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Review

# UVA Radiation, DNA Damage, and Melanoma

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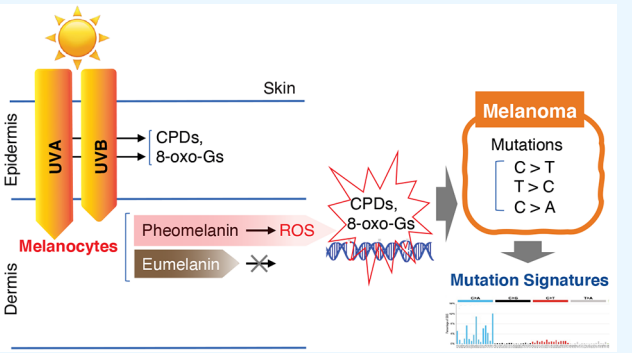
**ABSTRACT:** Melanoma is a lethal type of skin tumor that has been linked with sunlight exposure chiefly in fair-skinned human populations. Wavelengths from the sun that can reach the earth's surface include UVA radiation (320−400 nm) and UVB radiation (280−320 nm). UVB effectively induces the formation of dimeric DNA photoproducts, preferentially the cyclobutane pyrimidine dimers (CPDs). The characteristic UVB signature mutations in the form of C to T mutations at dipyrimidine sequences are prevalent in melanoma tumor genomes and have been ascribed to deamination of cytosines within CPDs before DNA polymerase bypass. However, evidence from epidemiological, animal, and other experimental studies also suggest that UVA radiation may participate in melanoma formation. The DNA damage relevant for UVA includes specific types of CPDs at TT sequences and perhaps oxidative DNA damage to guanine, both induced by direct or indirect, photosensitization-mediated chemical and biophysical processes. We summarize the evidence for a potential role of UVA in melanoma and discuss some of the mechanistic pathways of how UVA may induce mutagenesis in melanocytes.






## 1. EPIDEMIOLOGY OF MELANOMA

Cancer is a major public health concern in the United States, accounting for over 600,000 deaths in 2020. Skin cancer is believed to be the most frequent cancer in fair-skinned populations, with an increasing incidence rate worldwide. The main forms include melanoma originating from skin pigmenting melanocytes and basal cell carcinoma and squamous cell carcinoma arising from keratinocytes or their precursors. The incidence of keratinocyte skin cancer is poorly documented due to its low mortality rate. However, estimates suggest more than 1,000,000 cases per year in the US.[1] Incidence and mortality of melanoma have been well quantified. The most recent approximation places incidence of melanoma at around 96,000 cases per year and an annual percent change (APC) of 1.8% in males and 3.7% in females.[2]

Data collected from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results (SEER) combined database suggest an overall increase in incidence from 200.1 to 229.1 cases per million person-years over the past decade for which data are available (2006−2015). Increases in annual percent change were also consistent in both localized disease (APC, 1.9%) and distant metastatic disease (APC, 4.8%).[3] The same data suggested that an increased incidence of melanoma is largely associated with adults aged 40 and above.

In comparison, incidence of melanoma in children, adolescents, and young adults is trending downward. Notably, incidence rates have remained low and consistent among children aged 0−9, while rates in adolescent (age, 10−19) and young adults (age, 20−29) reached a peak incidence around 2004−2005 and began to decrease thereafter.[3] The downward trend in melanoma incidence for individuals aged 30 and below is likely due to screening efforts and public education that has been ongoing for the past 20 years. Limited exposure to ultraviolet (UV) radiation is expected to play a role in children, as UV radiation has a robust association with melanoma.[4]

Data obtained from population-based cancer registries in the US, UK, Norway, Sweden, Denmark, Australia, and New Zealand found that women consistently had higher rates of melanoma than men in early life until the approximate age of 50 years when higher rates prevail in men. The same study found significant differences in specific anatomic sites between sexes, with women demonstrating higher rates of lower limb melanoma and higher rates of head and neck melanoma in men.[5] Sex differences in melanoma incidence among anatomic location has been credited to behavioral differences such as differences in attire and time outdoors. Recent studies demonstrate that high naevus count on lower limbs for

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women is in part under genetic control. This finding suggests that specific genetic influences on naevus count at different sites may explain differences in site-specific melanoma incidence.[6]

Investigators have speculated that the increased incidence of melanoma can be attributed to an increase in diagnostic scrutiny. Analysis on data from SEER demonstrated the 2.5-fold increase in biopsy rate to be significantly contributing to the increased 2.4-fold incidence of melanoma, while mortality remained stable, suggesting potential overdiagnosis.[7] Increased diagnoses during the study period were of early stage melanoma and not contributing to mortality, leading investigators to conclude that increased scrutiny of lesions may lead in some cases to potentially unnecessary biopsies. While many have suggested an increased incidence of melanoma to be related to a rise in screening, others have hypothesized that there is a true increase in melanoma incidence. Investigators using data from SEER concluded that individuals of low socioeconomic status (SES), believed to have limited access to screening, demonstrated the highest increases of melanoma compared to their high SES counterparts.[8]

Data compiled by the Centers for Disease Control and Prevention demonstrate melanoma mortality rates have remained constant from 1982 to 2011. In 2011, the overall age-adjusted melanoma death rate was 2.7 per 100,000.[9] More recently, mortality rates for melanoma have declined by 2% per year in adults aged 50 and older and 4% per year in individuals under 50 (Cancer Facts and Figures, 2019. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2019.html). Five-year survival rates for metastatic melanoma have drastically improved from 15% to 30% over the last 20 years, secondary to successful developments in targeted therapy and immunotherapy.[10]

## 2. RISK FACTORS FOR MELANOMA

Clinical and epidemiological evidence supporting a relationship between solar UV radiation and melanoma is overwhelming. Incidence of melanoma is higher in fair-skinned people, especially those who sunburn easily, when compared to people with darker complexions. Higher melanoma occurrence rates in fair-skinned individuals support the notion that greater penetration of UV radiation into skin increases the possibility of malignancy. In black populations, incidence of melanoma is only 1/10 of that among whites, often occurring in areas not under heavy UV exposure such as the foot and nailbed, supporting the idea that dark skinned individuals are at risk for atypical forms of melanoma, but are otherwise well protected.[11]

It has been estimated that at least 60−70% of malignant melanomas are caused by UV radiation exposure.[12] Association between UV radiation exposure and melanoma occurs at a dose−response rate with greater exposure related to greater occurrence of disease. Population-based cohort studies demonstrate a greater association for melanoma on the trunk and lower limbs compared to upper limbs and head and neck melanoma at higher exposure rates.[13] The same studies have demonstrated inconsistency in association between the number of nevi and site of melanoma, contradicting the divergent pathway hypothesis which suggests people with an inherently high propensity for melanocytic proliferation require moderate UV exposure to initiate melanoma on body sites with many nevi such as the legs in women.[13,14] This finding may reflect the complexity of association between melanoma, UV radiation, sun exposure, number of nevi, and age.

Epidemiological studies have demonstrated a strong association between UV radiation and melanoma risk. UV light is a type of electromagnetic radiation emitted by the sun. The UV spectrum is conventionally subdivided into UVA radiation (320−400 nm), UVB radiation (280−320 nm), and UVC radiation (100−280 nm), although slightly different definitions also exist (i.e., 280−315 nm for the UVB range and 315−400 nm for UVA). Only UVA radiation and a portion of the UVB spectrum (above approximately 300 nm) can reach the surface of the earth. Both UVA and UVB may contribute to melanoma.

Of particular importance is the correlation between heavy sunbed use and melanoma occurrence.[15,16] The majority of commercially available, canopy style tanning beds primarily emit UVA radiation with energy 10−15 times higher than from sunlight. Recent estimates attribute 50−80% of sunbed associated erythema and inflammation to UVA radiation, which is a 3-fold increase compared to solar UVA radiation.[17] Deleterious exposure to UVA radiation in sunbed use has been linked to increased incidence of melanoma. Between 1990 and 2006, there was a significant increase in truncal melanoma occurrence in Icelandic women which was tied to rising sunbed use.[15] Various studies have confirmed the correlation between sunbed use and heightened melanoma risk.[18] Of additional concern, the application of UVB sun blockers that do not shield the skin effectively from UVA rays increases the exposure of a person to much higher doses of UVA radiation because they can spend more time in the sun without experiencing painful UVB-induced sunburns.

## 3. ANIMAL MODELS OF UVA CARCINOGENESIS

In animal models, UVB radiation has long been known to effectively induce squamous cell carcinomas of the exposed skin. This has been demonstrated convincingly in hairless mouse models.[19,20] Using similar mouse models, estimations suggested that the UVA fraction of sunlight may be about 4−10-times less effective than the UVB component in inducing nonmelanoma skin tumors after exposure with a simulated sunlight irradiation source.[21] In early experiments with pigmented hairless mice, the authors found that UVA induces squamous cell carcinomas effectively, but they did not observe melanomas.[22−24]

Melanoma mouse models are more difficult to implement with standard hairless mice because most melanocytes in mouse skin are localized in the hair shafts. Therefore, other vertebrate animal models have served as useful tools in melanoma research. In different animal models, however, when investigators tested the role of pure UVA wavelengths with little or no UVB contribution, a quite complicated and sometimes controversial picture has emerged. Earlier studies using the *Xiphophorus* (platyfish or swordtails) fish model has shown that UVA radiation can induce melanoma-like lesions.[25] However, a later study came to the conclusion that there was no significant difference in melanoma frequencies between UVA-irradiated and untreated fish.[26] Another study used *Monodelphis domestica* (opossums) for irradiation with UVB or UVA. After 81 weeks of exposure, the authors found that UVA was only weakly effective in producing nonmelanoma skin tumors, but almost as equally potent as UVB in producing melanocytic hyperplasia, a presumed precursor lesion of


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melanoma.[27] Later, the use of a hepatocyte growth factor/scatter factor-transgenic mouse melanoma model produced a different result. The hepatocyte growth factor mice have extrafollicular melanocytes in the dermal to epidermal junctions and in the epidermis of the trunk of the animals, which more closely resembles human skin. In this model, UVA (320−400 nm) was ineffective in inducing cutaneous melanoma.[28] Yet, in the same model, but using pigmented mice, it was later shown that UVA can induce melanoma in a melanin-initiated, pigment-dependent pathway, but not in albino mice.[29] These mice also have the HGF signaling pathways constitutively activated in melanocytes, which is probably different from normal human melanocytes but predisposes these mice to melanoma.

Other useful mouse models for melanoma consist of BRAF or NRAS mutant transgenic mice in which the mutant alleles are expressed in their melanocytes.[30] Using these models, which also carried a conditional *CDKN2A* knockout allele, investigators tested the potency of UVB and UVA irradiation for promoting melanoma formation. While a single dose of 4.5 kJ/m$^2$ UVB dramatically accelerated melanoma onset, UVA at the chosen dose of 70 kJ/m$^2$ produced only a modest, yet significant reduction in tumor latency as compared with control mice that were not irradiated.[31] While the authors readily identified C to T UV signature mutations in the UVB-induced melanomas, they were not able to identify a specific signature for UVA-induced tumors. In these very sensitive mouse models, a substantial fraction of the mice developed melanomas even in the absence of radiation, making it more difficult to identify specific UV radiation-induced changes.

## 4. DNA DAMAGE INDUCED BY UVA RADIATION

The stability of DNA is a most critical requirement for its function in cells. UV radiation induces various types of DNA damage in irradiated cells directly or indirectly. The photoreactivity of DNA allows direct absorption of UV photons onto pyrimidine bases and causes dimerization of two adjacent pyrimidines as the major type of direct DNA damage in skin cells. UV photons, especially the high energy ones of the UVB and UVC spectrum, can alter the structure of the DNA bases, predominantly of thymines at dipyrimidine sequences through the formation of electronic excited states.[32] These UV-induced photolesions are a replication-blocking type of DNA damage. The most frequent dipyrimidine photoproduct induced by the higher-energy wavelength UVB range (280−320 nm) is the *cis-syn* cyclobutene pyrimidine dimer (CPD) formed by connecting C5 and C6 of two adjacent pyrimidines through singlet/triplet excitation (Figure 1A). The pyrimidine (6−4) photoproduct [(6−4)PP] is the next most frequent DNA lesion created by stable bonding between positions C6 and C4 of two neighboring pyrimidines in double-stranded DNA. This lesion is less frequent than the CPD and is repaired preferentially by nucleotide excision repair.[33,34] Some of (6−4)PPs can be converted to a photoisomerization product, the Dewar valence isomer, by subsequent absorption of photons around the 320 nm wavelength to form a third type of relevant DNA photoproduct initiated in the UVB range.[35] However, current data show that the levels of the (6−4) photoproducts are extremely low in the UVA range compared to the higher levels induced by UVB or by nonphysiological UVC radiation.[36−38]

CPDs are responsible for up to 80% of the UVB-induced mutations in a mammalian cell model in which either CPDs or

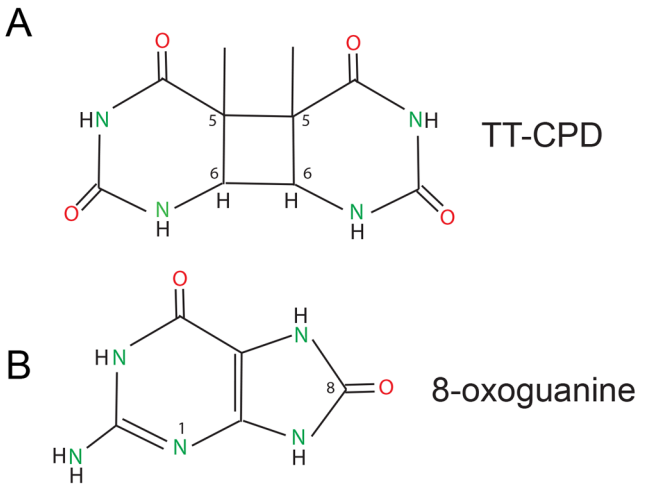


**Figure 1.** Major types of DNA damage induced by UVA radiation. (A) Cyclobutane pyrimidine dimer (CPD) at 5′TT sequences. (B) 8-Oxoguanine (8-oxoG).

(6−4)PPs are removed selectively by photolyase activities prior to mutation scoring.[39] The formation of UVB-induced CPDs in genomic DNA occurs mainly at 5′TT and 5′TC sequences followed by 5′CC and 5′CT dinucleotide sequences at genome-wide levels of about 53%, 34%, 8%, and 5%, respectively.[40] However, UV radiation-induced skin cancers predominantly accumulate C > T mutation at 5′TC and 5′CC dipyrimidine sequences, and these mutations rarely occur at 5′TT sites.[41] The prevalent model for the origin of UVB-specific C > T mutations involves deamination of cytosines (or 5-methylcytosines) within CPDs.[41,42] The deamination event, which occurs within a few hours after UVB irradiation, produces uracil bases (or thymines) within the CPDs.[43,44] Such dimers are copied by incorporation of adenines opposite the deaminated bases by DNA polymerase eta (POLH), eventually leading to a C > T mutation at the deaminated CPD of dipyrimidines. Using our newly developed method of circle-damage-sequencing (CD-seq) to map DNA damage genome-wide and at single base resolution, we recently showed that melanoma mutational signatures are indeed highly correlated with the preferred sequence positions of UVB-induced, cytosine-deaminated CPDs.[40]

UVA can also induce CPDs, either directly or indirectly. The direct CPD formation through absorbance of UVA photons was confirmed by showing the formation of CPDs in purified DNA after UVA irradiation.[45,46] However, it also has been shown that UVA can promote the indirect formation of CPDs as a result of transfer of triplet energy from an excited photosensitizer molecule to dipyrimidines.[37,47−49] UVA-induced CPDs are formed at a lower frequency than after UVB exposure, but they predominantly occur at TT dipyrimidine sequences (TT-CPDs).[37,48] Since UVA-induced TT-CPDs may arise by a unique photochemistry involving endogenous photosensitizers, the possibility exists that the distribution of these UVA CPDs along the genome differs from that of UVB-induced TT-CPDs.

In addition to CPD photoproducts, the formation of 8-oxoguanine (8-oxoG) (Figure 1B) by UVA exposure has been studied extensively. It was shown that UVA indirectly induces reactive oxygen species (ROS) by the excitation of various cellular chromophores, photosensitizers, like for example flavins, melanin, riboflavin, porphyrins, and 6-formylindolo-


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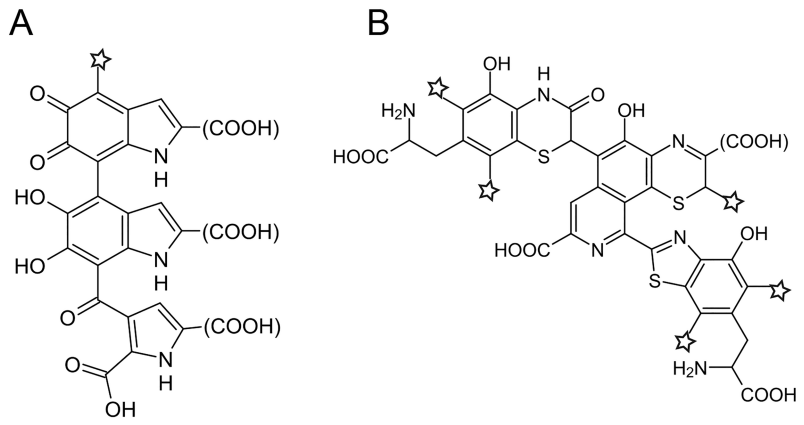


**Figure 2.** Eumelanin and pheomelanin. (A) Eumelanin. (B) Pheomelanin. Shown are subunits (monomers) of the two types of melanin. Adapted with permission from ref 65. Copyright 2008 Wiley. The stars indicate the sites of the molecules where conjugation of additional subunits (polymerization) can occur. The positions with carboxyl groups in brackets may contain either H or COOH. Those positions may also form attachment to other monomers. The benzothiazine units in pheomelanin may participate in photosensitization reactions.

[3,2-*b*]carbazole.[50−52] ROS can generate single-strand DNA breaks, oxidized pyrimidines and oxidized purines, the most frequently oxidized base being 8-oxoG, in mammalian cells.[53]

## 5. MECHANISMS OF DNA DAMAGE FORMATION IN MELANOCYTES

UVA penetrates dermal stroma, whereas UVB is mostly absorbed by the epidermis,[54] and UVA photon energy can be delivered at a 100-fold higher level than UVB photon energy into the lower epidermis and upper dermis regions near melanocytes.[52] Furthermore, the UVA photolesions are mainly mediated by the chemical properties and cellular location of endogenous photosensitizers in layers of skin cells.[55] When comparing UV-induced DNA damage in primary cultures of keratinocytes and melanocytes, Mouret et al. observed no difference in the frequency of CPDs in both UVB and UVA ranges, but the level of 8-oxoG was higher in the melanocytes by as much as 2.2-fold compared to UVA irradiation of keratinocytes.[56] UVA-dependent melanoma formation may be exacerbated by the presence of melanin in melanocytes acting as an endogenous photosensitizer and leaving a characteristic DNA damage footprint.[57]

It has been known that endogenous melanin plays a role chiefly as a photoprotective agent because melanin can absorb UV photons and scavenge ROS[58] but may also be involved as a carcinogenic photosensitizer producing CPDs and/or oxidative DNA damage as a downstream result of ROS generation.[59,60] This controversy about the photoprotective versus the photosensitization effects of melanin can potentially be explained by the two types of melanin, eumelanin and pheomelanin, which coexist at a defined ratio in the same melanocytes,[61−63] which determines the color of hair and skin. The two types of melanin differ in their chemical structure, most notably by the addition of cysteine molecules to DOPA-quinone, a process which selectively leads to the formation of pheomelanin (Figure 2). Both melanin derivatives are derived from cyclization of the initial modified tyrosine, partial polymerization, and formation of a complex with proteins. Eumelanin is a good radical scavenger.[64] However, pheomelanin is not. Instead, the benzothiazole units of pheomelanin[65] can act as photosensitizers leading to the formation of ROS.[62,66,67]

The activity of melanocortin 1 receptor (MC1R) is involved in setting up the ratio of these two melanin synthesis pathways in melanocytes. Upregulated MC1R induced by alpha-melanocyte stimulating hormone (alpha-MSH) secreted from UV-irradiated keratinocytes stimulates the production of brown eumelanin by inducing tyrosinase activity. Eumelanin accumulation leads to the transfer of melanin granules (melanosomes) to keratinocytes and acts as a natural sunscreen against UVB and UVA, due to its strong UV absorbing, UV-scattering and ROS scavenging properties.

On the other hand, downregulation of MC1R function with agouti signaling protein, an inverse agonist opposing alpha-MSH, induces the synthesis of red/yellow pheomelanin in the skin.[63,68] The incidence of melanoma is increased in fair-skinned, and particularly in red-haired individuals, who have loss-of-function variants in the *MC1R* gene, compared to black- and brown-haired individuals.[59,69] In a study from Queensland, individuals with red hair and more than 20 naevi had a melanoma odds ratio of 10 compared with individuals with dark brown hair and 0−4 naevi.[70] This risk is likely due to decreased production of eumelanin, or a shift in the pheomelanin to eumelanin ratio, and perhaps also due to reduced $\alpha$-MSH mediated repair of DNA damage.[71]

The lower energy of UVA radiation relative to UVB has led to the proposal that the formation of DNA damage by UVA involves endogenous photosensitizers, although direct excitation without photosensitizers may produce CPDs at very high doses of UVA as well.[47] However, it has remained unclear what the nature of these endogenous photosensitizers might be and how effective they are in forming either TT-CPDs and/or oxidized guanines. Besides direct absorption of photons, CPDs can form by photosensitization via the so-called triplet−triplet energy transfer pathway. The photosensitizer, for example, the synthetic chemical acetophenone, is excited by the absorption of UV photons and converted into its triplet state via intersystem crossing. If the energy is high enough and the photosensitizer molecule is close to a DNA dipyrimidine, it may transfer its triplet energy to the DNA to generate a CPD.

As a DNA-damaging photosensitizer, pheomelanin is not only involved in the production of ROS and may lead to the formation of 8-oxoG, but it also has been suggested that pheomelanin can produce a chemiexcitation reaction that mediates CPD formation. This unusual pathway leads to the


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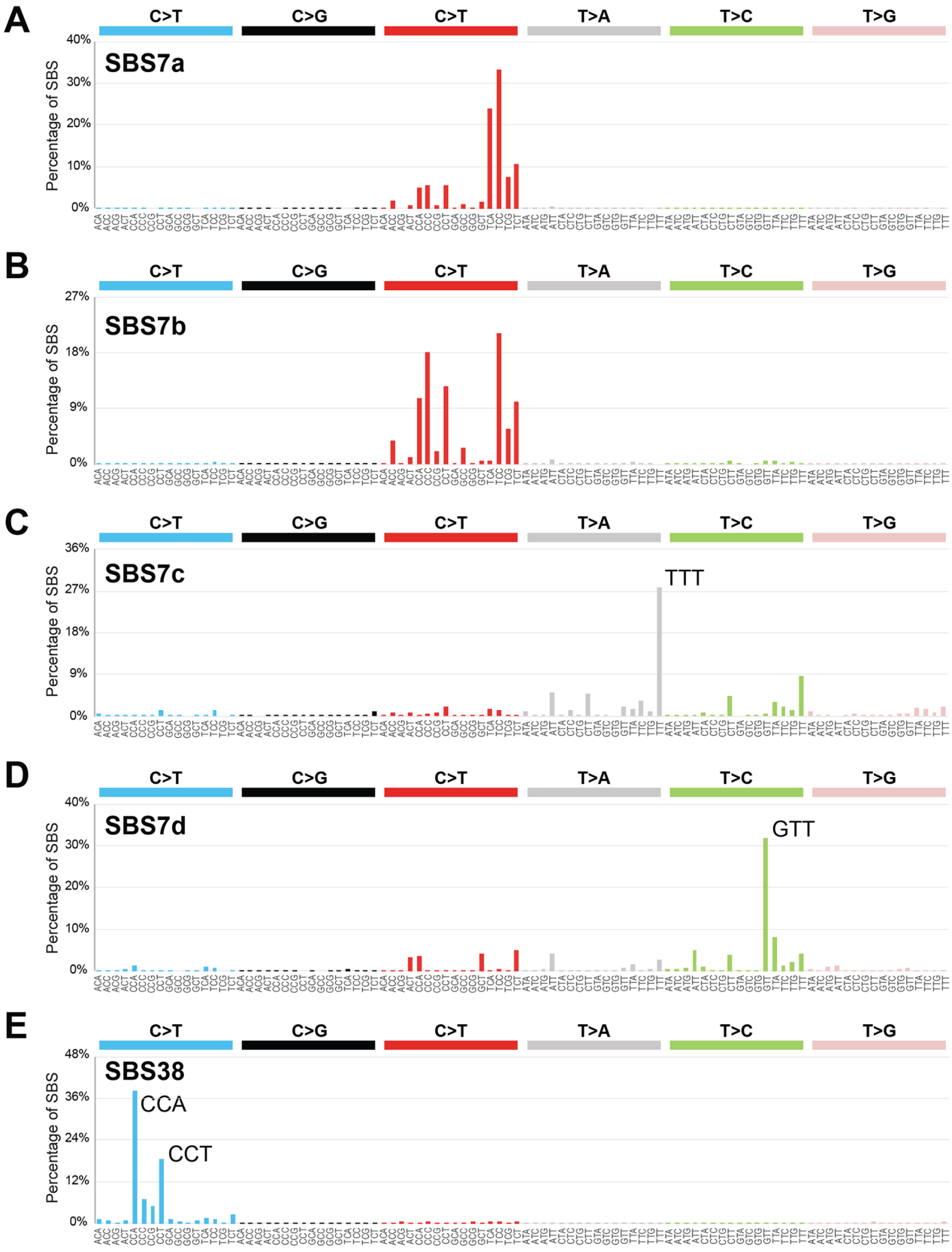


**Figure 3.** Mutational signatures enriched in melanoma genomes. (A) SBS7a. There are many mutations at 5′TC dinucleotides. (B) SBS7b. These mutations are seen at 5′CC and 5′TC sequences. (C) SBS7c. This signature is dominated by T > A and T > C mutations with a strong bias toward TTT trinucleotides. (D) SBS7d. The signature shows T > C mutations and the strongest occurrence of these events at 5′GTT sequences. (E) SBS38. The signature is characterized by C > A/G > T mutations in certain sequence contexts.



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formation of so-called "dark CPDs" within a few hours of incubation of the cells in the dark after initial UVA irradiation.[72,73] This dark CPD generating process is more prominent in the pheomelanin containing melanocytes and is mediated by the formation of the oxidant peroxynitrite produced by reaction of nitric oxide ($^{\bullet}NO$) and superoxide anion ($O2^{\bullet-}$), which leads to electron-excited melanin monomers.[72] Peroxynitrite in the nucleus can react with melanin monomers to form high-energy melanin dioxetane and carbonyl compounds which may have photosensitizing properties.[74] An excited triplet carbonyl, when close to DNA, can transfer its energy to DNA forming a CPD. Based on this recently accumulating data, the levels of DNA damage ascribed to UVA may be more substantial than previously assumed.

## 6. REPAIR OF UVA-INDUCED DNA DAMAGE

The dimeric DNA photoproducts, the CPDs and the (6−4)PPs, are repaired by the nucleotide excision repair (NER) pathway. This pathway is defective in the cancer-prone human syndrome xeroderma pigmentosum (XP).[75] The incidence of melanoma is greatly (>1000-fold) increased in XP patients relative to the normal population, directly proving that photodamage repaired by NER is mechanistically linked to melanoma.[76,77] Several gene products participating in NER, including DNA damage recognition proteins, helicases, and excision nucleases, are mutated in XP, and the classification of their specific mutations has led to the establishment of different subcategories or complementation groups of the disease (XP-A to XP-G). The detailed mechanisms of NER have been summarized, and the reader is referred to other review articles on this topic.[78,79]

One class of XP carries mutations in DNA polymerase eta (XP-variant cases), a polymerase that participates in error-free bypass of CPDs.[80,81] At a genome-scale level, CPDs are removed with much slower kinetics than (6−4)PPs because the (6−4) lesion is more helix distorting and more easily recognized by the NER pathway. However, one specific subpathway of NER, transcription-coupled repair removes CPDs effectively from transcribed sequences of the genome with a preference for repair of the transcribed DNA strand[82] and also with a preference for repair of sequences near transcription start sites.[83] This selective repair leads to a strand bias of C to T mutations at dipyrimidines in melanoma genomes with more of these mutations found on the nontranscribed DNA strand (Catalogue of Somatic Mutations in Cancer (COSMIC) database). It is not known, however, if UVA-induced and UVB-induced CPDs are repaired by different kinetics, perhaps because of the different sequence contexts at which the damage is formed.

ROS-induced single base lesions, including 8-oxoG, are repaired primarily by the base excision repair pathway. This repair process is initiated by a DNA glycosylase recognizing a damaged base, which removes the modified base by cleaving the N-glycosylic bond. The resulting abasic site is then processed by AP endonucleases and by AP lyase enzymes which remove the deoxyribose residues so that gap filling by a DNA polymerase can restore the original sequence. For the 8-oxoG lesion, the initial repair DNA glycosylase enzyme is OGG1, but this base may also be recognized by a few other DNA glycosylases as potential backup systems.

## 7. MUTATIONS INDUCED BY UVA AND UVB

The mutational patterns of some cancer types reflect the footprint of mutagen exposures throughout the genome.[84] Cutaneous melanoma is at the top of the list with the highest mutation burden of any cancer type.[85] Most melanoma genomes carry UV-specific mutation patterns, described as UV signature mutations.[41] In early research on UV-specific mutations, the involvement of UV mutagenesis in skin cancer genomes was characterized by comparison of the relevant mutation patterns at specific gene loci, for example, in the *TP53* tumor suppressor gene. Comparison between skin cancers and internal malignancies showed UV-related *TP53* point mutations in 58% of the tested squamous cell carcinomas of the skin.[86] *TP53* is not frequently mutated in melanomas. In addition, gene-specific mutation data are generally not rich enough to see a broad mutational landscape of melanoma genomes.

With the application of new genome-wide analytical tools and high-throughput sequencing, melanoma whole genome or exome sequencing studies have shown unambiguously that melanomas carry very well-characterized genomic variants in the form of specific C > T and CC > TT UV signature mutations.[87] Exome sequencing of 147 melanomas also clearly showed the UV-related C > T mutational signature being associated with melanomas from sun-exposed body sites but not from sun-shielded sites.[88] A study from The Cancer Genome Atlas (TCGA) consortium of whole-exome sequencing of 318 primary and metastatic melanomas showed that more than 75% of melanomas carried the typical UV mutation signature, representing over 60% of C > T transitions at dipyrimidine sites and about 5% of CC > TT transitions.[89,90] In vitro studies using UVB-irradiated cells as a model system also consistently show the typical UV signature. For example, from mutation reporter gene analysis with CPDs induced by UVB irradiation, most mutations were C > T transitions at dipyrimidine sites (65%) and about 9.3% of mutations were CC > TT tandem events.[91] Broadly, these data and data from many other studies of UVB-exposed cells, support a major role of DNA damage by UV light in melanomagenesis. The C > T signature mutations at dipyrimidine sites, which show a preference for the 3′ bases of 5′TC and 5′CC sequences, are a hallmark of UVB-induced mutagenic effects and considered as a defining general UV signature.[41,89]

In 2013, Alexandrov et al. analyzed many cancer genomes and derived over 30 single-base substitution (SBS) signatures as listed in the COSMIC database (version 2).[92] The SBS7 reflects the UV (UVB) signature with predominant C > T mutations at 5′TC and 5′CC sequences and with fewer T > N mutations at dipyrimidine sequences in skin cancer.[93] In 2019, the classical signature SBS7 has been subdivided into four subsignatures (SBS7a, SBS7b, SBS7c, and SBS7d) to reflect the presence of multiple submutational processes induced by UV light (Figure 3).

SBS7a and SBS7b reflect the C > T mutation in the TCN trinucleotides context (the mutated base is underlined) and C > T mutation at CCN in melanoma genomes, respectively (Figure 3A,B). The doublet-base substitution (DBS1) in COSMIC v. 3 reflects CC > TT tandem mutations and associates well with SBS7a and SBS7b,[85] suggesting that these three signatures are closely linked to UVB exposure. We recently showed that these two newly classified signatures, SBS7a and SBS7b, which show typical C > T mutations at


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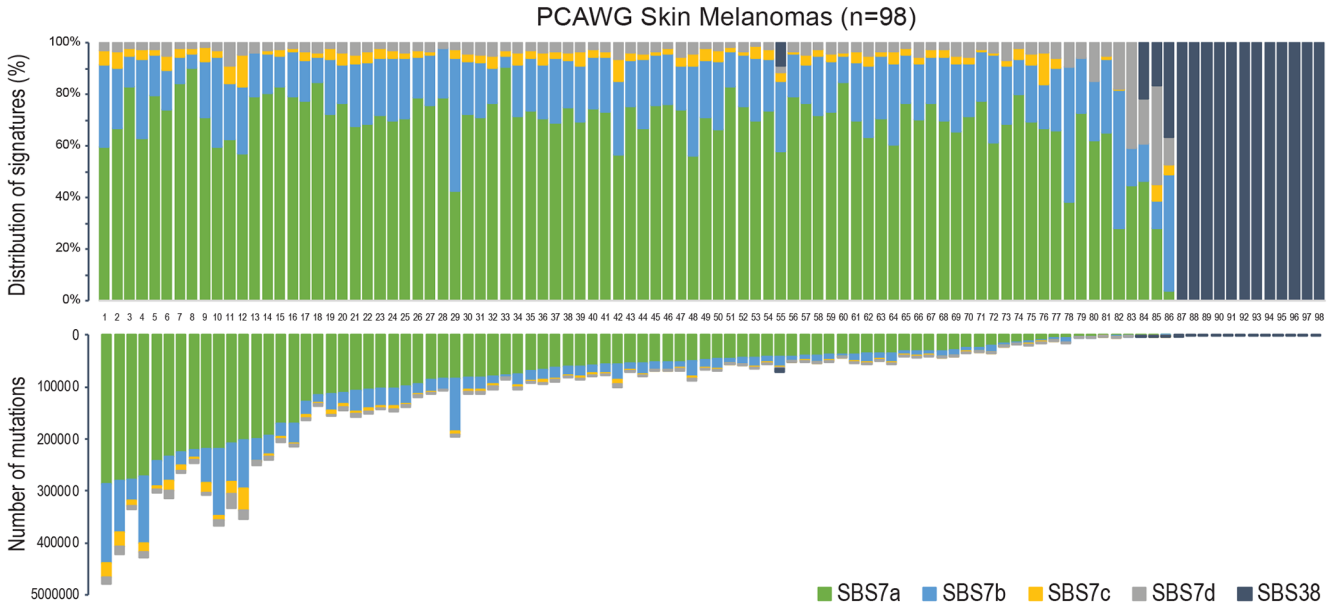


**Figure 4.** Mutational signatures of melanoma genomes from the PCAWG study. Top graph: The contribution of five mutational signatures to the collection of genome mutations in 98 individual melanoma tumors is shown. The signatures SBS7a−d and SBS38 are indicated by the color code. The data have been obtained by whole genome sequencing. In some melanomas with low mutation load, no signature could be identified. These are not displayed here. Bottom graph: The load of mutations in 98 individual melanoma tumors is shown. Data are sorted from left to right according to the frequency of signature SBS7a. The same samples from the top and bottom panels are matching and shown stacked vertically. The distributions of mutational signatures of skin melanomas from PCAWG and TCGA were obtained from the International Cancer Genome Consortium data portal link: https://dcc.icgc.org/releases/PCAWG/mutational_signatures/Signatures_in_Samples/SP_Signatures_in_Samples. The frequencies of signatures SBS7a−d and SBS38 from skin melanoma genomes (PCAWG: $n$ = 107 and TCGA: $n$ = 412) were extracted from the downloaded data sets and were generated with the SigProfiler framework.[85] The samples with a minimal score of >5 in any signature were displayed in the graphs of Figures 4 and 5.

dipyrimidine sequences, are likely caused by UVB-induced CPDs that have undergone hydrolytic deamination before DNA replication.[40]

SBS7c shows predominantly T > A mutations at NTT (58% of mutations) with half of them in the TTT trinucleotide context (Figure 3C). SBS7d shows mainly T > C mutations at NTT (55.7% of total mutations), and 32% of these mutations are at GTT trinucleotides (Figure 3D). SBS7c and SBS7d have been predicted as the result of the misincorporation of T or G opposite to UV photoproducts, which are most enriched at TT dipyrimidine sequences,[85] but the detailed mechanism is unknown. The distribution of the signatures in cutaneous melanomas was dominant from around 100% to 40% of the mutation factions for SBS7a, followed by SBS7b and SBS7c[94] (Figures 4 and 5). In addition, from COSMIC v. 3, the signature SBS38 shows elevated levels of C > A (G > T) mutations at CCN (68% of total mutations) with 38% at CCA and 18% at CCT from the total mutations in that category (Figure 3E). This signature was only found in melanomas and not in other tumors.

Given its sequence specificity and mutation types, it may be hypothesized that SBS38 is derived from UV-induced oxidative DNA damage to guanines. The SBS38 signature is present in a limited number of melanoma genomes (Figures 4 and 5). Curiously, in the Pan-Cancer Analysis of Whole Genomes (PCAWG) data set derived from whole genome sequencing, the SBS38 mutations are most prevalent in those tumors with a very low mutation load and rarely coincide with SBS7a−d mutations (Figure 4, see right side of the figure panels). However, in the TCGA data obtained by exome sequencing, SBS38 mutations also occur in a set of tumors with higher mutation load (Figure 5, see right side of the figure panels). In this set of cases, as well, SBS38 mutations do not co-occur with mutations from signature SBS7a−d. Although these data are intriguing, the origin of the melanoma-specific mutation signature SBS38 remains unknown and awaits to be proven experimentally. There are also a few cases of PCAWG melanoma genomes which lack the UV signatures SBS7a−d and lack SBS38, but show SBS2 and SBS13, which are likely caused by APOBEC-induced cytosine deamination.

Regarding the potential role of UVA in cutaneous melanoma, however, the overall interpretation of the melanoma signatures mainly reflects UVB signatures. Yet, it is thought that theoretically, C > T mutations may also be caused by UVA-induced CPDs through either a direct absorption of UVA photons or via indirect photosensitization reactions.[46,47,72,95] A defined UVA-specific mutational signature is not clearly characterized yet, for several reasons: (1) There is a limited availability of UVA-induced mutation data experimentally obtained in human melanocyte cell culture models. Mutation patterns in UVA-irradiated melanocytes should more closely reflect the correct cellular environment of melanomagenesis. (2) There is obscure understanding of mutagenic processes initiated by UVA damage. UVA-induced mutagenic pathways are much more complicated than UVB-induced mutagenesis, which directly induces large numbers of DNA photoproducts and causes mutations by a well-defined mechanism.[40] UVA- and UVB-induced mutations may originate from the same types of DNA photoproducts, CPDs and/or 8-oxoGs, as premutagenic lesions.[96] To obtain a correct etiology of UV signatures in melanoma genomes, one should be able to distinguish between UVA- and UVB-induced


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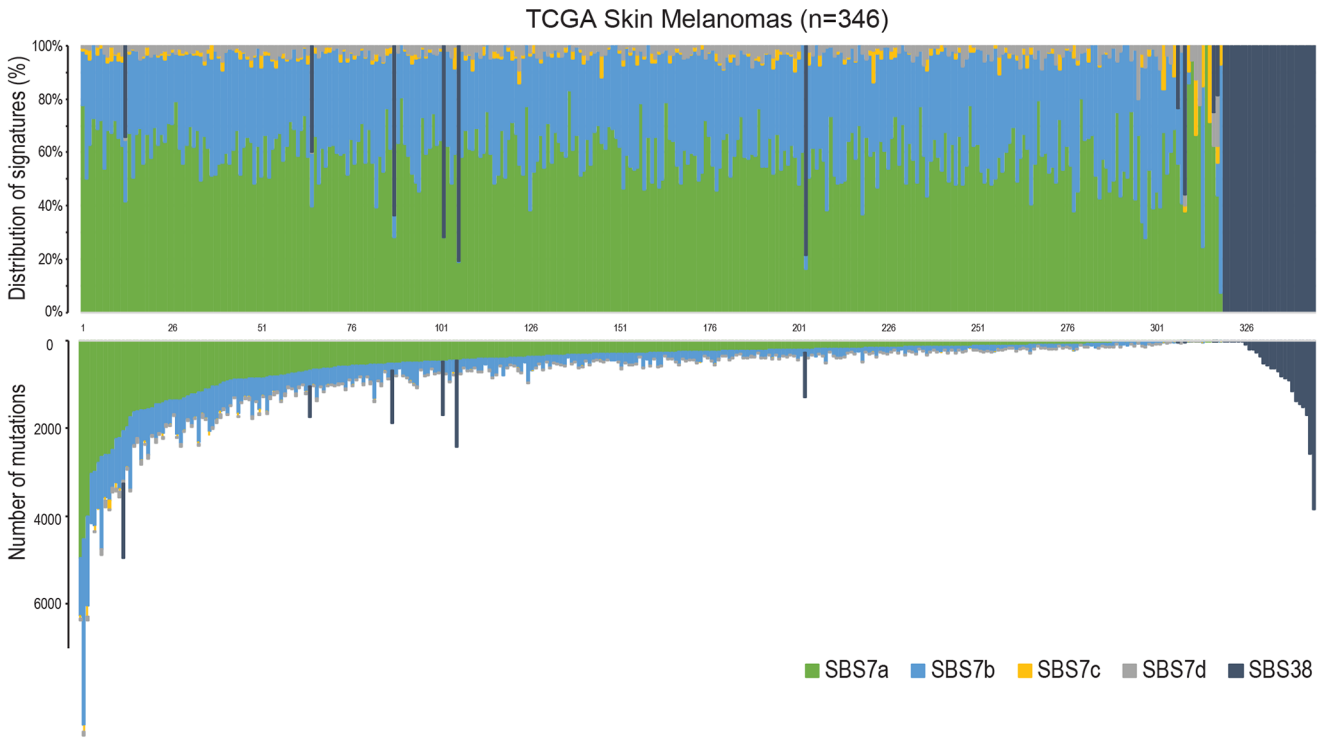


**Figure 5.** Mutational signatures of melanoma genomes from TCGA study. Top graph: The contribution of five mutational signatures to the mutations in 346 individual melanoma tumors is shown. The signatures SBS7a–d and SBS38 are indicated by the color code. These data are from exome sequencing. In some melanomas with low mutation load, no signature could be identified. These are not displayed here. Bottom graph: The load of mutations in 346 individual melanoma tumors is shown. Data are sorted from left to right according to the frequency of SBS7a. The same samples from the top and bottom panels are matching and shown stacked vertically.

mutations through the definition of divergent mutagenic processes initiated by the two types of UV exposure. (3) There is a lack of technically advanced methods to detect UVA-induced photoproducts at single-base resolution and genome-wide with high accuracy and of methodologies to reliably map the presumably rare UVA-induced mutations. DNA photoproducts are major premutagenic lesions, and mapping of the photoproduct lesions genome-wide is essential to understand cellular mutational mechanisms of cancer and to define etiology of melanoma by comparing with melanoma mutational spectra.[40,97] (4) Furthermore, the level of DNA damage and mutations induced by UVA is expected to be lower than that of UVB-induced lesions, giving another layer of challenge to obtain UVA signatures.

Despite of the relatively low levels of CPD formation by UVA exposure, several UVA damage studies have revealed that UVA-induced CPDs are most enriched at TT dipyrimidine sequences (TT-CPD) with around 90% of all pyrimidine dimers forming at that sequence, while UVB induces only around 50% of all CPDs as TT-CPDs.[37,40,41,47,98] TT-CPDs may be recruited as a fingerprint for a UVA mutational signature which is distinguished from UVB signatures. In addition, while it has been considered that the CPD is a dominant photoproduct induced by UVA exposure, and the frequency of CPDs may be similar in both keratinocytes and melanocytes,[46,48] UVA can more efficiently produce the oxidative DNA damage product 8-oxoG mediated by melanin-derived photosensitizers in melanocytes rather than in keratinocytes and other cell types.[53,64,99,100] Thus, as one of the possible UVA signatures, the observation of G > T mutations in the melanoma-specific signature SBS38 suggests that the signature may be derived from the 8-oxoG photolesions in UVA irradiated melanocytes, and this knowledge can be used to define and characterize UVA mutational signatures.

UVA mutagenesis experiments have used different model systems including cell culture and mice with transgenic mutation reporter genes. These studies have produced various results ranging from a preponderance of G > T mutations in fibroblasts[101] to a dominance of C > T mutations at methylated CpG dinucleotides (mCG) in mouse skin.[102,103] It is possible that UVA may selectively induce CPDs at 5′TmCG sequences similar as it does at 5′TT. As a step toward recreating a UVA mutation signature, a recent study of UVA-irradiated XP variant fibroblast cells shows mutation data similar to SBS7a, SBS7b, and SBS7c and also similarity to SBS18 and SBS36, as possibly derived from oxidative damage to guanine.[104] However, to our knowledge, no study has used melanocytes to unravel the mutational potential and specificity of UVA.

## 8. MELANOMA-PROMOTING EFFECTS OF UVA RADIATION INDEPENDENT OF DIRECT DNA DAMAGE OR MUTATIONS

It has been believed that melanoma-promoting effects of UVA are dependent primarily on DNA damage and DNA mutations. Melanocytes are inherently more defective than keratinocytes in the repair of UV photoproducts and of 8-oxoG.[105] UVA-induced oxidative stress may also be indirectly linked to mutations in melanoma through its ability to impair DNA repair activities.[64,106]


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UV radiation induces the upregulation of many genes in a pathway termed the UV response.[107] However, upon cessation of radiation, most of these gene expression changes will return to baseline. For these reasons, it has been of interest to see if UVA radiation may mediate more permanent changes in the epigenome with potentially longer-lasting effects on gene expression patterns. Like most other types of cancer, melanomas are characterized by extensive genome-wide DNA hypermethylation events affecting hundreds or even thousands of CpG islands and genes.[108] The biological origin of these DNA methylation changes is undefined. In vitro and in vivo studies have demonstrated UVA's ability to modify transcription of oncogenic genes via epigenetic DNA and histone alterations.[109] These studies have demonstrated that UVA may induce hypermethylation of the promoter of the p16(*CDKN2A*) gene, a tumor suppressor. The same hypermethylation signature has been found in melanomas,[110,111] indicating a possible association between UVA-induced epigenetic changes and skin cancer. Similar events have been identified in dermal fibroblasts treated with repeat UVA exposure. UVA-treated fibroblasts developed hypermethylation in genes associated with cell defense and aging, including *FOXO1* and *RPTOR*.[112] Of note, methylation differences in UVA treated fibroblasts largely returned to control levels after 7 days, indicating that reversal of these epigenetic changes is possible after UVA exposure.

Epigenetic changes induced by UVB have also been documented. In vivo UVB radiation of mouse skin epidermis demonstrated an increase in CpG methylation in specific genes.[113] The same study also found demethylation in prooncogenic genes such as cyclin-dependent kinase 4 (*CDK4*) which resulted in higher RNA expression. However, an earlier study, using genome-wide DNA methylation analysis did not find any substantial changes in DNA methylation in UVB-exposed human keratinocyte cells.[114] Variations of histone methylation have been associated with expression differences in several genes in malignant melanoma.[115] Therefore, it is possible that UVA-induced epigenetic changes may also result in varying degrees of expression in genes that are relevant for the progression of melanoma.

Additionally, UVA has been found to induce genomic instability via single-strand and double-strand DNA breaks in addition to DNA damage-induced base substitution mutations. Genomic instability induced by DNA breaks results in chromosomal breakage and repair. Changes induced by the breakage and subsequent repair have been observed in various forms of skin cancer such as squamous cell carcinoma.[116] Similar studies using UVA radiation found tumorgenicity to be strongly correlated with gain of specific chromosomal markers.[117] Studies performed by Wischermann et al. and Boukamp et al. suggest a pathway by which UVA radiation contributes to skin cancer progression by inducing large chromosomal changes.[116,117] Melanoma genomes show frequent occurrence of chromothripsis, a chromosome shattering event of unclear etiology in which tens or hundreds of DNA breaks occur simultaneously at the same genomic regions and are then repaired by reassembly to result in localized genome rearrangements or deletions.[94,118]

UVA radiation, like UVB, has immunosuppressive effects in human and animal skin.[119,120] The mechanism by which this occurs is likely through a variety of molecular changes including DNA damage and direct activation of inflammatory mediators. A prominent theory in UVA-induced immunosuppression is that it involves the formation of ROS by UV radiation.[121,122] Protection from ROS via the reducing agent alpha-tocopherol prevented significant immunosuppression in mice.[123] Additional studies inhibiting the formation of ROS with a superoxide dismutase mimic have produced similar results, protecting mice from immunosuppression.[121] On the other hand, UVA radiation has been observed unexpectedly to induce resistance to UVB-induced immunosuppression in mice.[124] For these reasons, it may be difficult to isolate the specific effects of UVA on the immune system with solar radiation exposure. The contribution of UV-induced immune suppression to melanoma progression remains less well-defined.

## 9. CONCLUSIONS

From cancer genome sequencing data, it has become clear that 70% or more of cutaneous melanoma tumors carry a clear signature of UVB-induced mutations linked to dimerized pyrimidines containing cytosines. Although UVA radiation comprises 90% of the energy in terrestrial solar radiation, its contribution to DNA damage, mutagenesis, and melanoma initiation and progression has been controversial and unclear. However, UVA radiation does produce specific types of DNA damage in the form of CPDs at TT sequences and of 8-oxoguanine. We are still lacking a complete mechanistic understanding of how UVA exposure engages photosensitizing molecules and what exact chemical or biophysical pathways are involved in the formation of these DNA lesions. Using advanced sequencing and bioinformatics approaches, the spectrum of DNA damage and the consequences of the UVA-induced DNA damage for melanoma genome mutations is now beginning to emerge and eventually may become more quantifiable.

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### Notes

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## REFERENCES


(1) Leiter, U.; Keim, U.; Garbe, C. Epidemiology of Skin Cancer: Update 2019. *Adv. Exp. Med. Biol.* **2020**, *1268*, 123−139.

(2) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin* **2019**, *69* (1), 7−34.

(3) Paulson, K. G.; Gupta, D.; Kim, T. S.; Veatch, J. R.; Byrd, D. R.; Bhatia, S.; Wojcik, K.; Chapuis, A. G.; Thompson, J. A.; Madeleine,

M. M.; et al. Age-Specific Incidence of Melanoma in the United States. *JAMA Dermatol* **2020**, *156* (1), 57−64.
(4) Campbell, L. B.; Kreicher, K. L.; Gittleman, H. R.; Strodtbeck, K.; Barnholtz-Sloan, J.; Bordeaux, J. S. Melanoma Incidence in Children and Adolescents: Decreasing Trends in the United States. *J. Pediatr* **2015**, *166* (6), 1505−1513.
(5) Olsen, C. M.; Thompson, J. F.; Pandeya, N.; Whiteman, D. C. Evaluation of Sex-Specific Incidence of Melanoma. *JAMA Dermatol* **2020**, *156* (5), 553−560.
(6) Visconti, A.; Ribero, S.; Sanna, M.; Spector, T. D.; Bataille, V.; Falchi, M. Body site-specific genetic effects influence naevus count distribution in women. *Pigment Cell Melanoma Res.* **2020**, *33* (2), 326−333.
(7) Welch, H. G.; Woloshin, S.; Schwartz, L. M. Skin biopsy rates and incidence of melanoma: population based ecological study. *BMJ.* **2005**, *331* (7515), 481.
(8) Linos, E.; Swetter, S. M.; Cockburn, M. G.; Colditz, G. A.; Clarke, C. A. Increasing burden of melanoma in the United States. *J. Invest Dermatol* **2009**, *129* (7), 1666−1674.
(9) Guy, G. P., Jr.; Thomas, C. C.; Thompson, T.; Watson, M.; Massetti, G. M.; Richardson, L. C. Vital signs: melanoma incidence and mortality trends and projections - United States, 1982−2030. *MMWR Morb Mortal Wkly Rep* **2015**, *64* (21), 591−596.
(10) Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A. Cancer statistics, 2022. *CA Cancer J. Clin* **2022**, *72* (1), 7−33.
(11) Gilchrest, B. A.; Eller, M. S.; Geller, A. C.; Yaar, M. The pathogenesis of melanoma induced by ultraviolet radiation. *N Engl J. Med.* **1999**, *340* (17), 1341−1348.
(12) Sample, A.; He, Y. Y. Mechanisms and prevention of UV-induced melanoma. *Photodermatol Photoimmunol Photomed* **2018**, *34* (1), 13−24.
(13) Ghiasvand, R.; Robsahm, T. E.; Green, A. C.; Rueegg, C. S.; Weiderpass, E.; Lund, E.; Veierod, M. B. Association of phenotypic characteristics and UV radiation exposure with risk of melanoma on different body sites. *JAMA Dermatol* **2019**, *155* (1), 39−49.
(14) Caini, S.; Gandini, S.; Sera, F.; Raimondi, S.; Fargnoli, M. C.; Boniol, M.; Armstrong, B. K. Meta-analysis of risk factors for cutaneous melanoma according to anatomical site and clinico-pathological variant. *Eur. J. Cancer* **2009**, *45* (17), 3054−3063.
(15) Autier, P.; Dore, J. F.; Eggermont, A. M.; Coebergh, J. W. Epidemiological evidence that UVA radiation is involved in the genesis of cutaneous melanoma. *Curr. Opin Oncol* **2011**, *23* (2), 189−196.
(16) Autier, P.; Dore, J. F.; Lejeune, F.; Koelmel, K. F.; Geffeler, O.; Hille, P.; Cesarini, J. P.; Lienard, D.; Liabeuf, A.; Joarlette, M.; et al. Cutaneous malignant melanoma and exposure to sunlamps or sunbeds: an EORTC multicenter case-control study in Belgium, France and Germany. EORTC Melanoma Cooperative Group. *Int. J. Cancer* **1994**, *58*, 809−813.
(17) Sola, Y.; Lorente, J. Contribution of UVA irradiance to the erythema and photoaging effects in solar and sunbed exposures. *J. Photochem. Photobiol. B* **2015**, *143*, 5−11.
(18) Le Clair, M. Z.; Cockburn, M. G. Tanning bed use and melanoma: Establishing risk and improving prevention interventions. *Prev Med. Rep* **2016**, *3*, 139−144.
(19) de Gruijl, F. R. Photocarcinogenesis: UVA vs. UVB radiation. *Skin. Pharmacol. Appl. Skin Physiol.* **2002**, *15*, 316−320.
(20) de Gruijl, F. R.; Sterenborg, H. J.; Forbes, P. D.; Davies, R. E.; Cole, C.; Kelfkens, G.; van Weelden, H.; Slaper, H.; van der Leun, J. C. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res.* **1993**, *53*, 53−60.
(21) de Laat, A.; van der Leun, J. C.; de Gruijl, F. R. Carcinogenesis induced by UVA (365-nm) radiation: the dose-time dependence of tumor formation in hairless mice. *Carcinogenesis* **1997**, *18*, 1013−1020.
(22) van Weelden, H.; van der Putte, S. C.; Toonstra, J.; van der Leun, J. C. UVA-induced tumours in pigmented hairless mice and the carcinogenic risks of tanning with UVA. *Arch Dermatol Res.* **1990**, *282* (5), 289−294.
(23) Pathak, M. A. Ultraviolet radiation and the development of non-melanoma and melanoma skin cancer: clinical and experimental evidence. *Skin Pharmacol* **2004**, *4*, 85−94.
(24) Husain, Z.; Pathak, M. A.; Flotte, T.; Wick, M. M. Role of ultraviolet radiation in the induction of melanocytic tumors in hairless mice following 7,12-dimethylbenz(a)anthracene application and ultraviolet irradiation. *Cancer Res.* **1991**, *51* (18), 4964−4970.
(25) Setlow, R. B.; Grist, E.; Thompson, K.; Woodhead, A. D. Wavelengths effective in induction of malignant melanoma. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6666−6670.
(26) Mitchell, D. L.; Fernandez, A. A.; Nairn, R. S.; Garcia, R.; Paniker, L.; Trono, D.; Thames, H. D.; Gimenez-Conti, I. Ultraviolet A does not induce melanomas in a Xiphophorus hybrid fish model. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (20), 9329−9334.
(27) Ley, R. D. Ultraviolet radiation A-induced precursors of cutaneous melanoma in Monodelphis domestica. *Cancer Res.* **1997**, *57*, 3682−3684.
(28) De Fabo, E. C.; Noonan, F. P.; Fears, T.; Merlino, G. Ultraviolet B but not ultraviolet A radiation initiates melanoma. *Cancer Res.* **2004**, *64* (18), 6372−6376.
(29) Noonan, F. P.; Zaidi, M. R.; Wolnicka-Glubisz, A.; Anver, M. R.; Bahn, J.; Wielgus, A.; Cadet, J.; Douki, T.; Mouret, S.; Tucker, M. A.; et al. Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nat. Commun.* **2012**, *3*, 884.
(30) Viros, A.; Sanchez-Laorden, B.; Pedersen, M.; Furney, S. J.; Rae, J.; Hogan, K.; Ejiama, S.; Girotti, M. R.; Cook, M.; Dhomen, N.; et al. Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53. *Nature* **2014**, *511* (7510), 478−482.
(31) Bowman, R. L.; Hennessey, R. C.; Weiss, T. J.; Tallman, D. A.; Crawford, E. R.; Murphy, B. M.; Webb, A.; Zhang, S.; La Perle, K. M.; Burd, C. J. UVB mutagenesis differs in Nras- and Braf-mutant mouse models of melanoma. *Life Sci. Alliance* **2021**, *4* (9), e202101135.
(32) Markovitsi, D. UV-induced DNA Damage: The Role of Electronic Excited States. *Photochem. Photobiol.* **2016**, *92* (1), 45−51.
(33) Pfeifer, G. P. Formation and processing of UV photoproducts: effects of DNA sequence and chromatin environment. *Photochem. Photobiol.* **1997**, *65*, 270−283.
(34) Mitchell, D. L.; Nairn, R. S. The biology of the (6−4) photoproduct. *Photochem. Photobiol.* **1989**, *49*, 805−819.
(35) Douki, T.; Sage, E. Dewar valence isomers, the third type of environmentally relevant DNA photoproducts induced by solar radiation. *Photochem. Photobiol. Sci.* **2016**, *15* (1), 24−30.
(36) Besaratinia, A.; Yoon, J. I.; Schroeder, C.; Bradforth, S. E.; Cockburn, M.; Pfeifer, G. P. Wavelength dependence of ultraviolet radiation-induced DNA damage as determined by laser irradiation suggests that cyclobutane pyrimidine dimers are the principal DNA lesions produced by terrestrial sunlight. *FASEB J.* **2011**, *25*, 3079−3091.
(37) Douki, T.; Reynaud-Angelin, A.; Cadet, J.; Sage, E. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry* **2003**, *42* (30), 9221−9226.
(38) Yoon, J.-H.; Lee, C.-S.; O'Connor, T.; Yasui, A.; Pfeifer, G. P. The DNA damage spectrum produced by simulated sunlight. *J. Mol. Biol.* **2000**, *299*, 681−693.
(39) You, Y. H.; Lee, D. H.; Yoon, J. H.; Nakajima, S.; Yasui, A.; Pfeifer, G. P. Cyclobutane pyrimidine dimers are responsible for the vast majority of mutations induced by UVB irradiation in mammalian cells. *J. Biol. Chem.* **2001**, *276*, 44688−44694.
(40) Jin, S. G.; Pettinga, D.; Johnson, J.; Li, P.; Pfeifer, G. P. The major mechanism of melanoma mutations is based on deamination of cytosine in pyrimidine dimers as determined by circle damage sequencing. *Sci. Adv.* **2021**, *7* (31), No. eabi6508.
(41) Pfeifer, G. P. Mechanisms of UV-induced mutations and skin cancer. *Genome Instab Dis* **2020**, *1* (3), 99−113.
(42) Jiang, N.; Taylor, J.-S. In vivo evidence that UV-induced C-T mutations at dipyrimidine sites could result from the replicative bypass of cis-syn cyclobutane dimers or their deamination products. *Biochemistry* **1993**, *32*, 472−481.


https://doi.org/10.1021/acsomega.2c04424
*ACS Omega* 2022, 7, 32936−32948

(43) Lee, D. H.; Pfeifer, G. P. Deamination of 5-methylcytosines within cyclobutane pyrimidine dimers is an important component of UVB mutagenesis. *J. Biol. Chem.* **2003**, *278*, 16454.
(44) Tu, Y.; Dammann, R.; Pfeifer, G. P. Sequence and time-dependent deamination of cytosine bases in UVB-induced cyclobutane pyrimidine dimers in vivo. *J. Mol. Biol.* **1998**, *284*, 297−311.
(45) Jiang, Y.; Rabbi, M.; Kim, M.; Ke, C.; Lee, W.; Clark, R. L.; Mieczkowski, P. A.; Marszalek, P. E. UVA generates pyrimidine dimers in DNA directly. *Biophys. J.* **2009**, *96* (3), 1151−1158.
(46) Mouret, S.; Baudouin, C.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (37), 13765−13770.
(47) Ikehata, H.; Kawai, K.; Komura, J.; Sakatsume, K.; Wang, L.; Imai, M.; Higashi, S.; Nikaido, O.; Yamamoto, K.; Hieda, K.; et al. UVA1 genotoxicity is mediated not by oxidative damage but by cyclobutane pyrimidine dimers in normal mouse skin. *J. Invest Dermatol* **2008**, *128* (9), 2289−2296.
(48) Rochette, P. J.; Therrien, J. P.; Drouin, R.; Perdiz, D.; Bastien, N.; Drobetsky, E. A.; Sage, E. UVA-induced cyclobutane pyrimidine dimers form predominantly at thymine-thymine dipyrimidines and correlate with the mutation spectrum in rodent cells. *Nucleic Acids Res.* **2003**, *31* (11), 2786−2794.
(49) Perdiz, D.; Grof, P.; Mezzina, M.; Nikaido, O.; Moustacchi, E.; Sage, E. Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. Possible role of Dewar photoproducts in solar mutagenesis. *J. Biol. Chem.* **2000**, *275* (35), 26732−26742.
(50) Baptista, M. S.; Cadet, J.; Greer, A.; Thomas, A. H. Photosensitization Reactions of Biomolecules: Definition, Targets and Mechanisms. *Photochem. Photobiol.* **2021**, *97* (6), 1456−1483.
(51) Park, S. L.; Justiniano, R.; Williams, J. D.; Cabello, C. M.; Qiao, S.; Wondrak, G. T. The Tryptophan-Derived Endogenous Aryl Hydrocarbon Receptor Ligand 6-Formylindolo[3,2-b]Carbazole Is a Nanomolar UVA Photosensitizer in Epidermal Keratinocytes. *J. Invest Dermatol* **2015**, *135* (6), 1649−1658.
(52) Wondrak, G. T.; Jacobson, M. K.; Jacobson, E. L. Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochem. Photobiol. Sci.* **2006**, *5* (2), 215−237.
(53) Cadet, J.; Douki, T.; Ravanat, J. L. Oxidatively generated damage to cellular DNA by UVB and UVA radiation. *Photochem. Photobiol.* **2015**, *91* (1), 140−155.
(54) Agar, N. S.; Halliday, G. M.; Barnetson, R. S.; Ananthaswamy, H. N.; Wheeler, M.; Jones, A. M. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (14), 4954−4959.
(55) Hiraku, Y.; Ito, K.; Hirakawa, K.; Kawanishi, S. Photosensitized DNA damage and its protection via a novel mechanism. *Photochem. Photobiol.* **2007**, *83* (1), 205−212.
(56) Mouret, S.; Forestier, A.; Douki, T. The specificity of UVA-induced DNA damage in human melanocytes. *Photochem. Photobiol. Sci.* **2012**, *11* (1), 155−162.
(57) Zaidi, M. R.; De Fabo, E. C.; Noonan, F. P.; Merlino, G. Shedding light on melanocyte pathobiology in vivo. *Cancer Res.* **2012**, *72* (7), 1591−1595.
(58) Bustamante, J.; Bredeston, L.; Malanga, G.; Mordoh, J. Role of melanin as a scavenger of active oxygen species. *Pigment Cell Res.* **1993**, *6* (5), 348−353.
(59) Mitra, D.; Luo, X.; Morgan, A.; Wang, J.; Hoang, M. P.; Lo, J.; Guerrero, C. R.; Lennerz, J. K.; Mihm, M. C.; Wargo, J. A.; et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature* **2012**, *491* (7424), 449−453.
(60) Schuch, A. P.; Moreno, N. C.; Schuch, N. J.; Menck, C. F. M.; Garcia, C. C. M. Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. *Free radical biology & medicine* **2017**, *107*, 110−124.
(61) Inazu, M.; Mishima, Y. Detection of eumelanogenic and pheomelanogenic melanosomes in the same normal human melanocyte. *J. Invest Dermatol* **1993**, *100*, 172S−175S.
(62) Wenczl, E.; Van der Schans, G. P.; Roza, L.; Kolb, R. M.; Timmerman, A. J.; Smit, N. P.; Pavel, S.; Schothorst, A. A. (Pheo)melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes. *J. Invest Dermatol* **1998**, *111* (4), 678−682.
(63) Nasti, T. H.; Timares, L. MC1R, eumelanin and pheomelanin: their role in determining the susceptibility to skin cancer. *Photochem. Photobiol.* **2015**, *91* (1), 188−200.
(64) Douki, T. Oxidative Stress and Genotoxicity in Melanoma Induction: Impact on Repair Rather Than Formation of DNA Damage? *Photochem. Photobiol.* **2020**, *96* (5), 962−972.
(65) Ito, S.; Wakamatsu, K. Chemistry of mixed melanogenesis−pivotal roles of dopaquinone. *Photochem. Photobiol.* **2008**, *84* (3), 582−592.
(66) Napolitano, A.; Panzella, L.; Monfrecola, G.; d'Ischia, M. Pheomelanin-induced oxidative stress: bright and dark chemistry bridging red hair phenotype and melanoma. *Pigment Cell Melanoma Res.* **2014**, *27* (5), 721−733.
(67) Panzella, L.; Leone, L.; Greco, G.; Vitiello, G.; D'Errico, G.; Napolitano, A.; d'Ischia, M. Red human hair pheomelanin is a potent pro-oxidant mediating UV-independent contributory mechanisms of melanomagenesis. *Pigment Cell Melanoma Res.* **2014**, *27* (2), 244−252.
(68) Le Pape, E.; Wakamatsu, K.; Ito, S.; Wolber, R.; Hearing, V. J. Regulation of eumelanin/pheomelanin synthesis and visible pigmentation in melanocytes by ligands of the melanocortin 1 receptor. *Pigment Cell Melanoma Res.* **2008**, *21* (4), 477−486.
(69) Raimondi, S.; Sera, F.; Gandini, S.; Iodice, S.; Caini, S.; Maisonneuve, P.; Fargnoli, M. C. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int. J. Cancer* **2008**, *122* (12), 2753−2760.
(70) Duffy, D. L.; Lee, K. J.; Jagirdar, K.; Pflugfelder, A.; Stark, M. S.; McMeniman, E. K.; Soyer, H. P.; Sturm, R. A. High naevus count and MC1R red hair alleles contribute synergistically to increased melanoma risk. *Br J. Dermatol* **2019**, *181* (5), 1009−1016.
(71) Kadekaro, A. L.; Leachman, S.; Kavanagh, R. J.; Swope, V.; Cassidy, P.; Supp, D.; Sartor, M.; Schwemberger, S.; Babcock, G.; Wakamatsu, K.; et al. Melanocortin 1 receptor genotype: an important determinant of the damage response of melanocytes to ultraviolet radiation. *FASEB J.* **2010**, *24* (10), 3850−3860.
(72) Premi, S.; Wallisch, S.; Mano, C. M.; Weiner, A. B.; Bacchiocchi, A.; Wakamatsu, K.; Bechara, E. J.; Halaban, R.; Douki, T.; Brash, D. E. Photochemistry. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science* **2015**, *347* (6224), 842−847.
(73) Lawrence, K. P.; Delinasios, G. J.; Premi, S.; Young, A. R.; Cooke, M. S. Perspectives on Cyclobutane Pyrimidine Dimers-Rise of the Dark Dimers. *Photochem. Photobiol.* **2022**, *98* (3), 609−616.
(74) Premi, S.; Brash, D. E. Unanticipated role of melanin in causing carcinogenic cyclobutane pyrimidine dimmers. *Mol. Cell Oncol* **2016**, *3* (1), No. e1033588.
(75) Cleaver, J. E. Xeroderma pigmentosum: a human disease in which an initial stage of DNA repair is defective. *Proc. Natl. Acad. Sci. U. S. A.* **1969**, *63* (2), 428−435.
(76) Hanawalt, P. C.; Sarasin, A. Cancer-prone hereditary diseases with DNA processing abnormalities. *Trends Genet.* **1986**, *2*, 124−129.
(77) Kraemer, K. H.; Lee, M. M.; Andrews, A. D.; Lambert, W. C. The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. *Arch Dermatol* **1994**, *130* (8), 1018−1021.
(78) Marteijn, J. A.; Lans, H.; Vermeulen, W.; Hoeijmakers, J. H. Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat. Rev. Mol. Cell Biol.* **2014**, *15* (7), 465−481.
(79) Sugasawa, K. Molecular mechanisms of DNA damage recognition for mammalian nucleotide excision repair. *DNA Repair (Amst)* **2016**, *44*, 110−117.


https://doi.org/10.1021/acsomega.2c04424
*ACS Omega* 2022, 7, 32936−32948

(80) Johnson, R. E.; Kondratick, C. M.; Prakash, S.; Prakash, L. hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science* **1999**, *285*, 263−265.
(81) Masutani, C.; Kusumoto, R.; Yamada, A.; Dohmae, N.; Yokoi, M.; Yuasa, M.; Araki, M.; Iwai, S.; Takio, K.; Hanaoka, F. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* **1999**, *399*, 700−704.
(82) Hanawalt, P. C.; Spivak, G. Transcription-coupled DNA repair: two decades of progress and surprises. *Nat. Rev. Mol. Cell Biol.* **2008**, *9* (12), 958−970.
(83) Tu, Y.; Tornaletti, S.; Pfeifer, G. P. DNA repair domains within a human gene: selective repair of sequences near the transcription initiation site. *EMBO J.* **1996**, *15*, 675−683.
(84) Pfeifer, G. P. How the environment shapes cancer genomes. *Curr. Opin Oncol* **2015**, *27* (1), 71−77.
(85) Alexandrov, L. B.; Kim, J.; Haradhvala, N. J.; Huang, M. N.; Tian Ng, A. W.; Wu, Y.; Boot, A.; Covington, K. R.; Gordenin, D. A.; Bergstrom, E. N.; et al. The repertoire of mutational signatures in human cancer. *Nature* **2020**, *578* (7793), 94−101.
(86) Brash, D. E.; Rudolph, J. A.; Simon, J. A.; Lin, A.; McKenna, G. J.; Baden, H. P.; Halperin, A. J.; Pontén, J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10124−10128.
(87) Pleasance, E. D.; Cheetham, R. K.; Stephens, P. J.; McBride, D. J.; Humphray, S. J.; Greenman, C. D.; Varela, I.; Lin, M. L.; Ordonez, G. R.; Bignell, G. R.; et al. A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature* **2010**, *463* (7278), 191−196.
(88) Krauthammer, M.; Kong, Y.; Ha, B. H.; Evans, P.; Bacchiocchi, A.; McCusker, J. P.; Cheng, E.; Davis, M. J.; Goh, G.; Choi, M.; et al. Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. *Nat. Genet.* **2012**, *44* (9), 1006−1014.
(89) Brash, D. E. UV signature mutations. *Photochem. Photobiol.* **2015**, *91* (1), 15−26.
(90) Akbani, K. C.; et al. Genomic Classification of Cutaneous Melanoma. *Cell* **2015**, *161* (7), 1681−1696.
(91) You, Y. H.; Pfeifer, G. P. Similarities in sunlight-induced mutational spectra of CpG-methylated transgenes and the p53 gene in skin cancer point to an important role of 5-methylcytosine residues in solar UV mutagenesis. *J. Mol. Biol.* **2001**, *305*, 389−399.
(92) Alexandrov, L. B.; Nik-Zainal, S.; Wedge, D. C.; Aparicio, S. A.; Behjati, S.; Biankin, A. V.; Bignell, G. R.; Bolli, N.; Borg, A.; Borresen-Dale, A. L.; et al. Signatures of mutational processes in human cancer. *Nature* **2013**, *500* (7463), 415−421.
(93) Alexandrov, L. B.; Stratton, M. R. Mutational signatures: the patterns of somatic mutations hidden in cancer genomes. *Curr. Opin Genet Dev* **2014**, *24*, 52−60.
(94) Hayward, N. K.; Wilmott, J. S.; Waddell, N.; Johansson, P. A.; Field, M. A.; Nones, K.; Patch, A. M.; Kakavand, H.; Alexandrov, L. B.; Burke, H.; et al. Whole-genome landscapes of major melanoma subtypes. *Nature* **2017**, *545* (7653), 175−180.
(95) Runger, T. M. C−>T transition mutations are not solely UVB-signature mutations, because they are also generated by UVA. *J. Invest Dermatol* **2008**, *128* (9), 2138−2140.
(96) Kappes, U. P.; Luo, D.; Potter, M.; Schulmeister, K.; Runger, T. M. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. *J. Invest Dermatol* **2006**, *126* (3), 667−675.
(97) Jin, S. G.; Meng, Y.; Johnson, J.; Szabo, P. E.; Pfeifer, G. P. Concordance of hydrogen peroxide-induced 8-oxo-guanine patterns with two cancer mutation signatures of upper GI tract tumors. *Sci. Adv.* **2022**, *8* (22), No. eabn3815.
(98) Ikehata, H.; Ono, T. The mechanisms of UV mutagenesis. *J. Radiat Res. (Tokyo)* **2011**, *52* (2), 115−125.
(99) Denat, L.; Kadekaro, A. L.; Marrot, L.; Leachman, S. A.; Abdel-Malek, Z. A. Melanocytes as instigators and victims of oxidative stress. *J. Invest Dermatol* **2014**, *134* (6), 1512−1518.
(100) Cadet, J.; Douki, T. Formation of UV-induced DNA damage contributing to skin cancer development. *Photochem. Photobiol. Sci.* **2018**, *17* (12), 1816−1841.
(101) Besaratinia, A.; Synold, T. W.; Xi, B.; Pfeifer, G. P. G-to-T transversions and small tandem base deletions are the hallmark of mutations induced by ultraviolet a radiation in mammalian cells. *Biochemistry* **2004**, *43* (25), 8169−8177.
(102) Ikehata, H. Mechanistic considerations on the wavelength-dependent variations of UVR genotoxicity and mutagenesis in skin: the discrimination of UVA-signature from UV-signature mutation. *Photochem. Photobiol. Sci.* **2018**, *17* (12), 1861−1871.
(103) Ikehata, H.; Kudo, H.; Masuda, T.; Ono, T. UVA induces C−>T transitions at methyl-CpG-associated dipyrimidine sites in mouse skin epidermis more frequently than UVB. *Mutagenesis* **2003**, *18* (6), 511−519.
(104) Moreno, N. C.; de Souza, T. A.; Garcia, C. C. M.; Ruiz, N. Q.; Corradi, C.; Castro, L. P.; Munford, V.; Ienne, S.; Alexandrov, L. B.; Menck, C. F. M. Whole-exome sequencing reveals the impact of UVA light mutagenesis in xeroderma pigmentosum variant human cells. *Nucleic Acids Res.* **2020**, *48* (4), 1941−1953.
(105) Wang, H. T.; Choi, B.; Tang, M. S. Melanocytes are deficient in repair of oxidative DNA damage and UV-induced photoproducts. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (27), 12180−12185.
(106) Murray, H. C.; Maltby, V. E.; Smith, D. W.; Bowden, N. A. Nucleotide excision repair deficiency in melanoma in response to UVA. *Exp Hematol Oncol* **2015**, *5*, 6.
(107) Muthusamy, V.; Piva, T. J. The UV response of the skin: a review of the MAPK, NFkappaB and TNFalpha signal transduction pathways. *Arch Dermatol Res.* **2010**, *302* (1), 5−17.
(108) Jin, S. G.; Xiong, W.; Wu, X.; Yang, L.; Pfeifer, G. P. The DNA methylation landscape of human melanoma. *Genomics* **2015**, *106* (6), 322−330.
(109) Chen, I. P.; Henning, S.; Faust, A.; Boukamp, P.; Volkmer, B.; Greinert, R. UVA-induced epigenetic regulation of P16(INK4a) in human epidermal keratinocytes and skin tumor derived cells. *Photochem. Photobiol. Sci.* **2012**, *11* (1), 180−190.
(110) Castellano, M.; Pollock, P. M.; Walters, M. K.; Sparrow, L. E.; Down, L. M.; Gabrielli, B. G.; Parsons, P. G.; Hayward, N. K. CDKN2A/p16 is inactivated in most melanoma cell lines. *Cancer Res.* **1997**, *57* (21), 4868−4875.
(111) Gonzalgo, M. L.; Bender, C. M.; You, E. H.; Glendening, J. M.; Flores, J. F.; Walker, G. J.; Hayward, N. K.; Jones, P. A.; Fountain, J. W. Low frequency of p16/CDKN2A methylation in sporadic melanoma: comparative approaches for methylation analysis of primary tumors. *Cancer Res.* **1997**, *57* (23), 5336−5347.
(112) Tilburg, J.; Slieker, R. C.; Suchiman, H. E. D.; Heath, A.; Heemst, D. V.; Slagboom, P. E.; de Gruijl, F. R.; Gunn, D. A.; Heijmans, B. T. Repeat UVA exposure of human skin fibroblasts induces both a transitionary and recovery DNA methylation response. *Epigenomics* **2020**, *12* (7), 563−573.
(113) Yang, Y.; Wu, R.; Sargsyan, D.; Yin, R.; Kuo, H. C.; Yang, I.; Wang, L.; Cheng, D.; Wang, C.; Li, S.; et al. UVB drives different stages of epigenome alterations during progression of skin cancer. *Cancer Lett.* **2019**, *449*, 20−30.
(114) Lahtz, C.; Kim, S. I.; Bates, S. E.; Li, A. X.; Wu, X.; Pfeifer, G. P. UVB irradiation does not directly induce detectable changes of DNA methylation in human keratinocytes. *F1000Res.* **2013**, *2*, 45.
(115) Moran, B.; Silva, R.; Perry, A. S.; Gallagher, W. M. Epigenetics of malignant melanoma. *Semin Cancer Biol.* **2018**, *51*, 80−88.
(116) Wischermann, K.; Popp, S.; Moshir, S.; Scharfetter-Kochanek, K.; Wlaschek, M.; de Gruijl, F.; Hartschuh, W.; Greinert, R.; Volkmer, B.; Faust, A.; et al. UVA radiation causes DNA strand breaks, chromosomal aberrations and tumorigenic transformation in HaCaT skin keratinocytes. *Oncogene* **2008**, *27* (31), 4269−4280.
(117) Boukamp, P. UV-induced skin cancer: similarities−variations. *J. Dtsch Dermatol Ges* **2005**, *3* (7), 493−503.
(118) Campbell, P. J.; et al. Pan-cancer analysis of whole genomes. *Nature* **2020**, *578* (7793), 82−93.


https://doi.org/10.1021/acsomega.2c04424
*ACS Omega* 2022, 7, 32936−32948

(119) Clement-Lacroix, P.; Michel, L.; Moysan, A.; Morliere, P.; Dubertret, L. UVA-induced immune suppression in human skin: protective effect of vitamin E in human epidermal cells in vitro. *Br J. Dermatol* **1996**, *134* (1), 77−84.
(120) Bestak, R.; Halliday, G. M. Chronic low-dose UVA irradiation induces local suppression of contact hypersensitivity, Langerhans cell depletion and suppressor cell activation in C3H/HeJ mice. *Photochem. Photobiol.* **1996**, *64* (6), 969−974.
(121) Halliday, G. M.; Byrne, S. N.; Damian, D. L. Ultraviolet A radiation: its role in immunosuppression and carcinogenesis. *Semin Cutan Med. Surg* **2011**, *30* (4), 214−221.
(122) Iwai, I.; Hatao, M.; Naganuma, M.; Kumano, Y.; Ichihashi, M. UVA-induced immune suppression through an oxidative pathway. *J. Invest Dermatol* **1999**, *112* (1), 19−24.
(123) Yuen, K. S.; Halliday, G. M. alpha-Tocopherol, an inhibitor of epidermal lipid peroxidation, prevents ultraviolet radiation from suppressing the skin immune system. *Photochem. Photobiol.* **1997**, *65* (3), 587−592.
(124) Reeve, V. E.; Tyrrell, R. M. Heme oxygenase induction mediates the photoimmunoprotective activity of UVA radiation in the mouse. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96* (16), 9317−9321.





https://doi.org/10.1021/acsomega.2c04424
*ACS Omega* 2022, 7, 32936−32948