
Psoriasis, PUVA, and Skin Cancer – Molecular Epidemiology: The Curious Question of T→A Transversions

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Inspector Gregory – “Is there any other point to which you wish to draw my attention, Mr Holmes?”

Sherlock Holmes – “To the curious incident of the dog in the night time.”

Inspector Gregory – “But the dog did nothing in the night time!”

Sherlock Holmes – “That was the curious incident!”

from *Silver Blaze* by Sir Arthur Conan Doyle

Photochemotherapy with 8-methoxypsoralen and long wavelength ultraviolet radiation (PUVA) is commonly used to treat psoriasis and vitiligo. These vastly different diseases respond to the therapy by different mechanisms even though the immediate effects of the therapy – photoadduct formation – is the same for both. Because psoriasis is not cured by PUVA, patients receive many treatments over their lifetime and develop a significant risk for the development of skin cancers (primarily squamous cell carcinomas). In this review the basic aspects of psoralen photobiology are reviewed briefly. In addition the impact of the analysis of mutations in the tumor suppressor gene, p53, are summarized. An

unexpected mutation spectrum (very few T→A transversions and frequent UVB signature C→T transitions) suggest that effects other than direct DNA photoadduct formation may be at play. The roles of reactive oxygen species-induced base changes as well as other clastogenic factors are discussed. This analysis suggests that it may be possible to improve the therapeutic efficacy of PUVA by a careful evaluation of the mode of delivery. Key words: 5-methylcytosine/8-hydroxyguanine/melanoma/mutation/p53/photoadduct/psoriasis/PUVA/squamous cell carcinoma. Journal of Investigative Dermatology Symposium Proceedings 4:11–16, 1999

The widespread interest in tumor suppressor proteins, and p53 in particular, stems from their central role in carcinogenesis. The p53 gene was initially thought to be an oncogene because p53 was detected at elevated levels in transformed cells and overexpression seemed to result in transformation of normal cells (Linzer and Levine, 1979; Eliyahu *et al.*, 1984). p53, however, was later found to function as a tumor suppressor when additional studies revealed that transformed cells contained mutated p53, and that transfection of transformed cells with wild-type p53 resulted in cell death. As additional studies have elucidated its cellular activity various labels have been applied to the protein. The label “the guardian of the genome” (Lane, 1992) was indicative of its effects in DNA repair. The term “gatekeeper” came in to vogue more recently as the role of p53 in apoptosis and cell cycle regulation became evident (Levine, 1997). For a list of reviews of diverse functions p53 see **Table I**.

p53 functions to integrate cellular responses to stress. Depending on the nature of the stress, p53 will either cause cell cycle arrest, induce apoptosis, or activate cell differentiation. p53 is associated with the

repair of DNA damage and arrests cells at the transition from G₁ to S phase. p53 also regulates the transition from G₂ to M phase. One mechanism by which p53 regulates cell cycle arrest and apoptosis is through transcriptional activation of genes that promote apoptosis or growth arrest. Mutations in the DNA binding region (exons 5–8) of p53 have been found in approximately 50% of all human cancers. Mutations in this region alter p53 function as a transcription factor. Both p53 alleles are lost in most human tumors. Loss of heterozygosity (LOH) is commonly observed in one allele while the second contains a missense mutation that inactivates the gene. One model for skin cancer predicts that loss of p53 may result in a gain of function. Genotoxic damage from agents such as UV irradiation should result in death in cells containing wild-type p53; however, as long as the damage is not lethal, p53 null cells may survive and undergo clonal expansion. It appears that deleterious p53 mutations, usually found in the DNA binding region of the protein (exons 5–8), reduce its capacity to bind to damaged sites and hence replication occurs with reduced fidelity leading to cell transformations and cancer. More recently a proteolytic function for p53 has been described (see **Table I**).

In its role as a transcription factor it has also been suggested that mutated forms of p53 may interact with different sets of transcription sites, leading to the increased proliferation of cells characteristic of neoplasms (Dittmer *et al.*, 1993). In addition to being the most common genetic defect in human cancers, in the instances of cancers arising from definitive causative agents, the p53 mutational spectrum may display a characteristic fingerprint that can be correlated with the DNA damage specificity of that agent [*such as* UVB radiation (Brash *et al.*

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Table I. Recent p53 review articles and their focus

Cancer etiology, molecular pathogenesis (Greenblatt <i>et al.</i> , 1994)
Broad review describing multiple functions of p53 (Ko and Prives, 1996)
Growth control and neoplasia (Gottlieb and Oren, 1996)
p53, the cellular gatekeeper for growth and division (Levine, 1997)
p53 proteolysis (Okorokov <i>et al.</i> , 1997)
p53, the cellular gatekeeper for growth and division (Levine, 1997)
tp53 ubiquitination (Scheffner, 1998)

1991), aflatoxin (Hollstein *et al.*, 1993), and oxidative processes (Kreutzer and Essigmann, 1998)]. In a different kind of role, p53 expression and mutation have been found to occur in nontumorigenic indications such as rheumatoid arthritis and keloid formation (for a review see Gasparro, 1998).

The correlation between squamous cell carcinoma (SCC) incidence and mutations in p53 tumor suppressor gene has been well characterized. Signature mutations at pyrimidines and dipyrimidine sites observed by several independent investigators have been attributed to the UV-induced formation of pyrimidine dimers (Brash *et al.*, 1991 and references therein). Whereas the presence of a given mutation cannot be taken as any kind of proof of cause, the distribution of mutations from a group of specimens produces a mutation spectrum that can be indicative of important molecular events in that population. The tracing of possible causative events in this manner has led to the development of the field called molecular epidemiology. Although it may seem there could be no more objective analysis than this, it must be kept in mind that molecular events occurring earlier in the carcinogenesis process may not be evident in the tissue that is ultimately analyzed, namely cancerous tissue. Further complicating matters is the complex nature of a tumor specimen that usually contains a mixture of normal and neoplastic cells. Ren *et al.* (1997) have demonstrated the powerful amplification of signal to noise using a microdissection technique that distinguished different regions of tumors based on staining with anti-p53 antibodies.

A commonly accepted scheme for the development of tumors after exposure to a DNA damaging agent results in lesions that may or may not be repaired accurately. In addition, persisting adducts may lead to replication errors and or misincorporations. These events can result in mutations. If the latter are critical, the cell does not survive; however, a nonlethal mutation in a gene like p53 could provide a selective advantage for the proliferation of mutation-containing cells. Minimally the loss of normal p53 function would be akin to a speeding car with no brakes.

The foregoing could be a more or less complete picture for an internal tumor caused by an agent like aflatoxin; however, in the case of skin cancer, another effect of mutation-inducing UV radiation must be considered as it has also been shown to be a potent immune suppressive agent (Vink *et al.*, 1997). Thus, a p53 mutation that could confer a selective growth advantage for a particular cell may also occur on a background that includes reduced immune surveillance in the UV-exposed skin (for a recent and thorough review see Streilein, 1996). The potential effects of immune suppression in cancer induction are illustrated by the types of mutations observed in psoriasis patients who have developed SCC after extensive PUVA therapy.

p53 mutations in nonmelanoma skin cancer Molecular epidemiology offers a potent tool to elucidate contributing factors in the cause of cancers. As stated above mutations in the tumor suppressor protein p53 have been detected in more than 50% of all cancers. Furthermore, causative agents lead to distinctive mutational spectra. For example, in skin cancers from sun-exposed sites it is common to find C→T and CC→TT transitions at DNA sequences where pyrimidine photochemical events are expected to occur. Although it may be stated that it was known that sunlight caused skin cancers, the mutation spectrum in the p53 gene directly implicates DNA damage and mutated p53. Other effects such as UV-induced immune suppression may occur with a different action spectrum and contribute to the development of skin cancer. The wavelength dependence for immune suppression in human skin has not been characterized; however, the immune

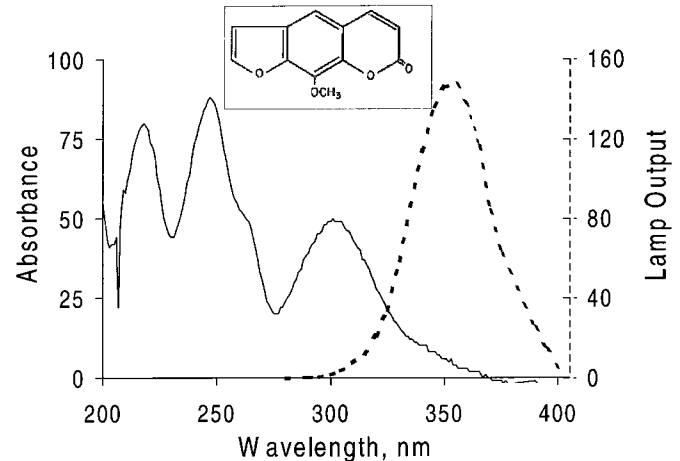


Figure 1. 8-MOP UV absorption spectrum, UVA Lamp output, and 8-MOP structure. The use of UVA to activate 8-MOP may appear less efficient than other wavelengths such as UVB (280–300 nm). The selection of UVA was based on the uncorrected excitation spectrum of 8-MOP (A. Lerner, personal communication). The reduced number of photons at the shorter UV wavelengths near 300 nm caused a reduction in 8-MOP excitation efficiency, resulting in a skewed maximum. Inset, 8-MOP structure.

suppressive effects of PUVA have been described (Kripke, 1984; Alcalay *et al.*, 1989).

SCC in PUVA-treated psoriasis patients In PUVA for psoriasis, genotoxic damage is intentionally inflicted on the skin (Parrish *et al.*, 1974). Thus, it was not too surprising to find in a study of the 1380 patients from the original U.S. multicenter PUVA trial, that a 2.6-fold increase in the incidence of cutaneous SCC was observed as early as 5 y after therapy began (Stern *et al.*, 1979). In the most recent report by Stern *et al.* (1998), the further analysis of skin cancers in this cohort is presented. For those patients without an SCC or BCC in the first decade, there was a strong dose-dependent increase in SCC in the second decade. A much less impressive effect was observed for basal cell carcinoma (BCC). High-dose patients (more than 337 PUVA treatments) carried a 68-fold increase in the overall risk of SCC with lower risks of 26- and 5-fold for medium- and low-dose exposures (160–336 and < 100 treatments, respectively), whereas only a 4-fold BCC increase was observed for all dosages. Whereas in early studies there appeared to be differences in European and American experiences with PUVA-induced incidence of SCC, the ongoing periodic re-analysis of each set of data with a longer follow-up period, now indicates comparable findings (Studniberg and Weller, 1993).

A recent report described the increased risk of malignant melanoma in the PUVA cohort (Stern *et al.*, 1997). Of the 1380 patients in the PUVA cohort, seven developed malignant melanoma at a period of 15 y. Patients who received 250 or greater PUVA treatments had a 5.5-fold increase in the relative risk of malignant melanoma, whereas those who received fewer treatments had only a 1.3-fold increase. The significance of these early data can only be confirmed by additional follow-up analyses; however, their occurrence so many years after the start of PUVA suggests the possibility of a different mechanism than for SCC development in the same PUVA cohort. Clues as to the etiology of melanomas in PUVA patients may be provided by the analysis of p53 mutations. In a recent analysis of 11 melanocytic lesions from 11 PUVA patients, we examined exons 5–8 and found one PUVA type mutation (A→T transversion at codon 139; Ren *et al.*, 1999a). This lesion was from a patient for whom we analysed four SCC and found seven mutations, including four separate T→G PUVA-type transversions at codon 205 (Ren *et al.*, 1999b). The relatively infrequent occurrence of mutations in melanomas compared with other cancers may be the result of the limited analysis of exons (typically 5–8). But it must not be forgotten that the role of p53 may not be as important as in SCC and another mutated gene may contribute to the development of melanomas.

Psoralen photochemistry and mutagenesis Psoralens are planar, tricyclic compounds, consisting of a furan ring fused to a coumarin moiety exhibiting absorption bands between 200 and 350 nm (Fig 1) (Moor and Gasparro, 1996). The planar structure and hydrophobicity of psoralens leads to their facile intercalation with nucleic acid base pairs. Upon photoactivation, different adducts (addition to DNA bases) can be formed (Fig 2). Based on *in vitro* photochemical analyses, the most frequently occurring photoadducts are expected to be the thymidine adducts, followed by cytosine adducts (10–20 times less efficiently) (Cimino *et al*, 1985) at 5'TpA dinucleotide sites. Although a photoaddition product to adenine has only been detected under *in vitro* conditions (Yun *et al*, 1992), no psoralen adduct with guanine has been reported; however, it is important to note that guanine often serves as a sink for oxidative damage that cannot be detected by conventional optical methods (see below). Furthermore, the production of oxygen radical mediated clastogenic factors (mixture of lipid peroxidation products, cytokines, and unusual nucleotides) by PUVA *in vivo* could lead to chromosomal instability that may contribute to the cancer risk in PUVA patients (Filipe *et al*, 1997). Furthermore, Lewis and Adams (1987) have shown that the induction of reactive oxygen species without 8-hydroxyguanine formation can induce DNA damage in keratinocytes.

In vitro studies in a wide range of cells have consistently demonstrated that UVA-activated psoralen leads to mutations at 5'TpA sites that are

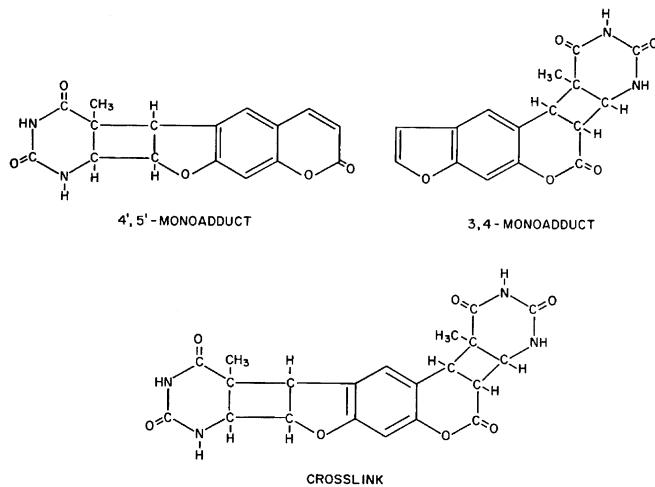
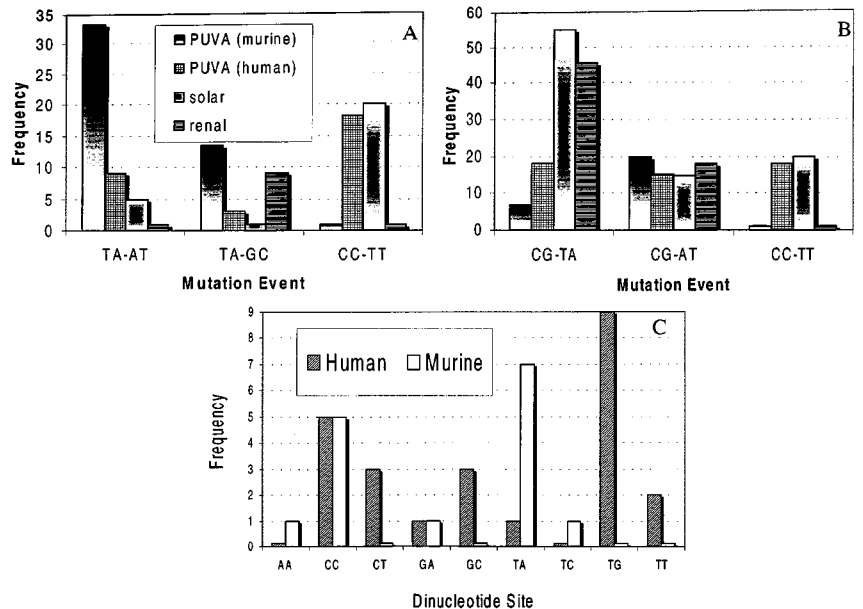


Figure 2. Photoadduct structures. 4',5'-monoadduct; 3,4-monoadduct; interstrand crosslink.

Figure 3. Comparison of PUVA and UV mutations in the p53 exons 5–8 from SCC. (A) T→A transversions; (B) C→T, CC→TT transitions (for SCC from mice treated with 8-MOP/UVA, psoriatic PUVA patients, over sun-exposed humans, renal transplant recipients, respectively). In (A) the prototypical T→A transversions are evident in the SCC from mice exposed solely to a PUVA regimen, but not in the human SCC from the PUVA-treated psoriasis patients (unexpected) nor in the other two groups of patients (expected). In (B), except for the lack of CC→TT transitions, the profile for the RTR is very similar to that for SCC from over-sun-exposed humans. (C) The dinucleotide sites for the mutation events in murine and human SCC are compared. Part (C) further illustrates the different effects of 8-MOP/UVA on murine and human epidermal DNA.



known from *in vitro* studies to be psoralen photochemical hot spots (Sage and Bredberg, 1991; Gunther *et al*, 1995). Whereas an *in vivo* study of SCC from mice chronically exposed to PUVA also exhibited this prototypical PUVA mutation spectrum in the p53 gene (Nataraj *et al*, 1996), the first analyses of p53 mutations in human skin cancers in PUVA-treated psoriasis patients yielded an unexpected mutation spectrum (Nataraj *et al*, 1997; Wang *et al*, 1997). Very few of the mutations were PUVA-type (T→A) at 5'TpA sites (see Fig 3A). Rather they were more characteristic of those resulting from solar exposure (C→T, CC→TT, see Fig 3B). In the report by Wang *et al* these clearly UVB-type events were most common. In the report by Nataraj *et al* (1997) mutations were observed at 5'TpG sites (nine out of 24 prevalence *versus* one at a 5'TpA site). It was argued that these could be a variation of mutations usually seen at 5'TpA sites seen in the *in vitro* PUVA studies. Another possibility, however, is that these sites are reflecting reactive oxygen species-mediated damage at G, C, and/or T (Wang *et al*, 1998). Further complicating the interpretation of mutation spectra is the observation that stable oxidation products of cytosine and thymine can induce C→T transitions (Wang *et al*, 1998). Whether this oxidative damage (and the subsequent mutations) is induced by the combination of 8-MOP and UVA or just UVA alone remains to be determined.

In a recent review several possible explanations were proposed for the unusual mutation spectrum in PUVA SCC (Gasparro *et al*, 1998). Although photoadducts may form at 5'TpA sites (note that this has yet to be demonstrated *in vivo*, see below for additional comments), these adducts may be processed with a high degree of accuracy, resulting in the low frequency of mutations at these sites. This unusual degree of fidelity might be traced to the so-called "A-rule" (Sagher and Strauss, 1983). When the DNA replication machinery is paused at a site of damage, there is an overwhelming tendency to insert an adenine. Thus photoadducts at thymines may be tolerated better than those at the other bases. It is also noteworthy that of all the p53 mutations characterized in human cancers (>7000), only 25% have been detected at A:T base pairs (Greenblatt *et al*, 1994). Furthermore, it is important to note that because early studies indicating a preponderance of p53 mutations occurred in exons 5–9, the examination of other exons may prove to be a fruitful area for additional studies.

The possible cause for SCC in psoriasis patients treated with high cumulative doses of PUVA may be explained by considering other effects of PUVA on cells. A psoralen photoadduct specific monoclonal antibody has been used to illustrate the presence of these photoadducts in the suprabasal keratinocytes of PUVA-treated skin (Fig 4). It has been assumed that SCC arise from these premutagenic photoadducts formed during photochemotherapy; however, Krueger *et al* have shown

that skin-infiltrating T cells in psoriasis patients are uniquely susceptible to the effects of low to modest PUVA doses, whereas keratinocytes appear to be unusually resistant (Johnson *et al*, 1996). The important role of T cells as mediators of psoriasis and a target cell for therapeutic intervention is also illustrated by the potency of cyclosporine and DAB-IL2 regimens (Gottlieb *et al*, 1995). Taken together, the therapeutic effects of PUVA appear to be due to clearing of T cells and/or alterations in cytokine production.

PUVA is also known to be immunosuppressive (Kripke, 1984). Repeated PUVA therapy could attenuate immune surveillance in skin and allow subpopulations of cells harboring preexisting p53 mutations to develop into tumors (Jonason *et al*, 1996; Ren *et al*, 1997). These tumors would be expected to express solar type mutations accumulated during the lifetime of solar exposure humans' experience. Such a series of steps could account for the "early" development of SCC in the PUVA cohort patients. These results also suggest that the epidemic of skin cancer so widely publicised may be due more to the immune suppressive effects of sunlight (as opposed solely to its direct genotoxic effects). UV and PUVA cause similar mutations even though different kinds of damage to cellular DNA are induced, the mechanism of SCC development could be different as well.

Recent studies describing p53 mutations in the skin cancers of renal transplant recipients (RTR) supports the possibility of immune effects in the development of SCC (McGregor *et al*, 1997). The p53 mutation spectrum obtained from the analysis of exons 5-9 of the p53 gene in the skin cancers from these chronically immune suppressed patients is remarkably similar to that for solar-induced skin cancer (Fig 3B). There was a striking similarity in the frequency of mutations at pyrimidines (primarily C), which was quite similar to that observed in the sun-induced skin cancers. An alternate viewpoint has been presented by Bennett *et al* (1997), who reported a lower incidence of p53 mutations in RTR SCC. In the latter study, SSCP analyses were not performed and it is possible that some mutations were not detected.

Other clinical observations, however, are consistent with an immune-effect hypothesis. Van de Kerkhof and de Rooij (1997) described the occurrence of multiple SCC in a psoriatic patient following 14 y of PUVA (2500 exposures). At the time cyclosporine therapy was started only three SCC had been detected. During 16 months of treatment with cyclosporine 21 SCC appeared (one on the lower lip, three on the arms, and 17 on the legs). In an animal study it had been previously shown that cyclosporine was able to promote the growth of transplanted UV-induced tumors (Servilla *et al*, 1987). Others described very similar cases in which PUVA patients with SSC have been found to harbor the human papillomavirus (Weinstock *et al*, 1995 and Rübber *et al*, 1996). Both suggested that PUVA-induced immune suppression increased the susceptibility to both HPV and SCC as a result of wild-type p53 being inactivated by the HPV-produced E6 protein.

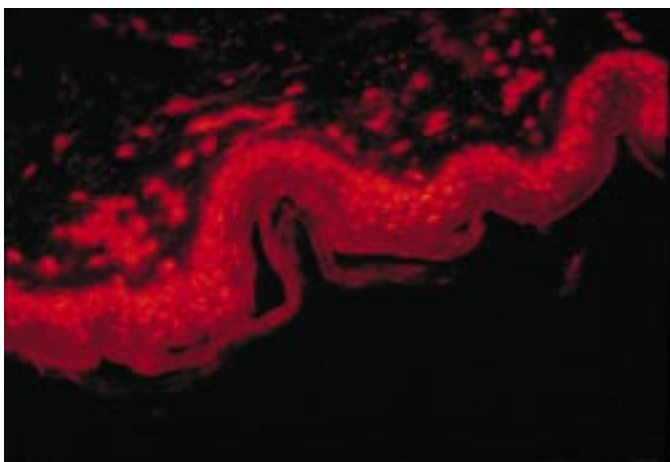


Figure 4. Section of human skin treated with 8-MOP and UVA and then stained with 8G1. 8G1 is a monoclonal antibody recognizing 8-MOP photoadducts in DNA (Santella *et al*, 1985). The binding of 8G1 to suprabasal keratinocytes is reflected by the rhodamine-tagged goat-antimouse IgG.

Any other effective therapy for psoriasis will very likely need to have similar effects on T cells. It is possible that repeated treatments with other therapies (either systemic or topical) to clear psoriasis, could also lead to an increased incidence of SCC (as in the case report on cyclosporine described above). Although these alternate therapies may not appear to act through direct genotoxic events (as in PUVA), they may generate reactive oxygen species that can lead to the formation of oxidative damage like 8-hydroxyguanine, with all of its concomitant effects (Kvam and Tyrrell, 1997) or the release of clastogenic factors (Filipe *et al*, 1997).

Understanding *in vivo* DNA photochemistry Although thymine dimers are considered the most common DNA photoproduct, mutations are rarely observed at T. There may be two contributing factors. The first (the "A" rule) has already been discussed. The second is that photoproduct prevalence based on laboratory studies does not reflect the actual photochemistry that occurs in epidermal DNA. Virtually all laboratory studies of DNA photochemistry have employed UVC radiation (254 nm). Of course human skin is never exposed to UVC because it does not penetrate the ozone layer (cut off near ~290 nm). When combined with the transmission properties of human skin, it becomes clear that DNA in epidermal cells is exposed to a range of wavelengths significantly different from those commonly employed to study DNA photochemistry. In addition there may be strong microenvironmental effects on DNA photochemistry that could predispose base damage at specific DNA sequences (Ross and Yu, 1988). Using solar radiation, Tomassi *et al* (1997) showed that p53 mutation hot spots containing 5-methylcytosine (5 mC) within a dipyrimidine sequence are 15-fold more susceptible to pyrimidine dimer formation. These findings were confirmed by Drouin and Therrien (1997) and Denisshenko *et al* (1997), who showed a correlation between UVB-induced cyclobutane dimer formation and skin cancer mutational hot spots in p53.

The absorption curve for 5 mC is red-shifted by 6 nm (compared with cytosine, see Fig 5), which results in a 5-15-fold higher extinction for 5 mC versus C between 300 and 315 nm (Pfeifer and Denisshenko, 1998). Thus, a selective absorption of photons by 5 mC could account for the occurrence of C→T and CC→TT mutations at dipyrimidine sites containing 5 mC bases. This preference for photoproduct formation at 5 mC sites may also explain the unusual action spectrum for the induction of p53 recently reported by Deck *et al* (1998). A maximum at 275 nm was reported and it was suggested that this might reflect a non-DNA chromophore (peak not at 260 nm).

The relevance of the preceding discussion to PUVA resides in the complex nature of 8-MOP photochemistry (see above). There may be unique photochemical events at selected DNA sequences (a micro-environmental effect). In addition, oxidative processes may produce a

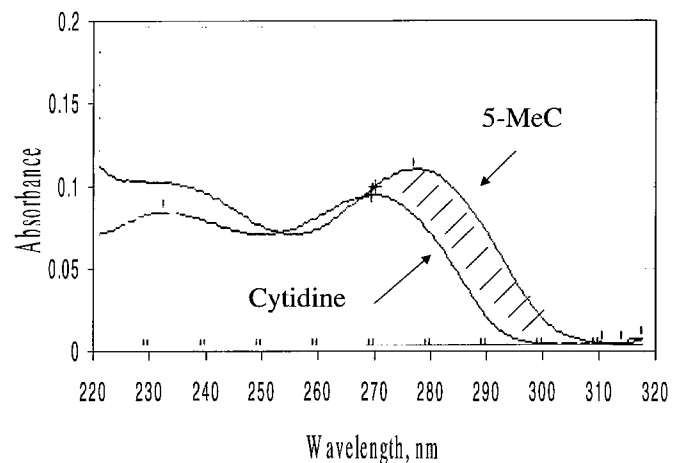


Figure 5. UV absorption spectra for 5-methylcytosine and cytosine. The spectrum for 5 mC is red shifted 6 nm. It should also be noted that the spectrum for C itself is red-shifted compared with that for thymine (λ_{\max} ~ 265 nm). The shaded region illustrates the additional absorptive properties of 5 mC at longer UVB wavelengths.

mixture of clastogenic factors (Filipe *et al.*, 1997; Przybyszewski *et al.*, 1998) that could skew the *in vivo* mutation spectrum. While it may be tempting to interpret the observed C→T mutations in PUVA SCC as evidence of previous actinic damage (Gasparro *et al.*, 1998), the possibility that they arise by some other process cannot be discounted.

CONCLUSIONS

It is evident that molecular epidemiology has made significant contributions to our understanding of skin cancer caused by solar exposure and/or PUVA. Clearly there is a need for more information. To establish that a specific mutation is caused by a particular photochemical reaction in cellular DNA, 8-MOP photoadduct mapping studies should be performed to verify the location of photoadduct formation (or other damage) under *in vivo* conditions.

For example, although it is known that 8-MOP photoadducts form in cells treated with 8-MOP and UVA, their locations at specific DNA sequences have not been determined. The extent of 8-MOP photoadduct formation is reduced by three orders of magnitude for *in vivo* treated cells (compared with *in vitro* treated DNA; Ross and Yu, 1988). It is commonly assumed that the *in vivo* sites of photoadduct formation will be the same as those found *in vitro* (Sage & Bredberg 1991). As illustrated above for pyrimidine dimer formation at 5 mC-containing sites, however, *in vitro* and *in vivo* results may differ significantly. The common tendency of AT rich transcription factor binding sites to be occluded by nucleosomal assemblies may preclude or at least reduce 8-MOP photoadduct formation at these sites (Klemm *et al.*, 1994). Furthermore, other sites where DNA damage is not detected *in vitro* may become new hot spots as a result of nucleosome-induced structures that provide new binding sites for 8-MOP at sequences not considered hot spots in naked DNA.

For technical reasons it has been difficult to detect the formation of base oxidation photoproducts (e.g. 8-hydroxyguanine); however, evidence of its formation in epidermal DNA may be evident in the observed p53 mutation spectrum from nonmelanoma skin cancers. G→C and G→T transversions can be derived from 8hG (Palmer *et al.*, 1997). It is also possible for some C→T transitions to arise from other base oxidation photoproducts. Wang *et al.* (1998) have shown that stable oxidation products of cytosine and thymine can induce C→T transitions. It is interesting to note that the distinctive action spectrum for the photosensitized induction of 8hG extends far into the near UV (long wavelength UVA). Although the endogenous sensitizing agent (possibly NADH or porphyrins) remains to be determined, the occurrence of photoproducts by any means utilizing longer wavelengths indicates the importance of studying these wavelengths for their potential carcinogenic effects.¹

The reviewed studies of p53 mutations shows that assumptions about the causes of skin cancer need to be considered carefully. PUVA is a well-tolerated efficacious therapy for psoriasis with well-characterized and controllable side-effects. High dose patients at risk for SCC are recognizable and any SCC that develop can be treated. It is appreciated that this may not apply to PUVA melanomas. If the incidence of melanoma in these patients continues to increase the dire concerns about the continued use of PUVA would be justified. The current PUVA management of psoriasis had been refined as the result of nearly 25 y of clinical experience. Before newer therapies are advocated to replace PUVA, comparable efficacy and safety must be demonstrated. The concern about the hazards of PUVA may be warranted but these hazards may not be unique to PUVA.

The insights provided by the studies described here augur well for the future of molecular epidemiology in augmenting our understanding of skin cancer development, and possibly, prevention. For potential

PUVA patients it may be possible to use a p53 mutation profile of normal appearing skin (but containing actinic damaged-induced p53 mutations) as a pre-PUVA screen for psoriasis patients likely to develop skin cancers. Other studies might also include the analysis of p53 mutations in malignant melanomas detected in PUVA patients (Stern *et al.*, 1997). It will be important to know whether these mutations have distinctive features of PUVA-induced DNA damage or whether they are more like the less common p53 mutations typically found in melanomas (Albino *et al.*, 1994; Weiss *et al.*, 1995).² In this regard it will be important to consider all types of UV-induced mutations – both the prototypical C→T and the CC→TT transitions as well as mutations arising from the newly appreciated significance of oxidized DNA bases. In evaluating the results of these kinds of studies, it should be kept in mind that carcinogenesis is a complex process and that there are redundant molecular controls of proliferation, mutations in p53 could be a central event, perhaps even a driving force, in one malignancy and not another.

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REFERENCES

- Albino AP, Vidal MJ, McNutt NS, *et al.*: Mutation and expression of the p53 gene in human malignant melanoma. *Melanoma Res* 4:35–45, 1994
- Alcalay J, Ullrich SE, Kripke ML: Local suppression of contact hypersensitivity in mice by a monofunctional psoralen plus UVA radiation. *Photochem Photobiol* 50:217–220, 1989
- Bennett MA, O'Grady A, Kay EW, Leader M, Murphy GM: P53 mutations in squamous cell carcinomas from renal transplant recipients. *Biochem Soc Trans* 25:342–345, 1997
- Brash DE, Rudolph JA, Simon JA, *et al.*: A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* 88:10124–10128, 1991
- Cimino GD, Gamper HB, Isaacs ST, *et al.*: Psoralens as photoactive probes of nucleic acid structure and function: organic chemistry, photochemistry, and biochemistry. *Ann Rev Biochem* 54:1151–1193, 1985
- Deck A, Blydes J, Hupp T, Gibbs NK: An ultraviolet action spectrum for p53 activation *in vitro* indicates that DNA may not be the primary chromophore. *Photochem Photobiol* 67:54S, 1998
- Denissenko MF, Chen JX, Tang M-S, Pfeifer GP: Cytosine methylation determines hot spots of DNA damage in the human p53 gene. *Proc Natl Acad Sci USA* 94:3893–3898, 1997
- Dittmer D, Pati S, Zambetti G, *et al.*: Gain of function mutations in p53. *Nature Gen* 4:42–46, 1993
- Drouin R, Therrien JP: UVB-induced cyclobutane pyrimidine dimer frequency correlates with skin cancer mutational hot spots in p53. *Photochem Photobiol* 66:719–726, 1997
- Elyahu D, Raz A, Gruss P, Givol D, Oren M: Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature* 312:646–649, 1984
- Filipe P, Emerit I, Youssef AA, Levy A, Carnjavski L, Freitas J, Cirne de Castra JL: Oxylradical-mediated clastogenic plasma factors in psoriasis. Increases in clastogenic activity after PUVA. *Photochem Photobiol* 66:497–501, 1997
- Gasparro FP: p53 in Dermatology. *Arch Dermatol* 134:1029–1032, 1998
- Gasparro FP, Liao B, Foley PJ, Wang XM, McNiff JM: Psoralen photochemotherapy clinical efficacy: photomutagenicity, the role of molecular epidemiology in minimizing risks. *Environ Mol Mutagenesis* 31:105–112, 1998
- Gottlieb TM, Oren M: p53 in growth control and neoplasia. *Biochim Biophys Acta* 1287:77–102, 1996
- Gottlieb S, Gilleaudeau P, Johnson R, Estes L, Woodworth TG, Gottlieb AB, Krueger JG: Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggest a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1:442–447, 1995
- Greenblatt MS, Bennett WP, Hollstein M, Harris CC: Mutations in p53 tumor suppression gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 54:4855–4878, 1994
- Gunther EJ, Yeasky TM, Gasparro FP, Glazer PM: Mutagenesis by 8-methoxypsoralen and 5-methylangelicin photoadducts in mouse fibroblasts: mutations at cross-linkable sites induced by of adducts as well as cross-links. *Cancer Res* 55:1283–1288, 1995
- Hollstein MC, Wild CP, Bleicher F, Chutimataewin S, Harris CC, Srivatanakul P, Montesano R: p53 mutations and aflatoxin B1 exposure in hepatocellular carcinoma patients from Thailand. *Int J Cancer* 53:51–55, 1993
- Johnson R, Staiano-Coico L, Austin L, Cardinale I, Nabeya-Tsukifuji R, Krueger JG:

¹This observation also highlights the potential need for protection against these wavelengths by sunscreens. An adequate UVB absorbing sunscreen could easily prevent erythema while allowing the penetration of large amounts of shorter visible radiation wavelengths and hence the accumulation of premutagenic 8hG lesions or other oxidized bases (Tsurudrome *et al.*, 1998). Thus, it is important that studies evaluating sunscreen efficacy should employ full spectrum solar radiation (actual or simulated) and not so-called sun lamps.

²Few (if any) studies distinguish between the different manifestations of melanoma. It may well be that some are light related and that others are not. Collecting all melanoma p53 data in one file may be misleading.

- PUVA treatment selectively induces a cell cycle block and subsequent. *Apoptosis Human T-Lymphocytes Photochem Photobiol* 63:566-571, 1996
- Jonason AS, Kunala S, Price GJ, et al: Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA* 93:14025-14029, 1996
- Klemm JD, Rould MA, Aurora R, Herr W, Pabo CO: Crystal structure of the October-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. *Cell* 77:21-32, 1994
- Ko LJ, Prives C: p53: puzzle and paradigm. *Genes Dev* 10:1054-1072, 1996
- Kreutzer DA, Essigmann JM: Oxidized, deaminated cytosines are a source of C→T transitions *in vivo*. *Proc Natl Acad Sci USA* 95:3578-3582, 1998
- Kripke ML: Effects of methoxsalen plus near-ultraviolet radiation or mid-ultraviolet radiation on immunologic mechanisms. *Natl Cancer Inst Monogr* 66:247-251, 1984
- Kvam E, Tyrrell RM: Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation. *Carcinog* 18:2379-2384, 1997
- Lane DP: Cancer. p53, guardian of the genome. *Nat* 358:15-16, 1992
- Levine AJ: p53, the cellular gatekeeper for growth and division. *Cell* 88:323-331, 1997
- Lewis JG, Adams D: Inflammation, oxidative DNA damage and carcinogenesis. *Environ Health Perspect* 76:19-27, 1987
- Linzer DI, Levine AJ: Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 17:43-52, 1979
- McGregor J, Berkpout RJM, Rocyska M, ter Schegget J, Bavinck JNB, Brooks L, Crook T: p53 Mutations implicate sunlight in post-transplant skin cancer irrespective of human papilloma virus status. *Oncogene* 15:1737-1740, 1997
- Moor ACE, Gasparro FP: Biochemical aspects of psoralen photochemotherapy. *Clinics Dermatol* 4:353-356, 1996
- Nataraj AJ, Black HS, Ananthswamy HN: Signature p53 mutations at DNA cross-linking sites in 8-methoxypsoralen and ultraviolet A (PUVA)-induced murine skin cancers. *Proc Natl Acad Sci USA* 93:7961-7965, 1996
- Nataraj AJ, Wolf P, Cerroni L, Ananthswamy HN: P53 mutations in squamous cell carcinomas from psoriasis patients treated with psoralen + UVA (PUVA): Relative frequency of PUVA- versus UV-signature mutations. *J Invest Dermatol* 109:238-243, 1997
- Okorokov AL, Ponchel F, Milner J: Induced N- and C-terminal cleavage of p53: a core fragment of p53, generated by interaction with damaged DNA, promotes cleavage of the N-terminus of full-length p53, whereas ssDNA induces C-terminal cleavage of p53. *EMBO J* 16:6008-6017, 1997
- Palmer CM, Serafini DM, Schellhorn HE: Near ultraviolet radiation (UVA and UVB) causes a formamidopyrimidine glycosylase-dependent increase in G to T transversions. *Photochem Photobiol* 65:543-549, 1997
- Parrish JA, Fitzpatrick TB, Tannenbaum L, Pathak MA: Photochemotherapy of psoriasis with oral methoxsalen and long-wave ultraviolet light. *N Engl J Med* 291:1207-1211, 1974
- Pfeifer GP, Denissenko MF: Formation and repair of DNA lesions in the p53 gene: relation to cancer mutations. *Environ Mol Mutagen* 31:197-205, 1998
- Przybyszewski J, Box HC, Kulesz-Martin M: Induction of reactive oxygen species without 8-hydroxydeoxyguanosine formation in DNA of initiated mouse keratinocytes treated with 12-O-tetradecanoylphorbol-13-acetate. *Carcinogenesis* 19:1467-1474, 1998
- Ren ZP, Ahmadian A, Ponen F, et al: Benign clonal keratinocyte patches with p53 mutations show no genetic link to synchronous squamous cell precancer or cancer in human skin. *Am J Pathol* 150:1791-1803, 1997
- Ren ZP, McNiff J, Gasparro FP: p53 mutations at psoralen photochemical hot spots - rare but significant events in PUVA induced skin cancers. *J Invest Dermatol* 112:657, 1999a
- Ren ZP, Peritz AE, Douglass MC, McNiff J, Gasparro FP: Analysis of p53 mutations based on microdissection of tumor biopsies from psoriasis patients treated with PUVA. *Photochem Photobiol* 69S:29S-30S, 1999b
- Ross PM, Yu HS: Interstrand crosslinks due to 4,5',8'-trimethylpsoralen and near ultraviolet light in specific sequences of animal DNA. Effect of constitutive chromatin structure and of induced transcription. *J Mol Biol* 201:339-351, 1988
- Rübben A, Traidl C, Baron JM, Grussendorf-Conen E-I: Demonstration of human papillomavirus type 16-related DNA and absence of detectable p53 gene mutations in widespread cutaneous squamous cell carcinoma after oral psoralen with UV-A treatment. *Arch Dermatol* 132:1257-1259, 1996
- Sage E, Bredberg A: Damage distribution and mutation spectrum: the case of 8-methoxypsoralen and UVA in mammalian cells. *Mutat Res* 263:217-222, 1991
- Sagher D, Strauss B: Insertion of nucleotides opposite apurine/apyrmidinic sites in deoxyribonucleic acid during *in vitro* synthesis: uniqueness of adenine nucleotides. *Biochem* 22:4518-4526, 1983
- Santella RM, Dharmaraja N, Gasparro FP, Edelson RL: Monoclonal antibodies to DNA modified by 8-methoxypsoralen and ultraviolet A light. *Nucl Acids Res* 13:2533-2544, 1985
- Scheffner M: Ubiquitin, E6-AP, and their role in p53 inactivation. *Pharmacol Ther* 78:129-139, 1998
- Servilla KS, Burnham DK, Daynes RA: Ability of cyclosporin to promote growth of transplanted ultraviolet-induced tumors in mice. *Transplan* 44:291-295, 1987
- Stern RS, Thibodeau LA, Kleinerman RA et al: Risk of cutaneous carcinoma in patients treated with oral methoxsalen photochemotherapy for psoriasis. *N Engl J Med* 300:809-813, 1979
- Stern RS, Nichols KT, Vákevá LH: for the PUVA follow-up study. Malignant melanoma in patients treated for psoriasis with methoxsalen (psoralen) and ultraviolet A radiation (PUVA). *N J Engl Med* 336:1041-1045, 1997
- Stern RS, Nichols KT, Vákevá LH: and the PUVA follow-up study. Oral psoralen and ultraviolet-A light (PUVA) treatment of psoriasis and persistent risk of nonmelanoma skin cancer. *J Natl Cancer Inst* 90:1278-1284, 1998
- Streilein JW: Photoimmunology of non-melanoma skin cancer. *Cancer Surv* 26:207-217, 1996
- Studniberg HM, Weller P: PUVA, UVB, psoriasis and nonmelanoma cancer. *J Am Acad Dermatol* 29:1013-1022, 1993
- Tomassi S, Denissenko MF, Pfeifer GP: Sunlight induces pyrimidine dimers preferentially at 5-methylcytosine bases. *Cancer Res* 57:4727-4730, 1997
- Tsurudome Y, Hirano T, Kamiya H, Yamaguchi R, Asami S, Itoh H, Kasai H: 2-hydroxyadenine, a mutagenic form of oxidative DNA damage, is not repaired by a glycosylase type mechanism in rat organs. *Mut Res* 408:121-127, 1998
- Van de Kerkhof PCM, de Rooij MJM: Multiple squamous cell carcinomas in a psoriatic patient following high-dose photochemotherapy and cyclosporin treatment: response to long-term acitretin maintenance. *Br J Dermatol* 136:275-278, 1997
- Vink AA, Moodycliffe AM, Shreedhar V, Ullrich SE, Roza L, Yarosh DB, Kripke ML: The inhibition of antigen presenting activity of dendritic cells resulting from UV irradiation of murine skin is restored by *in vitro* photorepair of cyclobutane pyrimidine dimer. *Proc Natl Acad Sci USA* 94:5255-5260, 1997
- Wang XM, McNiff JM, Klump V, Asgari M, Gasparro FP: An unexpected spectrum of p53 mutations from squamous cell carcinomas in patients treated with PUVA. *Photochem Photobiol* 66:294-299, 1997
- Wang D, Kreutzer DA, Essigmann JM: Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. *Mutat Res* 400:99-115, 1998
- Weinstock MA, Coulter S, Bates J, Bogaars HA, Larson PL, Burmer GC: Human papilloma virus and widespread cutaneous carcinoma after PUVA photochemotherapy. *Arch Dermatol* 131:701-704, 1995
- Weiss J, Heine M, Arden KC, Korner B, Pilch H, Herbst RA, Jung EG: Mutation and expression of TP53 in malignant melanomas. *Rec Results Cancer Res* 139:137-154, 1995
- Yun MH, Choi SJ, Shim SC, A: novel photoadduct of 4,5',8-trimethylpsoralen and adenosine. *Photochem Photobiol* 55:457-460, 1992