial pathways. Moreover, this phytochemical selectively targets proliferative cells more efficiently than differentiated cells.[65]

*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. Tissue injury and resultant inflammatory response result in generation of cytokines and growth factors which activate transcription factors, such as AP-1 and NF-κB. 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.[18] The upregulation of matrix metalloproteinases, which promotes invasiveness of the tumor, and expression of cell adhesion molecules (ICAM-1), which allows tumor anchorage and vascular invasion, are intimately involved in tumor metastases.

Curcumin, has been shown to have antimetastatic effects, including antiproliferation, suppression of NF-κB activation, downregulation of anti-apoptotic proteins, inhibition of both tyrosine kinase-dependent pathways (receptor-mediated MAP-kinase pathways), and serine/threonine kinase pathways (IκBα kinase, mitogen activated kinases (MAP3K and MAP2K, and Akt survival kinase).[66] Curcumin also down-regulates the expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9).[67,68] 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, enabling the tumor cells to invade into the dermis, as well as penetrate blood vessels. In addition, curcumin has been observed to inhibit angiogenic differentiation,[67] which functions to promote meta-

 

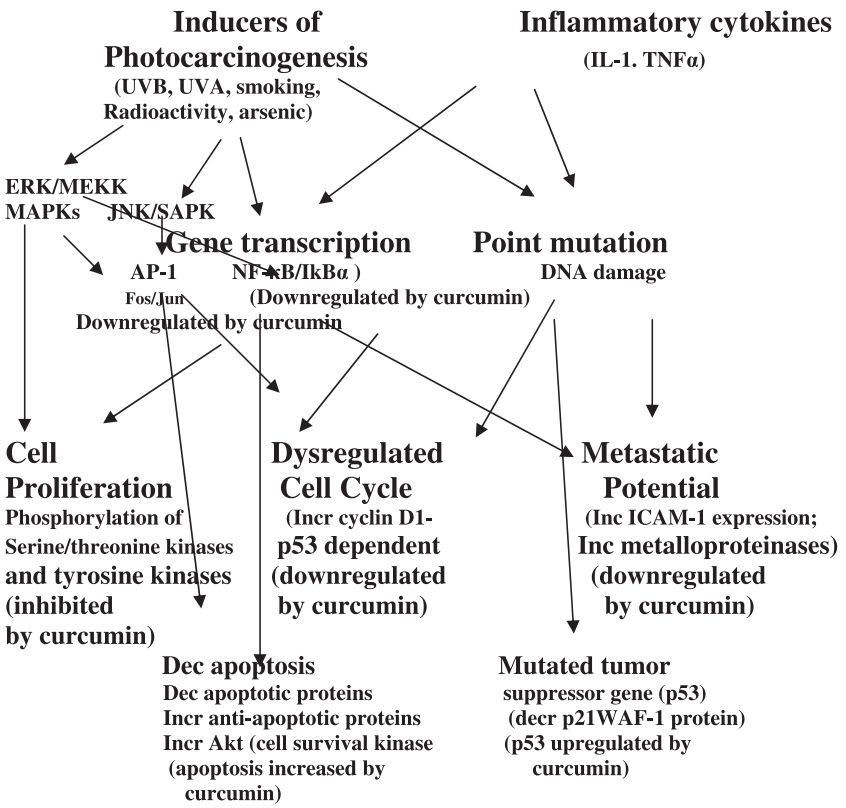


**Figure 1** Curcumin targeted signaling pathways

static spread of tumor cells. Metalloproteinase-2 (MMP-2) expression has been shown to correlate with aggressiveness of cutaneous squamous cell carcinomas.[69] Downregulation of these metalloproteinases by curcumin may reduce the potential for tumor invasion and metastases.

## Curcumin: a selective inhibitor of phosphorylase kinase

The anti-carcinogenic properties of curcumin have been extensively reviewed by Aggarwal *et al.*[60] As detailed above, curcumin appears to block carcinogenesis in a multi-targeted fashion (Fig. 1), which may be confusing at first glance because of its complexity. We propose a unifying concept which may explain the multifaceted effects of curcumin in inflammation, photoaging and photocarcinogenesis through its selective inhibitory activity on phosphorylase kinase - a protein kinase with multiple specificities attributable to its unique structural properties.

### Phosphorylase kinase: a protein kinase with multiple specificities

Protein kinases catalyze the transfer of high energy phosphate bonds from ATP to either serine/threonine or tyrosine residues,[70] but usually not both. This is because protein kinases, with the exception of phosphorylase kinase, allow only one configuration at its substrate binding site. By contrast, phosphorylase kinase has the ability to alter both the size and the shape of its substrate binding site. This is accomplished by the presence of a hinge joint between the subunits, which allow changes in size of the substrate binding site. In addition, the substrate binding site can be made to swivel in one plane by bind-

**Phosphorylase Kinase (PhK)**

**(a) STRUCTURE:**

1. Molecule is tetramer of 4 subunits (αβγδ)4
2. The δ subunit is calmodulin
3. Hinge joint between subunits allows for change in size of substrate binding site
4. Ion binding (Mg or Mn) allows change in shape of substrate binding site

**(b) FUNCTION: Blocked by Curcumin, a Selective PhK inhibitor**

1. Phosphorylase kinase (PhK) is also known as ATP- phosphorylase b phosphotranferase, breaking down glycogen to ATP
2. PhK links multiple calcium-calmodulin dependent signaling pathways involved in cell proliferation, cell migration cell cycling, and inhibition of apoptosis to ATP generation and phosphorylation events
3. The ability to change the shape and size of its substrate-binding site allow for dual specificity, and ability to phosphorylate both serine-threonine kinases MAP3K, MAP2K, IκBα kinase; JNK, Akt; and tyrosine kinases (growth factor receptor kinases; p38 kinase, p42/ p44 kinase)

**Figure 2** Phosphorylase kinase: structure and function

ing to Mn, or in another plane by binding to Mg. In this way, phosphorylase kinase is able to phosphorylase 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.*[71] 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 and the ability to alter the shape of the substrate binding site by metal ion (Mg or Mn) specificity.[72] This flexibility enables phosphorylase kinase to take part in a multiplicity of phosphorylation reactions. The ability of phosphorylase kinase subunits to adapt to different enzyme configurations allow for phosphorylase

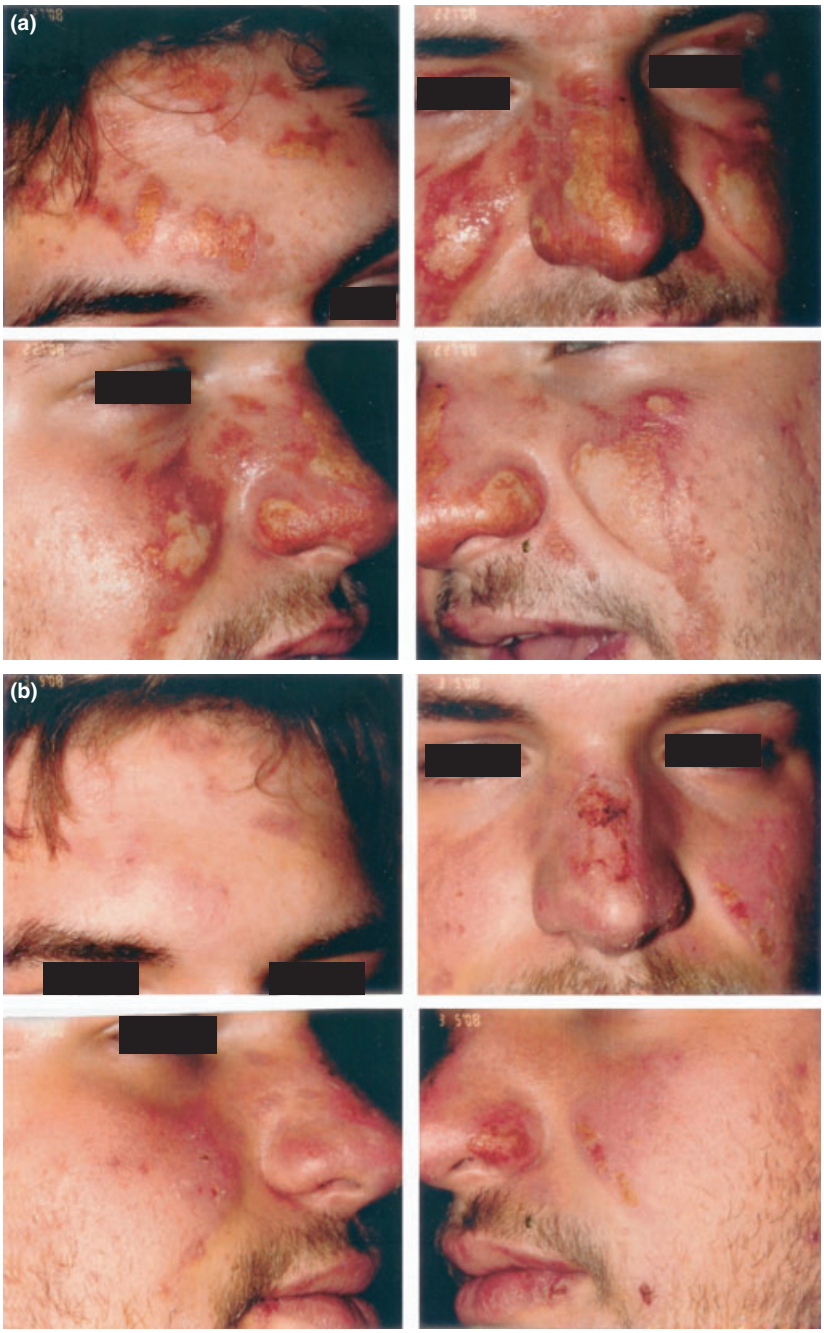


**Figure 3** (a) Burns on face from burning alcohol 3 d after injury. Note at least second degree burns with necrotic tissue in many areas of his forehead cheeks and nose before curcumin treatment. (b) Same patient one week after oral prednisone 60 mg daily with topical curcumin gel twice daily, showing marked healing of the necrotic areas, and minimal or no residual scarring

kinase to accept many substrates, including serine/threonine kinases, tyrosine kinase and phosphatidylinositol kinase, among others.

#### Phosphorylase kinase: an ATP generator

As far as it is known, phosphorylase kinase is the only known enzyme which catalyzes the phosphorylation of glycogen phosphorylase b (inactive) to glycogen phosphorylase a (active) in glycogen phosphorylase to generate ATP supplies from the breakdown of glycogen. Phosphorylase kinase is a tetramer of four subunits $(\alpha\beta\gamma\delta)_4$, with binding sites for ATP, glycogen as well as Types I and II cAMP protein kinases. The δ subunit is calmodulin. Also known as ATP-phosphorylase b phosphotransferase, phosphorylase kinase integrates multiple calcium-calmodulin dependent signaling pathways triggered by cAMP-dependent protein kinases while coupling these reactions to glycogenolysis and ATP-dependent phosphorylation. Thus, both the ATP content in the tissues and the signaling pathways activated by phosphorylase kinase may be controlled by the selective phosphorylase kinase inhibitor, curcumin. The structural and functional properties of phosphorylase kinase are summarized in Fig. 2. These functions are blocked by curcumin, a selective phosphorylase kinase inhibitor.

## Curcumin: a selective phosphorylase kinase inhibitor

In photoaging and photocarcinogenesis, curcumin has been found to inhibit two kinds of pathways: serine/threonine kinase-dependent pathways and tyrosine kinase-dependent pathways. This includes inhibition of NF-κB dependent gene transcription, cell cycling and apoptosis by inhibiting IκBα kinase (a serine/threonine kinase); inhibition of cell proliferation by inhibition of extracellular signal-regulated MAP kinases (MEKK/MAP3K and MAP2K (both serine/threonine kinases); promoting apoptosis of photodamaged cells by inhibition of Akt cell survival kinase (a serine/threonine kinase); and inhibiting growth and proliferation of photodamaged cells by inhibition of growth factor-dependent tyrosine kinases (a series of MAP kinases, including p38, p42 and p44 kinases). In addition, curcumin induces apoptosis in deregulated cyclin D1 expressed cells at the G2 phase of the cell cycle in a p53-dependent manner.[49,64] Through inhibition of NF-κB activation, curcumin promotes apoptosis of photodamaged cells, thus retarding photoaging and the development of skin malignancies. In solar induced photoaging and photocarcinogenesis, the signaling pathways targeted by curcumin are summarized in Fig. 1. By blocking these pathways, curcumin is able to minimize UV-induced damage, thereby enhancing repair of photodamaged skin. In addition, by inducing apoptosis of the DNA damaged cells, curcumin protects against further damage to the DNA, in particular to the p53 suppressor gene, and promotes p53-dependent cell regulation, thus inhibiting tumor transformation.

By inhibiting phosphorylase kinase, curcumin has the unique biochemical property of blocking both the serine/threonine kinase-dependent pathways and the tyrosine kinase-dependent pathways at the same time. The importance of this effect is emphasized by the results of a recent study which demonstrated that blocking the serine/threonine-dependent pathways alone resulted in potentiation of tyrosine kinase-dependent pathways. The investigators[36] observed that abrogating the serine/threonine kinase pathway by deletion of a double-stranded RNA dependent serine/threonine protein kinase (PKR)

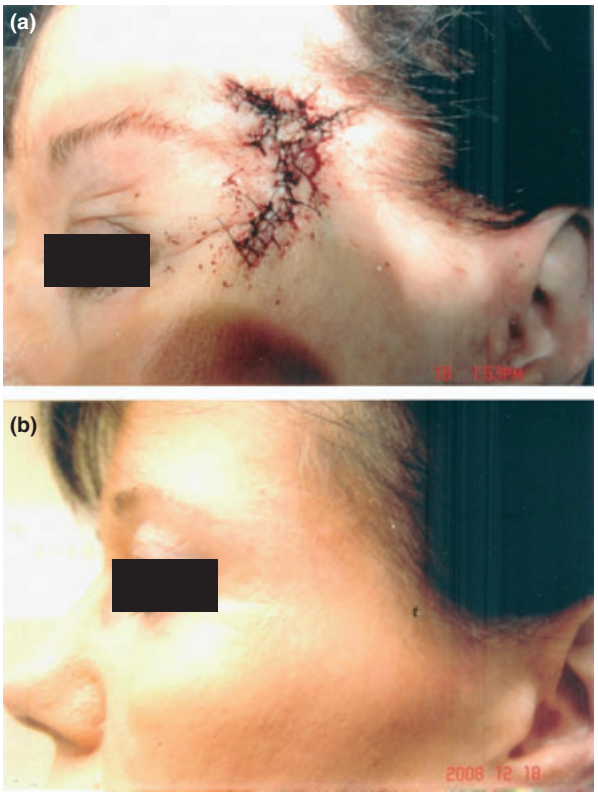


**Figure 4** (a) Sclerosing basal cell carcinoma excised from superior forehead of a 58-year-old Caucasian female. The wound was closed with tissue transferred from a donor site on the forehead. The graft was stitched in place with 60 ethilon sutures. The stitches from the graft were removed 4 weeks later; those from the donor site and remaining wound in 2 weeks. After stitch removal, the wound was treated with extra-strength curcumin gel, massaged into the scar twice daily with the fingers. (b) After 8 weeks of curcumin gel twice daily, minimal to no scarring was observed at the surgical site

abrogated TNFα-induced serine/threonine kinases including IκBα kinase, JNK, Akt and serine/threonine MAP kinases in cell proliferation, but resulted in potentiation of tyrosine kinases such as p38 MAPK and p42/p44 MAPK. This phenomenon may explain the rebound phenomenon observed with the use of corticosteroids. Curcumin, by inhibiting phosphorylase kinase, has the ability to block both pathways simultaneously, without observation of a "rebound" when treatment is discontinued.

## Clinical implications

### Topical versus oral curcumin: problems with tissue delivery

Unfortunately, curcumin does not seem to be well absorbed when taken orally, and high doses of curcumin

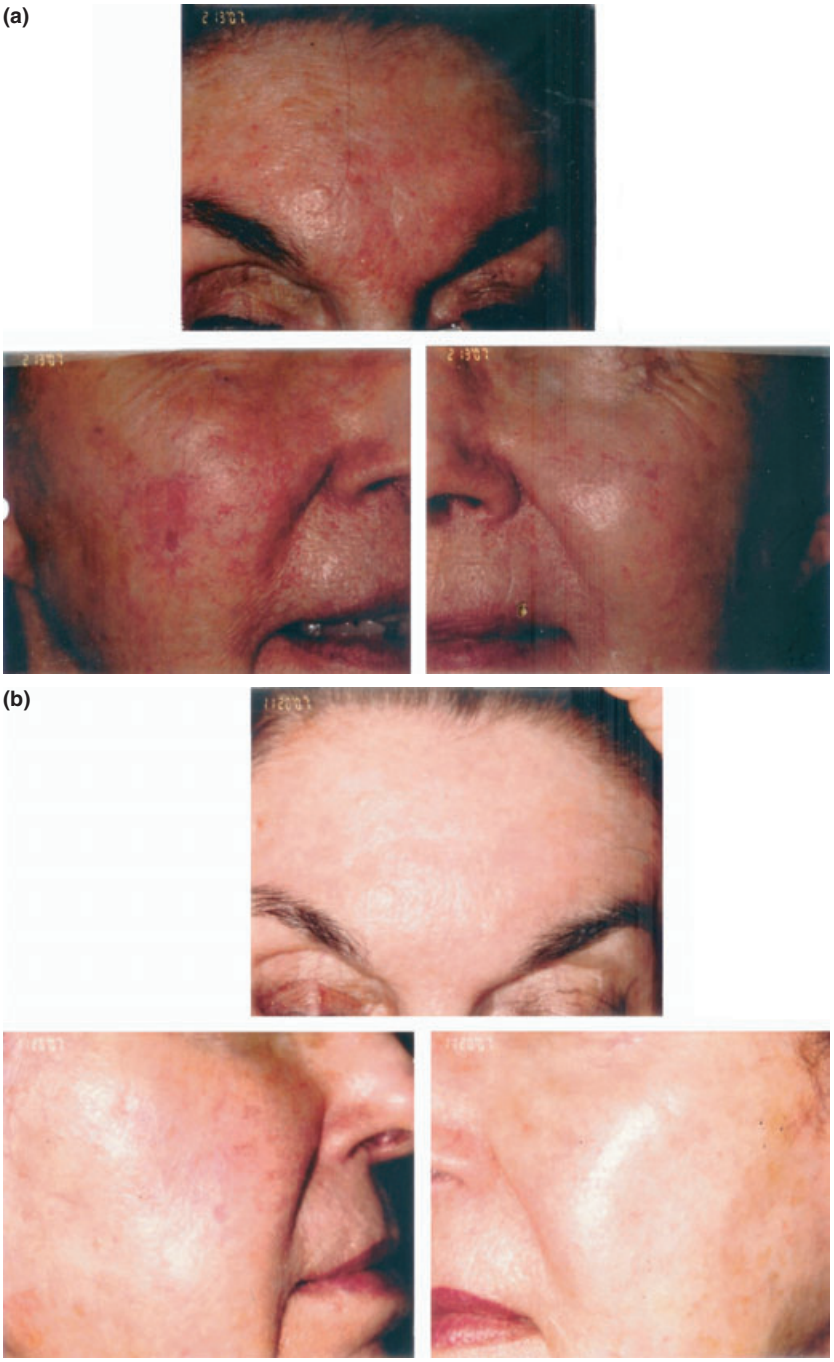


**Figure 5** (a) Photodamaged skin with marked photosensitivity before curcumin gel teatment. Note marked erythema, telangiectasia and wrinkling of skin around the malar cheeks and eyes. (b) Erythema is significantly decreased with the use of curcumin gel twice daily, together with sunscreen applied over the curcumin gel during the day. Note improvement of erythema, telangiectasia and wrinkling of skin 10 months later

have apparently failed to produce clinically or pharmacologically relevant blood levels.[60] The curcumin molecule is metabolized to curcumin glucuronate and curcumin sulfate, and these metabolites have been shown to be absorbed into the blood stream. These metabolites, which are water soluble, do not have anti-phosphorylase kinase activity (Heng MCY unpublished data, 2009), and may be less relevant to potential cutaneous anti-photoaging and anti-carcinogenic therapy. On the other hand, they are anti-inflammatory in that they possess the ability to inhibit histamine, prostaglandins and leukotrienes, and may have relevance in suppressing inflammation in certain systemic diseases. However, recent advances in systemic delivery of unconjugated curcumin has been explored by the use of encapsulated curcumin packaged in liposomes,[73] making it possible to treat systemic cancers such as colorectal[74] and pancreatic cancer.[75]

Topical delivery of curcumin has also been fraught with difficulty. We have tested a specialized formulation of topical curcumin in the form of a gel preparation in psoriasis. Psoriasis is a genetic disease associated with elevated phosphorylase kinase activity with failure to switch-off the elevated phosphorylase kinase induced by injury. We have previously reported that topical curcumin in a gel preparation inhibited phophosrylase kinase activity in psoriatic skin,[31] supporting the belief that the curcumin gel preparation is capable of penetrating psoriatic skin. We have subsequently treated 647 consecutive psoriatic patients with topical curcumin in a protocol which includes the use of topical steroid preparations, and meticulous avoidance of precipitating factors and treatment of infections. We have observed complete resolution of psoriasis in over 70% of psoriatic patients at 16 weeks of treatment with this protocol (Heng MCY *et al.*, 2009. Manuscript in preparation). Further support of the penetrability of topical curcumin to deeper tissues in injured skin was obtained in a study of 220 patients in which a higher concentration of curcumin gel used as the sole therapeutic agent, prevented or decreased scar tissue formation following surgical trauma (Heng MCY *et al.*, 2009. manuscript in preparation). This data suggests that topical curcumin gel is capable of sufficient dermal penetration to achieve modulation of fibroblastic and excessive inflammatory activity necessary for scar prevention and resolution. It should be noted, however, that the topical curcumin was applied to injured skin.

### Inflammatory skin disease caused by injury and scar prevention

We have previously shown that inhibition of phosphorylase kinase by curcumin gel is associated with decreased T lymphocyte populations in inflammatory disease.[31] In addition, curcumin gel has been observed clinically to benefit many skin lesions induced by injury, including burns/scalds (Fig. 3a) and surgical wounds (Fig. 4a). Curcumin gel has been observed to decrease inflammation (redness, swelling, and pain), to minimize the deleterious effects of injury from burns and scalds, promoting rapid healing with minimal or no residual scarring (Fig. 3b). In postsurgical wounds, the use of extra-strength curcumin gel also allows for healing of the wounds with minimal or no scarring (Fig. 4b).

### Photodamaged skin

In photodamaged skin, curcumin gel applied once or twice daily has been observed to improve the texture of photodamaged skin, resulting in decreased appearance of wrinkle formation (Fig. 5a,b). Improvement is usually not seen before 3–6 months, and may take 15 months or more.

It has been observed that the more damaged the skin, the greater the improvement, with patients with mini-

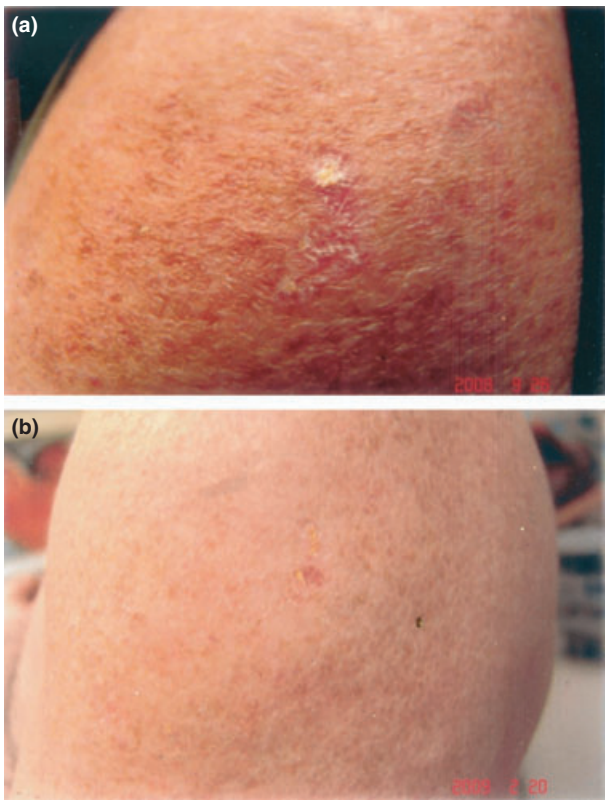


**Figure 6** (a) Photodamaged skin of the deltoid shoulder and upper arm showing advanced actinic keratosis surrounded by photodamaged skin before curcumin treatment. (b) Same patient showing resolution of actinic keratoses as well as repair of surrounding photodamaged skin as shown by improvement of skin appearance and texture after less than 6 months of curcumin gel application twice daily together with sunscreen

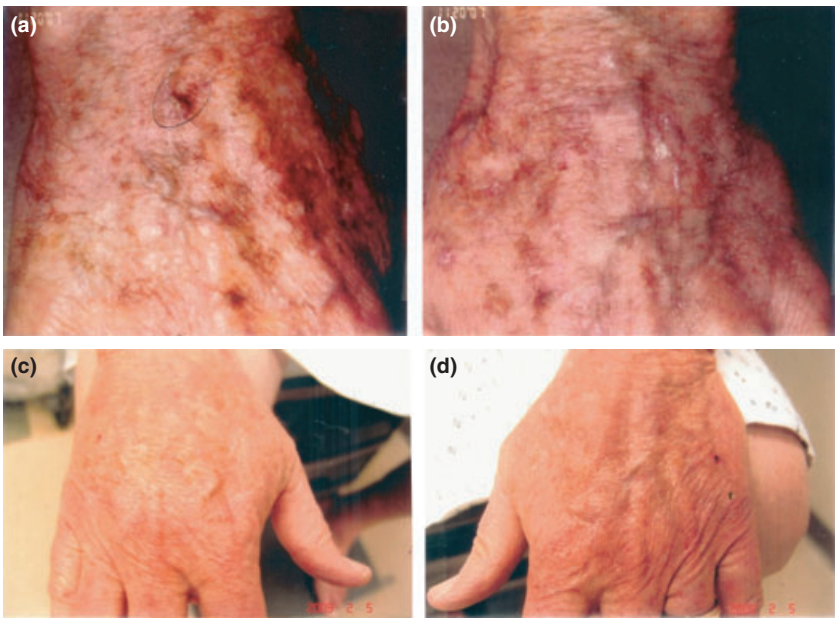


**Figure 7** (a,b) Marked photodamaged skin on the dorsum of hands, with multiple solar lentigenes and scattered actinic keratoses prior to curcumin gel therapy. The patient had been using sunscreen for years with no improvement. (c,d) Marked improvement in skin texture, with resolution of both solar lentigenes and actinic keratoses after 15 months of topical curcumin gel twice daily, with sunscreen during the day

mally damaged skin showing the least improvement. It is believed that topical curcumin does not possess sunscreen properties. Therefore, curcumin gel should be applied together with a sunscreen, which is applied over the curcumin gel when dry.

Topical curcumin has been observed to be clinically effective in decreasing solar-induced erythema in photosensitivity and rosacea, and in improving solar-induced telangiectasia (Fig. 5a,b). In photodamaged skin with actinic keratoses and solar lentigenes (Figs 6a and 7a,b), curcumin gel has been observed to induce repair of these lesions (Figs 6b and 7c,d). One of the advantages of using noninvasive therapy, such as curcumin gel, over surgical procedures in photodamaged skin is the capability of curcumin gel to repair large areas of skin (Figs 5b, 6b and 7c,d), compared with limited areas improved by surgical procedures. Moreover, the curcumin-treated skin more closely resembles the appearance and texture of normal skin (Fig. 7c,d), without the scarring and pigmentary changes which frequently accompanies surgical procedures. Both solar lentigenes and actinic keratoses are observed to benefit from curcumin gel therapy (Fig. 7c,d).

We have previously demonstrated that our topical curcumin preparation inhibited phosphorylase kinase activity in psoriatic skin and induced apoptosis of cells expressing the proliferating cell nuclear antigen (PCNA) as detected by the Ki-67 immunocytochemical marker.[31] Although proliferating cell nuclear antigen (PCNA) is also expressed in both pre-malignant (actinic keratoses, solar lentigenes) and malignant lesions (basal cell carcinoma, squamous cell carcinoma, malignant melanoma), it is cautioned that beause of the uncertainties of delivery of unconjugated curcumin into tissues including cutaneous tissue, topical curcumin gel may only be beneficial for pre-malignant lesions such as photodamaged skin, actinic keratoses and solar lentigens, and not indicated as a sole therapy for malignant lesions. In a personal series of over a hundred patients with photodamaged skin, it is not uncommon to find that in any one patient, multiple actinic keratoses resolve in this manner usually within 6 months, while a few keratotic lesions fail to resolve or continue to enlarge. It is recommended that actinic keratoses that fail to improve or resolve within a 6-month period of treatment be biopsied to rule out early squamous cell carcinomas.

While curcumin gel may be capable of causing apoptosis in pre-malignant lesions such as actinic keratoses and solar lentigenes, problems with topical penetration may limit its use in malignant tumors. It is of interest that curcumin encapsulated in liposomes for systemic delivery has been reported to have potent antiproliferative and proapoptitic effects on melanoma cells.[76]

## Conclusion

Topical curcumin, a phosphorylase kinase inhibitor, has a potential role in inhibiting the effects of cutaneous injury, including ultraviolet radiation induced injury, and in enhancing repair of cutaneous lesions by minimizing postinjury inflammation. Its apoptotic effects may be used to assist in the removal of damaged and pre-malignant skin cells, thus promoting repair of photodamaged skin. The above clinical data refers to an

 

anecdotal series. A controlled study needs to be carried out and peer-reviewed before any valid conclusions can be made.

## CME questions

1. Which rays in the ultraviolet spectrum are mainly responsible for squamous cell carcinomas?
   a. UVB
   b. UVA
   c. Both
   d. Neither
2. Which rays in the ultraviolet spectrum are mainly responsible for malignant melanomas and basal cell carcinomas?
   a. UVB
   b. UVA
   c. Both
   d. Neither
3. Sunscreens block mainly
   a. UVB
   b. UVA
   c. Both
   d. Neither
4. Redness, burning, and blistering are caused by
   a. UVB
   b. UVA
   c. Both
   d. Neither
5. Solar elastoses and wrinkles are caused mainly by
   a. UVA
   b. UVB
   c. Both
   d. Neither
6. Mark TRUE (T) or FALSE (F) for each of the following statements:
   a. Skin malignancies are caused by exposure to both UVA and UVB radiation.
   b. UVA radiation generates cyclobutane pyrimidine dimers that differ from those generated by UVB.
   c. The thymidine-thymidine cylobutane pyrimidine dimers generated by UVA radiation correlate with mutagenesis because thymidine is mainly found in DNA.
   d. The cyclobutane pyrimidine dimers generated by UVB radiation are more difficult to remove and therefore more mutagenic.
   e. The bystander effect is seen with UVA but not UVB exposure.
7. Mark TRUE (T) or FALSE (F) for each of the following statements:
   a. Actinic keratoses are the result of breaks in the promoter DNA sequence.
   b. Squamous cell carcinomas are caused by damage or deletion of the p53 suppressor gene.
   c. Point mutations are caused by UVB mutations alone.
   d. Point mutations are caused by UVB and UVA radiation.
   e. Secretion of excessive metalloproteinases enhance tumor invasion.
8. Phosphorylase kinase catalyzes the transfer of high energy phosphate bonds to
   a. serine/threonine residues
   b. tyrosine residues
   c. Both
   d. Neither
9. Curcumin is
   a. The principal active ingredient in turmeric
   b. Curcumin has poor biovailability with oral absorption
   c. Curcumin is a phosphorylase kinase inhibitor
   d. All of the above
10. Curcumin
    a. Downregulates NFκB
    b. Induces apoptosis in DNA damaged cells
    c. Upregulates p53 expression
    d. All of the above

## Answers to Questions

1 a.
2 b.
3 a.
4 a.
5 a.
6a T.
6b T.
6c T.
6d F.
6e T.
7a T.
7b T.
7c F.
7d T.
7e T.
8 c.
9 d.
10 d.

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