Review

Toxic effects of ultraviolet radiation on the skin

Yasuhiro Matsumura* and Honnavara N. Ananthaswamy

Department of Dermatology, Kansai Medical University, Osaka 570-8507, Japan
Department of Immunology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA

Received 10 August 2003; accepted 27 August 2003

Abstract

Ultraviolet (UV) irradiation present in sunlight is an environmental human carcinogen. The toxic effects of UV from natural sunlight and therapeutic artificial lamps are a major concern for human health. The major acute effects of UV irradiation on normal human skin comprise sunburn inflammation (erythema), tanning, and local or systemic immunosuppression. At the molecular level, UV irradiation causes DNA damage such as cyclobutane pyrimidine dimers and (6-4) photoproducts, which are usually repaired by nucleotide excision repair (NER). Chronic exposure to UV irradiation leads to photoaging, immunosuppression, and ultimately photocarcinogenesis. Photocarcinogenesis involves the accumulation of genetic changes, as well as immune system modulation, and ultimately leads to the development of skin cancers. In the clinic, artificial lamps emitting UVB (280–320 nm) and UVA (320–400 nm) radiation in combination with chemical drugs are used in the therapy of many skin diseases including psoriasis and vitiligo. Although such therapy is beneficial, it is accompanied with undesirable side effects. Thus, UV radiation is like two sides of the same coin—one side, it has detrimental effects, and on the other side, it has beneficial effects.

© 2003 Elsevier Inc. All rights reserved.

Keywords: Ultraviolet radiation; Photoproducts; Skin cancer

Introduction

Sunlight is composed of a continuous spectrum of electromagnetic radiation that is divided into three main regions of wavelengths (Fig. 1): ultraviolet (UV), visible, and infrared. UV radiation comprises the wavelengths from 200 to 400 nm, the span of wavelengths just shorter than those of visible light (400–700 nm). UV radiation is further divided into three sections, each of which has distinct biological effects: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). UVC is effectively blocked from reaching the Earth’s surface by the stratospheric ozone layer, although accidental exposure could occur from man-made sources, such as germicidal lamps. UVA and UVB radiation both reach the Earth’s surface in amounts sufficient to have important biological consequences to the skin and eyes. Wavelengths in the UVB region of the solar spectrum are absorbed into the skin, producing erythema, burns, and eventually skin cancer. Although UVA is the predominant component of solar UV radiation to which we are exposed, it is supposed to be weakly carcinogenic, and cause aging and wrinkling of the skin.

The incidence of skin cancer has been increasing at an astonishing rate over the past several decades, and it is estimated that over one million new cases of non-melanoma skin cancer (NMSC) occur each year in the United States (Gloster and Brodland, 1996). The relevance of sunlight exposure to NMSC epidemic is well known (Strom, 1996). The skin responds to sun exposure by tanning and skin thickening, which provides some protection from further damage by UV irradiation. The degree of pigmentation in the skin and the ability to tan are important risk factors in skin cancer development, and the risk of NMSC is highest in people who sunburn easily and suntan poorly (Gloster and Brodland, 1996).

UV irradiation, on the other hand, has been used as a therapeutic agent for various skin diseases. Topical application of extracts, seeds, and parts of plants that contain natural psoralens followed by exposure to sunlight had been used as a remedy for vitiligo thousands of years ago in Egypt. In modern medicine, the first clinical studies in vitiligo with topical and oral psoralens were reported in 1948 (El Mofty, 1948). In 1974, it was shown that orally
administered 8-methoxypsoralen (8-MOP) and subsequent irradiation with artificial UVA were a highly effective treatment for psoriasis, and this photochemotherapy, termed PUVA (psoralen plus UVA), has shown remarkable effects on a variety of skin disorders including psoriasis, mycosis fungoides, vitiligo, and atopic dermatitis (Honigsmann et al., 1999). UVB, another type of UV irradiation, has also been used since the 1920s in the treatment of psoriasis. In recent years, the availability of new fluorescent bulbs with an emission spectrum (311–312 nm) that closely conforms to the peak of the action spectrum for clearing psoriasis has improved the efficacy of UVB phototherapy for psoriasis, making it as efficient as PUVA therapy. However, those phototherapies are associated with acute and chronic side effects on human skin, which cannot be separated from the beneficial effects of UV irradiation. The purpose of this article is to discuss the toxic effects of UV irradiation derived from natural sunlight and artificial lamps in terms of molecular mechanisms and clinical findings.

Short-term effects of UV on human skin

Acute UV irradiation (a single exposure) induces DNA lesions such as pyrimidine dimers and (6-4) photoproducts, which could lead to DNA mutations if they are not repaired. To prevent DNA mutations, cells are equipped with DNA repair mechanisms.

UV-induced DNA lesions and the repair mechanisms

UV irradiation from 245 to 290 nm is absorbed maximally by DNA (Tornaletti and Pfeifer, 1996). UV irradiation is able to induce mutagenic photoproducts or lesions in DNA among adjacent pyrimidines in the form of dimers (Ananthaswamy, 1997). These dimers are of two main types: cyclobutane dimers (CPDs) between adjacent thymine (T) or cytosine (C) residues, and pyrimidine (6-4) photoproducts among adjacent pyrimidine residues. CPDs are formed between the C-4 and C-5 carbon atoms of any two adjacent pyrimidines; the double bonds become saturated to produce a four-membered ring (Kanjilal and Ananthaswamy, 1996). Similarly, (6-4) photoproducts are formed between the 5-prime 6 position and the 3-prime 4 position of two adjacent pyrimidines, most often between TC and CC residues (Tornaletti and Pfeifer, 1996). CPDs are produced three times as often overall as (6-4) photoproducts (Tornaletti and Pfeifer, 1996). Both lesions occur most frequently in areas of tandem pyrimidine residues, which are known as ‘hot spots’ of UV-induced mutations (Kanjilal and Ananthaswamy, 1996). Although both lesions are potentially mutagenic, CPDs are supposed to be the major contributor to mutations in mammals (Tornaletti and Pfeifer, 1996); (6-4) photoproducts are repaired much more quickly than CPDs in mammalian cells (Mitchell and Nairn, 1989).

If not repaired, UV-induced DNA lesions can lead to mutations in the DNA sequences. These mutations are in the form of C to T and CC to TT transitions, known as UV ‘signature’ mutations. The ‘A rule’ has been proposed to explain how UV signature mutations arise from DNA lesions (Tessman et al., 1992). According to the A rule, DNA polymerase-eta inserts adenine (A) residues by default opposite lesions that it cannot interpret. A mutation is then created upon DNA replication of the strands containing base-pair changes. The TT cyclobutane dimers do not result in mutations because A normally is paired with T, and no mutation would result from insertion of A residues by default opposite the dimer. However, with CC CPDs, a CC to TT transition occurs, because two A residues are placed opposite the dimer by default in the place of two guanine (G) residues. In (6-4) photoproducts between a pyrimidine and a C residue, the 5-prime residue base-pairs correctly, but the 3-prime C residue resembles a nonstructural site (Kanjilal and Ananthaswamy, 1996). A C to T mutation occurs because an A residue is placed opposite the C residue by default.

All mammalian cells are equipped with several DNA repair systems, which are able to protect the cell from the effects of DNA damaging compounds by removing DNA lesions (Wood, 1996). Depending upon the primary DNA
lesion, one or more repair pathways become active such as direct repair, base excision repair, mismatch repair, double-stranded break repair, and nucleotide excision repair (NER). CPD and (6-4) photoproducts generated by UV irradiation are primarily repaired by NER, which removes bulky DNA damage in two distinct subpathways (Lehmann, 1995); damages existing in actively transcribed genes are removed by a quick mechanism called transcription-coupled repair (TCR), and the damages prevailing in other parts of the genome are removed in a slower fashion called global genome repair (GGR). The two subpathways are different only in the first step of NER, that is, DNA damage recognition. In GGR, the protein complex XPC/HHR23B is involved in damage recognition, although in TCR, a stalled RNA polymerase II itself is the damage recognition signal and CSA and CSB proteins are supposed to facilitate this process. The following stages are the same in GGR and TCR; (B) unwinding of the DNA helix surrounding the DNA lesion, (C) dual incision of the damaged DNA strand, and (D) excision of the damaged stretch and de novo DNA synthesis.

**p53 protein, cell cycle arrest, and apoptosis**

The clinical aspects of human skin after acute UV irradiation include sunburn and immunomodulation (Fig. 2). Despite the ability of human cells to repair UV-induced DNA damage, some damage will remain. The cells of the skin contain mechanisms to prevent such DNA damage from leading to skin carcinogenesis. One of these mechanisms is growth arrest followed by DNA repair, and the other is cell death by apoptosis (Matsumura and Ananthaswamy, 2002). Both of these mechanisms prevent the transmission of mutations to daughter cells that can lead to transformation and carcinogenesis. The p53 protein is important in both these mechanisms.

Upon DNA damage by acute UV irradiation, p53 transcription is upregulated, and p53 protein is activated by phosphorylation of Ser15 and Ser20 (Prives and Hall, 1999). The accumulation of activated p53 protein induces a cell-cycle arrest at the G1 phase, which allows the repair of DNA damage before its replication in the S phase (Huang et al., 1996). In this pathway, p21/WAF1/CIP1 was discovered as an inhibitor of cyclin-dependent kinase (CDK), whose induction is associated with the expression of p53 (Harper et al., 1993). p21/WAF1/CIP1 inactivates the CDK–cyclin complex by competitively forming complex with CDK, thus leading the cell into G1 arrest.

If the DNA damage caused by UV irradiation is too severe and cannot be repaired, apoptotic pathways are activated to eliminate damaged cells. p53 also plays a leading role in the apoptotic pathways. As a transactivator of transcription, p53 protein can induce apoptosis by upregulating the expression of apoptosis-promoting genes such as Bax, Fas/Apo-1, or by downregulating the expression of apoptosis-suppressing genes such as Bcl-2 (Mullauer et al., 2001). In addition, wild-type p53 protein can activate the Fas gene by binding to the transcriptional activation site within Fas gene as well as to its promoter region (Muller et al., 1998). Recent studies have shown that Fas/Fas-L interactions are essential for the induction of sunburn cells in UV-irradiated mouse skin (Hill et al., 1999).

p53 also contributes to the maintenance of genomic stability (Lane, 1992), promotes proper DNA replication and repair via GADD45 gene (Kastan et al., 1992), and inhibits angiogenesis via TSP1 and BAI1 genes which in a critical factor in progression to malignancy (Dameron et al., 1994; Nishimori et al., 1997). In this way, p53 plays a pivotal role in the protection of genome, cells, and skin tissue from UV irradiation.

**Sunburn inflammation and tanning**

The major acute clinical effects of UV irradiation on normal human skin are sunburn inflammation (red skin; erythema) and tanning (enhanced melanogenesis). The histological changes following UV irradiation include thickening of stratum corneum, epidermis and dermis, as well as

---

Fig. 2. Effects of acute UV irradiation on the skin.
intercellular and perivascular swelling (edema) in the dermis, and perivascular infiltration. The individual erythemal and tanning responses of human skin are largely genetically determined. Recent studies suggest a possible role for polymorphism in the melanocyte-stimulating hormone (MSH) receptor (Valverde et al., 1995), although other genes are also likely to be involved.

Sunburn erythema is the most conspicuous and well-recognized acute cutaneous response to UV irradiation, particularly in fair-skinned individuals. The molecules responsible for light absorption (chromophores) that initiates sunburn inflammation have not been precisely identified. However, the action spectrum of erythema is consistent with the hypothesis that UV interactions with DNA are of major importance (Parrish et al., 1982), suggesting that the principal event would be direct damage to DNA by UVB and short UVA wavelengths. Indirect oxidative damage might also occur secondarily to endogenous photosensitization reactions at longer wavelengths.

The skin-pigmentation response following UV irradiation is biphasic, comprising the immediate pigment darkening (IPD) and the delayed formation of new melanin (McGregor and Hawk, 1999). IPD is maximal seconds after UV exposure and apparently results from the alteration and redistribution of melanin moieties already present in the skin. IPD is thought to provide protection against damage to epidermal basal cell nuclei by forming ‘nuclear caps’ (McGregor and Hawk, 1999). Delayed tanning (DT) following UV exposure is the result of both UVB and UVA irradiation. Delayed tanning from UVB exposure is associated with an increase in the activity and number of melanocytes. Single exposures increase only activity, although subsequent doses are required to increase numbers of melanocytes. Melanocyte tyrosinase activity also increases, melanocyte dendrites elongate and branch, and melanosome numbers and sizes increase (Pathak, 1976). Accelerated melanin transfer to keratinocytes then results in a large increase in melanin granules in the epidermis. UVA-initiated tanning has distinct effects that are wavelength dependent. UVA irradiation between 320 and 340 nm increases the synthesis density localized to the basal cell layer, whereas UV A irradiation between 340 and 400 nm increases melanin granules in the epidermis (Fitzpatrick, 1986).

The increase in the dose of UV irradiation required to sunburn in chronically irradiated subjects is not only the result of tanning but also of hyperplasia of the stratum corneum, epidermis, and dermis. UV-induced hyperplasia results from increased epidermal and dermal mitotic activity about 24–48 h after acute UV exposure and is also associated with increased synthesis of DNA, RNA, and proteins (Epstein, 1970). This occurs after a transient period of cell-cycle arrest regulated by the activity of nuclear p53 tumor suppressor protein, which plays a crucial role in DNA repair and in protecting the genome from potentially deleterious mutations before mitosis begins (Hall, 1993; Lane, 1992).

All of the histological changes mentioned above are temporary and, in the absence of further UV exposure, the skin cells return to normal within 1–2 weeks.

**Immunological responses**

The skin contains Langerhans cells (LCs) that serve as antigen-presenting cells (APCs) and are capable of communicating with T, and probably non-T, lymphocytes. In addition, keratinocytes produce several cytokines that might also participate in immune recognition in the skin. These cells, together with the regional draining lymph nodes that serve them, have been labeled ‘skin-associated lymphoid tissues’ (SALT) (Streilein and Tigelaar, 1983). UV irradiation induces immunosuppression by affecting this system, including suppressing contact hypersensitivity (CHS) (Noonan et al., 1981) and delayed-type hypersensitivity (DTH) (Molendijk et al., 1987).

DNA damage is proposed to initiate UV-induced immunosuppression by the following evidence: (1) UV-induced suppression of CHS in American opossum, whose DNA damage is repaired by visible light-activated photoreactivating enzyme, was completely prevented by exposing opossum skin to visible light immediately after UVB irradiation (Applegate et al., 1989); (2) topical application of T4N5 (bacteriophage T4 endonuclease V, an excision repair enzyme for CPDs in DNA) to UVB-irradiated mouse skin prevented UVB-induced suppression of DTH and CHS responses and induction of suppressor T cells (Kripke et al., 1992), and (3) IL-10, which is shown to be responsible for systemic immune suppression, is produced by cultured keratinocytes after UV irradiation, but not by keratinocytes pretreated with T4N5, suggesting that UV-induced DNA damage may trigger the production of soluble immunosuppressive mediators such as IL-10 from keratinocytes (Nishigori et al., 1996).

The involvement of the immune system in human skin carcinogenesis is suggested by the increased risk of malignancy in patients undergoing immunosuppressive therapies. The increased risk of skin cancer in renal transplant patients is approximately 7-fold (Hoxtell et al., 1977), and patients treated with immunosuppressive chemotherapeutic agents also appear to be at increased risk for the development of skin cancer (Hill, 1976). By analogy, the immunosuppressive state caused by UV irradiation could lead to an increased risk of skin cancers.

**Long-term UV effects on human skin**

Long-term and recurrent exposure to sunlight causes the gradual deterioration of cutaneous structure and function. It apparently occurs as a result of cumulative DNA damage resulting from recurrent, acute DNA injury, and from the effects of chronic inflammation. Those actinic damages could ultimately lead to the development of skin cancers,
which is a multistep process involving induction of mutations and escape from immune surveillance (Fig. 3).

Skin photoaging

Skin photoaging is the result of chronic sun exposure. The clinical symptoms include dryness (roughness), irregular pigmentation [freckling, flat patches of increased pigmentation (lentigines), persistent hyperpigmentation], wrinkling, elastosis (fine nodularity or coarseness), and telangiectasia (dilation of preexisting blood vessels creating small focal red lesions). These features are predominantly observed in fair-skinned whites with a history of ample past sun exposure and usually involve the face, neck, or extensor surface of the upper extremities. Although UVB photons are much more energetic than UVA photons and are mostly responsible for sunburn, suntanning, and photocarcinogenesis, UVA is also suspected of playing a substantial role in photoaging (Kochevar, 1995). UVA induces the formation of reactive oxygen species that readily react with membrane lipids and amino acids. Membrane damage results in the release of arachidonic acid and leads to activation of secondary cytosolic and nuclear messengers that activate UV-response genes. Human skin exposed daily for 1 month to suberythemic doses of UVA alone demonstrated epidermal hyperplasia, stratum corneum thickening, LC depletion, and dermal inflammatory infiltrates with deposition of lysozymes on the elastic fibers (Lavker et al., 1995). These changes suggest that frequent casual exposure to sunlight while wearing a UVB-absorbing sunscreen may eventually result in damage to dermal collagen and elastin in ways expected to produce photoaging.

Skin carcinogenesis of non-melanoma skin cancer (NMSC)

Carcinogenesis is usually thought of as a multistep process involving initiation, promotion, and progression. UV irradiation causes mutations and increases cellular proliferation; it is therefore able to cause skin cancer without additional initiators or promoters being present and is thus termed a “complete carcinogen.”

Carcinogenesis by UV radiation often involves the inactivation of one or more tumor suppressor genes or the overactivation of growth-stimulatory protooncogenes. Tumor suppressor genes are negative growth regulators and usually are recessive in that they require both copies of the gene to be inactivated before loss of control of cell growth occurs. Accumulation of proteins that bind to and sequester tumor suppressor proteins can also make the cell more susceptible to further mutations. Activation of oncogenes is dominant in that a change in only one copy of the gene is required to have an effect. Protooncogenes, the normal versions of oncogenes, act to control cell proliferation and differentiation, and are divided into three groups: growth factors and growth factor receptors, signal transduction proteins, and nuclear factors (Kanjilal and Ananthaswamy, 1996). Carcinogenesis can result either from expression of a mutant or altered gene product. Several genes have been extensively studied that have important roles in skin carcinogenesis, including p53, patched, p19, and ras genes.

The p53 gene is the most frequent target of genetic alteration identified so far in human cancers (Hollstein et al., 1991). Loss or mutation of p53 has been demonstrated in approximately 50% of all human cancers examined, although the frequency of mutations varies greatly depending on the type of cancer. The basic function of p53 protein is to maintain a cell in normal status against various extracellular stress including UV irradiation, or to lead the cell into apoptosis when its DNA is severely damaged to prevent the carcinogenesis procedure. A number of investigators have detected p53 gene mutations in a large proportion of human SCCs, BCCs, and actinic keratoses (Matsumura and Ananthaswamy, 2002). Ziegler et al (1994) reported that p53 gene mutations in non-melanoma skin cancers were detected at a high frequency (about 50–90%) compared with those of internal malignancy, and the predominant alterations are C to T and CC to TT transitions at dipyrimidine sites. One report compared multiple SCCs with and without p53 mutations developed in the same patient and found that the former tend to exhibit more rapidly growing or histologically immature clinical features, suggesting that p53 gene mutations would bring more malignant characteristics to UV-induced skin cancers (Matsumura et al., 1995).

Statistical analysis of the distribution of BCCs in basal cell nevus syndrome (BCNS; hereditary BCC) patients suggested that tumors in the syndrome arise through a two-hit mechanism and that the underlying defect might be mutations in a tumor suppressor gene. This hypothesis was strongly supported by the identification of patched gene (Ptc in humans) to chromosome 9q22-31 and the demon-
stration that the exact same region was deleted in a high percentage of BCC and other tumors related to BCNS (Gailani et al., 1992). In addition to germline mutations in BCNS patients, patched mutations and allelic loss containing patched locus in sporadic BCCs have also been reported. patched mutations were detected in 12–40% of sporadic BCCs, and allelic loss was found in 42–69% of the examined specimens (Matsumura and Ananthaswamy, 2002). The mutational spectrum of patched in sporadic BCCs is rather diverse and only 41% (11 in 27 examined samples) exhibited the typical UV signature (Matsumura and Ananthaswamy, 2002). This result is different from the analyses of p53 gene mutations in sporadic BCCs, in which most of the mutations were presumably related to UV irradiation (Ziegler et al., 1994). The lower incidence of UV signature mutations in patched suggests that mutagenic events other than UV irradiation may also cause patched inactivation and trigger tumorigenesis.

CDK inhibitor 2A (CDKN2A) was identified on chromosome 9p21 and suspected to be involved in the carcinogenesis of SCCs and BCCs. Interestingly, the locus encoding CDKN2A gives rise to two distinct transcripts from different promoters: p16INK4a and p19ARF (p14ARF in humans) (Mao et al., 1995). p16INK4a is a CDK inhibitor that specifically inhibits progression through the G1 phase of the cell cycle in cells that express pRb (Serrano et al., 1993). p19ARF stabilizes p53 by inhibiting MDM2-dependent p53 degradation, thus specifically activating the p53 pathway (Scott et al., 1998). p16INK4a accumulates in HeLa cells after nonlethal UV irradiation and causes cell-cycle arrest, suggesting that an alteration in p16INK4a would constitute an important step in UV-induced carcinogenesis (Wang et al., 1996). p19ARF is also thought to be involved in skin carcinogenesis because the targeted disruption of p19ARF renders mice susceptible to SCC (Kamiyo et al., 1997). Kubo et al. (1997) reported that 3 out of 21 (14%) SCCs developed in a study of Japanese patients showed hemizygous mutations in the CDKN2A gene. All the mutations were found in exon 2, which is common to p16INK4a and p19ARF. Soufir et al. (1999) examined 20 human SCCs and found four different mutations. All four mutations led to amino acid substitutions. The mutational spectrum of p19ARF genes in 12 SCCs showed that 9 of them (75%) were UV signature mutations. No SCCs had simultaneous alterations of p53 and p16INK4a/p19ARF, confirming a reciprocal relationship among the genes (Pomerantz et al., 1998).

Among the various kinds of oncogenes that have been analyzed in human tumors, ras oncoproteins are most likely to be involved in carcinogenesis. The family of ras oncoproteins consists of three members, H-ras, K-ras, and N-ras, which encode 21-kDa guanosine triphosphate (GTP)-binding proteins located on the inner surface of the cell membrane (Barbacid, 1987). Most of the ras mutations found in various types of human cancers occur in codons 12, 13, and 61 (Bos, 1989), and result in the continuous activation of ras-mediated signal transduction. Activated ras genes may initiate papillomas (benign tumors of the epidermis) (Nelson et al., 1992) and, in cooperation with at least one other genetic alteration, they can induce malignant conversion (Greenhalgh et al., 1990). ras gene mutations were detected in about 20–40% of mouse skin cancers initiated by UV irradiation (Nishigori et al., 1994; Pierceall et al., 1992), however, the frequency is much lower in human non-melanoma skin cancers (Matsumura and Ananthaswamy, 2002). In contrast to the C to T transitions in p53 gene mutations in mouse skin cancers, transversions were frequently observed in ras mutations, suggesting the involvement of DNA damage other than pyrimidine dimers and (6-4) photoproducts, such as 8-hydroxydeoxyguanosine (8-OHdG) (Ito et al., 1993).

Malignant melanoma and UV irradiation

Malignant melanoma incidence and mortality rates are increasing in most countries throughout the world where they are being recorded (Marks, 2001). The likelihood of melanoma occurring in any individual is a combination of inherited or constitutional predisposition and exposure to environmental factors relevant to tumorigenesis. The major constitutional risk factor for melanoma is skin color and skin reaction against sunlight exposure; the fair-skinned people who burn only and never tan after exposure of sunlight have relatively higher incidence of melanoma (Evans et al., 1988). The only environmental risk factor that has been shown relevant to the development of melanoma is exposure to sunlight. A history of exposure to large doses of sunlight sufficient to cause sunburn in childhood is particularly important in the formation of melanoma which could occur many years later (Whiteman et al., 2001). Some studies also suggest that recreational activity leading to sunburn in adulthood is also associated with the risk of melanoma (Elwood and Jopson, 1997; Whiteman et al., 2001).

A great advance has been seen in the analyses of molecular mechanism of melanoma carcinogenesis in the recent decade. Linkage analysis of familial melanoma suggested presence of a melanoma susceptibility gene on chromosome 9p21 (Cannon-Albright et al., 1992; Foutain et al., 1992), and a few years later, p16 or CDKN2A gene at the locus was identified as a melanoma susceptibility gene (Kamb et al., 1994; Nobori et al., 1994). The germline mutations in p16/CDKN2A gene have been identified in familial melanomas (Gruis et al., 1995; Hussussian et al., 1994). To date, it is estimated that approximately 20% of melanoma families worldwide are linked to p16 mutations (Foulkes et al., 1997; Greene, 1999). Alternatively, p16 may still be involved with gene methylation, mutations in the promoter region of the gene, which may explain the lack of p16 mutations in 9p21-linked families (Harland et al., 2000). Melanoma has also been linked to chromosome1p36 by linkage and loss of heterozygosity (LOH) studies, and this locus has been implicated in other cancers which
suggests the presence of a new tumor suppressor gene in that locus (Smedley et al., 2000). The intragenic mutations of p16 gene in human sporadic melanomas were detected at frequencies of 0–26% (Matsumura and Ananthaswamy, 2002). UV signature mutations occupy approximately half of the whole mutations, implying that the causal role of UV in p16 gene mutation is not as clear cut as in p53 gene mutations in non-melanoma skin cancers.

Therapeutic UV exposure to human skin

PUVA (psoralen plus ultraviolet A) and UVB therapies are widely used and effective treatments in many skin disorders including psoriasis, atopic dermatitis, vitiligo, and cutaneous T-cell lymphoma. The rationale of PUVA is to induce remissions of skin diseases by repeated, controlled photocotoxic reactions. These reactions occur only when psoralens are photoactivated by UVA. Clinically, PUVA-induced photocotoxic reactions are characterized by a delayed sunburn-like erythema and skin inflammation that, upon overdosage, may progress to blistering and superficial necrosis. In case of repeated PUVA treatment with high cumulative UVA doses, cutaneous carcinogenicity becomes the major concern. UVB phototherapy has also been successfully used for the treatment of mainly inflammatory skin diseases. UVB irradiation is considered to exert its therapeutic effects through the inhibitory effect against cellular proliferation, apoptosis induction, and the systemic immune suppression.

PUVA photochemotherapy

The mechanism underlying the therapeutic effects of the combination of psoralen plus UVA (PUVA) is generally assumed that UVA-induced DNA-psoralen photoadducts impair cell replication (Honig et al., 1994). In fact, inhibition of cell proliferation is observed at psoralen concentrations and UVA doses, respectively, which do not affect cell viability (Luftl et al., 1998). On the other hand, higher doses cause irreversible cell damage, resulting in both apoptosis and necrosis (Johnson et al., 1998). Recently, Santamaria et al. (2002) revealed that PUVA treatment induces significantly less apoptosis in the epidermis of p53+/− mice compared to p53−/− mice, and Fas-L-deficient mice are completely resistant PUVA-induced apoptosis compared to wild-type mice, indicating that p53 and Fas–Fas ligand interactions are required in the process of PUVA-induced keratinocyte apoptosis. On the other hand, induction of cell death of lymphocytes by PUVA may be responsible for the anti-inflammatory effects of PUVA on lymphoproliferative diseases like cutaneous T-cell lymphoma.

Although PUVA clearly inhibits the immune system (Morison, 1984), the immunosuppressive activity has not been extensively studied in contrast to UVB. Kripke et al. (1983) first reported that PUVA suppressed the induction of contact hypersensitivity responses in a systemic fashion. It was proposed that PUVA inhibits gene transcription, which ultimately results in a shutdown of cytokine release or expression of accessory surface molecules. Neuner et al. (1994) studied the effect of PUVA on the release of the pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α from human peripheral blood mononuclear cells. This treatment resulted in a significant reduction of those cytokines, which would cause the anti-inflammatory activity of PUVA.

UVB phototherapy

UVB radiation in the range 290–320 nm (broadband UVB) has been successfully used for years to treat psoriasis, atopic dermatitis, and other inflammatory skin disorders. The biological effects of UVB radiation are located primarily within the epidermis due to the inability of UVB to penetrate the dermis. However, UVB exposure can also lead to systemic immunosuppression possibly via release of soluble mediators such as IL-1α, IL-10, tumor necrosis factor (TNF) α, and cis-urocanic acid from UVB-irradiated epidermis into the circulation (Ullrich, 2002). Among them, IL-10 has a great impact on immunoregulation, and the release of IL-10 from keratinocytes would be mediated via UV-induced DNA damage (Nishigori et al., 1996). UVB phototherapy produces long-lasting clinical remissions in patients suffering from extensive psoriasis, which is characterized by a skin infiltration of activated T cells and the hyperplasia and the incomplete differentiation of epidermal keratinocytes. After UVB phototherapy, most of the cellular and molecular changes that typify the psoriatic epidermis revert to normal (Beissert and Schwarz, 2001). Expression of the cell-cycle antigen Ki-67 decreases significantly and residual cell proliferation is appropriately confined to the basal layer. Epidermal thickening is strongly reduced on UVB exposure and a granular layer re-forms.

Within the last decade, a UVB irradiation source that emits mostly 311- to 312-nm radiation (narrow-band UVB) has been successfully introduced, especially for the therapy of psoriasis (Green et al., 1988). The primary mechanism of depletion of skin-infiltrating T cells was UVB-induced apoptosis as indicated by the TUNEL assay of UVB-exposed psoriatic skin specimens (Ozawa et al., 1999). Since UVB-treated keratinocytes have been reported to upregulate the death receptor ligand (CD95L), it is possible that UVB-induced lesional T-cell apoptosis is mediated indirectly by CD95L expression on neighboring keratinocytes (Leverkus et al., 1997). However, apoptosis is also induced by UVB 311-nm irradiation in vitro in pure T-cell populations cultured from psoriatic lesions, suggesting a direct cytotoxic effect of UVB (Aragane et al., 1998).

Acute adverse effects of UVB and PUVA therapy

To optimize therapy for psoriasis, most clinicians adopt a UV-dose incremental regimen to achieve mild but painless erythema. PUVA-induced erythema develops after about 24
h and peaks between 48 and 96 h (Van Praag et al., 1993).
On the other hand, the erythema develops 4–6 h after the
beginning of UVB, and the response peaks after 24–36 h.
UVB can be given as broadband or narrow-band UVB, and
the latter has a reduced incidence of burning episodes for
therapeutically equivalent regimens (Laube and George,
2001). A rare but well-recognized adverse effect is the
development of intermittent severe burning pain under the
skin 4–8 weeks after the onset of PUVA therapy (Norris et
al., 1987). It usually resolves spontaneously after several
weeks but treatment in the meantime is difficult.

**Chronic adverse effects of PUVA and UVB therapy**

The risk of SCC is related to the number of PUVA
therapy and cumulative UV dose, but the quantification
of the relative risk differs greatly among studies. Most
authors report that patients with 200 or more PUVA treat-
ments have a 10–30 times increased risk of developing
SCCs of the skin (Bruynzeel et al., 1991; Lindeloef et
al., 1991; Stern et al., 1998). Nataraj et al. (1997) reported p53
mutations in 65% of PUVA-induced SCCs, and the muta-
tional pattern was different from those in SCCs arising in the
genital population. Another recent report revealed high
frequency of ultraviolet mutations at the *INK4a-ARF*
locus in PUVA-induced SCCs developed on psoriatic patients
(Kreimer-Erlacher et al., 2003). An up to 5-fold increase of
BCCs has been observed in patients receiving 200 or
more PUVA treatments (Bruynzeel et al., 1991; Lindeloef et
al., 1991; Stern et al., 1998). Japanese studies report a much
lower incidence of non-melanoma skin cancers in psoriatic
patients treated with PUVA (Danno, 1999), which may be
explained by the protective effect of moderately pigmented
skin in Japanese, and that topical PUVA is preferred
beautician in Japan. Recently, Kreimer-Erlacher et al.
(2003) reported that most of the mutations detected at
*INK4a-ARF* locus in SCCs which developed in chronic PUVA-treated patients had either UVB fingerprint (58%) or
the UVB or PUVA fingerprint (16%), and no sample showed PUVA-specific mutation (5′ApTpG base substitu-
tion at non-dipyrimidine sites) which was reported in the
previous in vitro studies (Boyer et al., 1988; Sage and
Bredberg, 1991). This result would imply that the history of
therapeutic exposure to artificial UVB or natural sunlight
would be the direct cause of skin carcinogenesis, and PUVA
itself may play no direct role in the development of genetic
mutations but may promote tumor growth by nonmutational
effects such as tumor promotion or immunosuppression.

Sporadic cases of malignant melanoma (MM) have been
reported among patients treated with PUVA since 1980s
(Gupta et al., 1988). Several studies did not find an
increased incidence of MM after 10–16 years of follow-
up (Bruynzeel et al., 1991; Gupta et al., 1988; Lindeloef et
al., 1999). In contrast, Stern et al. (1997) reported that the
risk of MM is approximately 5-fold increased about 15
years after the first PUVA treatment, especially among
patients who have received more than 250 treatments. This
problem is still controversial and should be further investi-
gated upon the accumulated long-term follow-up cases.

In case of UVB therapy, its relationship with skin carci-
nogenesis has not been put much emphasis upon compared
with PUVA therapy, although UVB is an established carcni-
gen in both animals and human beings. A matched analysis
controlling for the level of exposure to PUVA revealed that
 exposure to more than 300 UVB treatments increased the
risk of genitonal SCC by about 4-fold (Stern et al., 1990). The
other reports, however, showed no significant increase in the
rate of skin cancer in long-term follow-up (Larko and
Swanbeek, 1982; Maughan et al., 1980; Studniberg and
Weller, 1993), implying that therapeutic UVB is associated
with minimal risk of cutaneous carcinogenesis even with
relatively high cumulative exposures. A systemic review of
the literature between 1980 and 1996 estimated an excess
risk of about 2% per year of non-melanoma skin cancers due
to UVB therapy (Pasker-de Jong et al., 1999).

**Summary**

It is well established that UV radiation present in sunlight
is a potent human carcinogen. UV radiation is termed a
“complete carcinogen” because it causes skin cancers
without additional initiators or promoters. The mutagenic
and carcinogenic effects of UV light can be attributed to the
induction of DNA damage and errors in repair and replica-
tion. Fortunately, cells are equipped with a variety of
mechanisms that constantly monitor and repair most of the
damage inflicted by UV light. Nucleotide excision repair
system prevents the DNA damages from leading to DNA
mutations, and finally, to skin carcinogenesis. In this pro-
cess, the *p53* gene plays a pivotal role by causing cell cycle
arrest to gain some time for DNA repair, or inducing cell
death by apoptosis when the DNA damages are too severe
to repair. Tanning leads to the reduction of DNA damage by
protecting the nuclei from UV irradiation. UV-induced
immunosuppression, on the other hand, seems to promote
skin carcinogenesis. Besides UVB radiation, UVA also
plays a substantial role in photoaging by inducing the
formation of reactive oxygen species. However, in contrast
to UVA, UVB is more active in inducing mutations in tumor
suppressor genes and oncogenes and in skin carcinogenesis.
Finally, therapeutic UV exposure for the treatment of skin
disorders causes unwarranted clinical side effects. A better
understanding of the interaction between UV radiation and
the skin should lead to harnessing the beneficial aspects of
UV irradiation and minimize the harmful effects.

**Acknowledgment**

This study was supported by NIH Grant R01-CA-46523
to H.N.A.
References


