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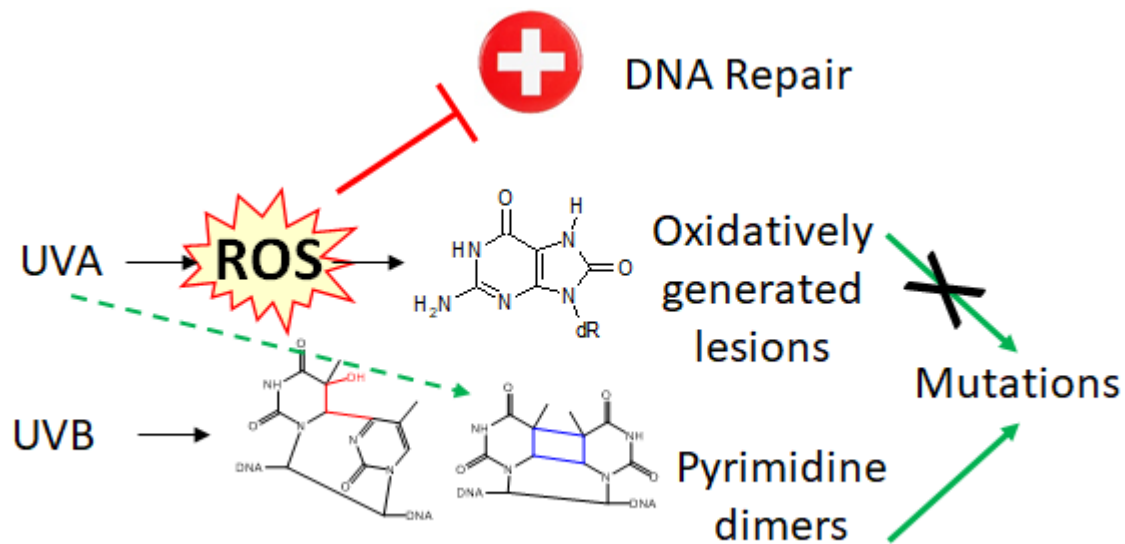
Oxidative Stress and Genotoxicity in Melanoma Induction: Impact on Repair Rather Than Formation of DNA Damage?

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GRAPHICAL ABSTRACT



Solar UV induces both pyrimidine dimers and oxidatively generated DNA lesions. The latter do not contribute to mutagenesis in melanoma in spite of the photosensitizing properties of melanin present in melanocytes. However, the intense oxidative stress mediated by melanin may hamper DNA repair and explain the contribution of UVA to the induction of melanoma.

ABSTRACT

Keratinocytes and melanocytes, two cutaneous cell types located within the epidermis, are the origin of most skin cancers, namely carcinomas and melanomas. These two types of tumors differ in many ways. First, carcinomas are almost 10 times more frequent than melanomas. In addition, the affected cellular pathways, the mutated genes and the metastatic properties of the tumors are not the same. This review addresses another specificity of melanomas: the role of photo-oxidative stress. UVA efficiently produces reactive oxygen species in melanocytes, which results in more frequent oxidatively generated DNA lesions than in other cell types. The question of the respective contribution of UVB-induced pyrimidine dimers and UVA-mediated oxidatively generated lesions to mutagenesis in melanoma remains open. Recent results based on next generation sequencing techniques strongly suggest that the mutational signature associated with pyrimidine dimers is overwhelming in melanomas like in skin carcinomas. UVA-induced oxidative stress may yet be indirectly linked to the genotoxic pathways involved in melanoma through its ability to hamper DNA repair activities.

INTRODUCTION

Skin cancers represent approximately one third of the overall burden of cancers. This large number is mostly explained by the occurrence of basal cell and squamous cell carcinomas. Melanomas are less frequent and represent less than 10% of all skin cancers. However, melanomas raise a major concern for public health since they constitute the most severe types of skin cancer with frequent metastases and a much higher death rate than carcinomas. Because of this severe health impact, melanoma is extensively studied and large amounts of information as well as open issues have been accumulated over the years, as summarized in a series of excellent recent reviews (1-6). Although located in the same tissue, melanomas and carcinomas exhibit strong differences. Melanoma arise from transformation of melanocytes and demonstrate *BRAF* and *NRAS* as the two most frequently mutated genes. In contrast, carcinomas, which derive from keratinocytes, most frequently show mutated *P53* and *PTCH1* genes. Differences have also been found in the basic photochemical events taking place in melanocytes and keratinocytes exposed to UV radiation. In particular, emphasis has been placed on the sensitivity of melanocytes to photo-oxidative stress and a possible role of oxidatively generated DNA damage in mutagenesis. The purpose of the present review is to first summarize current knowledge on the specificity of the nature and the yield of DNA damage in melanocytes. The respective involvement of pyrimidine dimers and oxidatively generated lesions in the induction of melanomas will then be discussed on the basis of recent mutational data obtained by next generation sequencing techniques. The final part of this

survey will focus on the negative impact of photo-oxidative stress on DNA repair and the role it may play in melanomagenesis.

WAVELENGTH-DEPENDENCE OF MELANOMA INDUCTION

A first clue to the specific genotoxic events leading to melanoma can be found in the wavelength specificity. While skin carcinomas have been unambiguously linked to UVB, a debate is still open concerning the portion of the solar spectrum at the origin of melanomas. Early experiments on fish led to the conclusion that UVA around 365 nm was almost equally efficient to UVB for inducing melanomas (7). Later works on *Monodelphis domestica* (gray short-tailed opossum), also showed that UVA could be at the origin of this type of tumors, although its efficiency compared to UVB was lower than in fish (8). It should be stressed that these two animal models express photolyases repair enzymes, in contrast to humans and most other mammals. The contribution of UVB-induced DNA damage may have thus been underestimated. These indications of a significant contribution of UVA to melanomagenesis were challenged by other data in fish (9, 10). The absence of induction of melanoma upon exposure to UVA was also reported in albino mice genetically engineered to mimic human melanoma (11). In this work, melanomas could be induced in approximately 25% of the animals with various UVB-containing sources, after single dose as well as repeated exposure. In contrast, less than 5% of the animals developed a tumor with pure UVA. In addition, metastases were observed in the UVB-exposed groups but not after UVA exposure. Interestingly, using mice with the same genetic background, the same group reported that UVA could be at the origin of melanoma in pigmented mice (12). While no melanoma-related death

was observed within 400 days in albino mice exposed to UVA, 50% of the pigmented mice were dead or sacrificed after 200 days because of induction of melanomas. With UVB, both strains had a similar median of melanoma-free survival at 150 days. A conclusion based on these animal data is that there may be two independent pathways leading to melanoma. One could be triggered by exposure to UVB while the other could involve exposure to UVA in the presence of melanin. The latter result is counter-intuitive because melanin is unambiguously associated with a decreased cancer rate in humans. This trend was observed not only for skin carcinomas but also for melanomas, which originate from melanocytes, the melanin producing cells in skin. The most solid data in that respect come from studies carried out in countries such as New Zealand (13) or South Africa (14) with inhabitants with either fair (phototype II) or dark (phototype VI) skin. It may be roughly considered that the solar exposure is similar for both fair- and dark-skinned people living in each of these countries. However, the ratios between occurrences of skin cancer in skin phototype II compared to phototype VI was 1 to 69, 20 and 20 for basal cell carcinoma, squamous cell carcinoma and melanoma, respectively. One has thus to reconcile the protection afforded by melanin with its putative deleterious properties when exposed to UVA (15, 3, 5).

One popular explanation to these unexpected observations is the induction of a photo-oxidative stress in melanocytes, mostly mediated by pheomelanin. As a matter of fact, melanin is not a single compound but a mixture of polymers of derivatives of dopaquinone (DOPA-Q), a metabolite of tyrosine (Fig. 1), which is subject to a series of biochemical transformations (16, 17). In the presence of cysteine, DOPA-Q is converted into cysteinyl-DOPA that polymerizes into pheomelanin, the “red melanin”. Another pathway converts DOPA-Q into indole

derivatives such as 5,6-dihydroxyindole 2-carboxylic acid, that are polymerized into eumelanin, the “black melanin”. Both types of melanin efficiently absorb UV radiation but they exhibit drastically different photochemical properties (18, 19). Eumelanin efficiently converts UV energy into heat and is a good radical scavenger. In contrast, the chemical structure of pheomelanin does not permit energy dissipation nor inhibition of reactive oxygen species (19). On the contrary, the benzothiazoles units of pheomelanin makes it a good photosensitizer (20-22). The production of both singlet oxygen (23, 24) and superoxide anion (25, 24) has been documented. Consequently, the sensitivity of melanocytes towards photo-oxidizing stress depends on the intracellular ratio between pheo- and eumelanin. The respective amounts of pheo- and eumelanin depend on the efficiency of the dark melanin pathway. In that respect, the Melanocortin-1 Receptor (MC1R) plays a major role since its activation triggers the biochemical activities at the origin of protecting eumelanin (26-28). In variants of this receptor protein leading to a slow biosynthesis of eumelanin, most DOPA-Q is used for the production of cysteinyl-DOPA. The resulting accumulation of pheomelanin is one parameter accounting for the photosensitivity of the skin of red-haired people who lack the protective eumelanin (29).

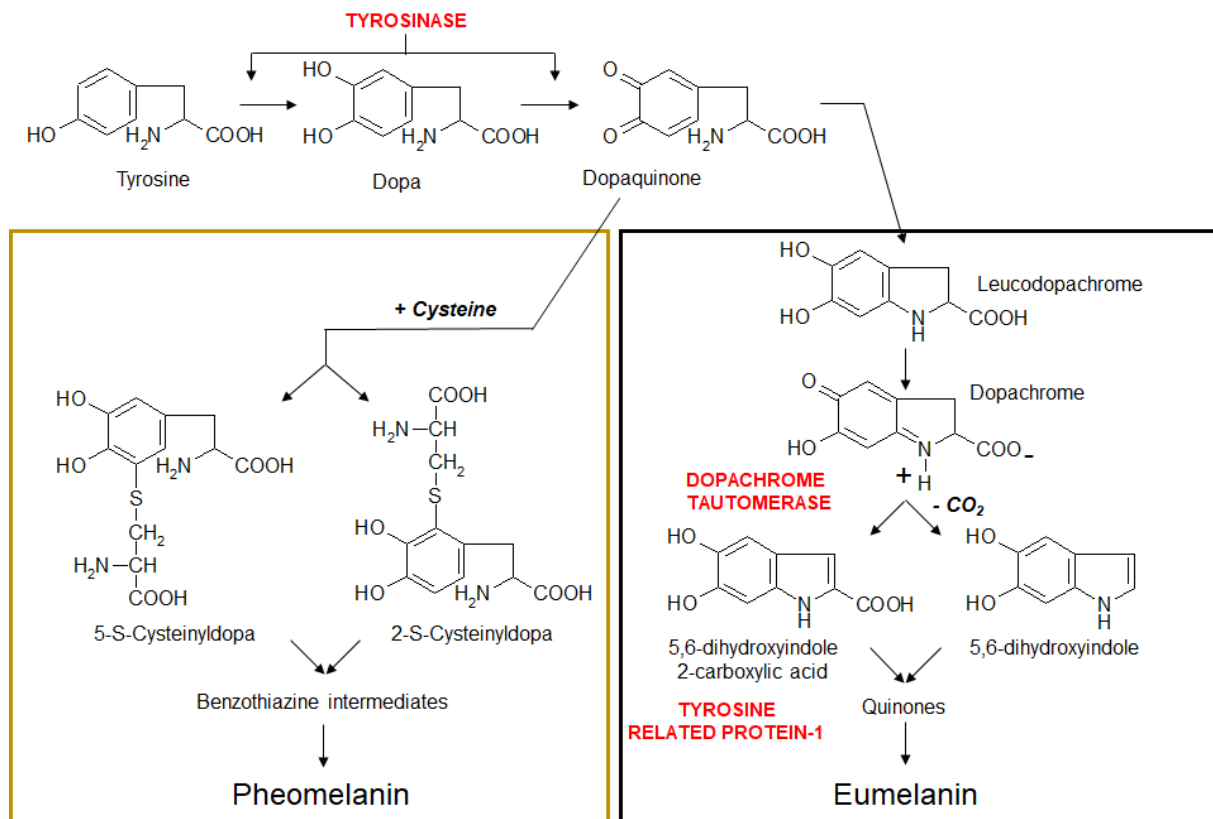


Figure 1. Biosynthesis of pheo- and eumelanin. A first common step converts tyrosine into dopaquinone (DOPA-Q). In a fast biotransformation, DOPA-Q is converted into Cysteinyl-DOPA in the presence of cysteine. The latter compound is at the origin of pheomelanin. Conversion of DOPA-Q into indole derivatives like dopachrome leads to the monomeric precursors that polymerize into eumelanin.

UV-INDUCED DNA DAMAGE IN MELANOCYTES

Formation of mutagenic DNA damage is a major event in the initiation step leading to the development of skin cancer. The chemical structures and the relative yields of UV-induced DNA damage is well understood and is known to be wavelength dependent (30). In the UVB range, damage is induced by the direct absorption of photons by DNA bases. The resulting excited states evolve through different pathways, including non-radiative deactivation,

intersystem crossing and photoreactivity (31). DNA photoproducts arising from dimerization reactions between the C5-C6 double bonds of adjacent pyrimidine bases (thymine, T, and cytosine, C) are overwhelming in cells exposed to UVB (32-35). The most frequent photoproducts are cyclobutane pyrimidine dimers (CPD, Fig. 2). They are produced in a sequence dependent manner with the following decreasing yield $TT > TC > CT > CC$ (36-40). The other types of UVB-induced DNA damage are pyrimidine (6-4) pyrimidone photoproducts (64PP) (32, 41, 35), which arise from the initial addition of the C5-C6 double bond of the 5'-end pyrimidine to the C4 position of the 3'-end base. 64PP can be photo-converted into their Dewar valence isomers upon further absorption of UVB or UVA photons (42, 43). The formation of CPD and 64PP was observed in all cutaneous cell types. Their yield of formation is similar in melanocytes and keratinocytes exposed to UVB (44, 45). An interesting feature is that melanocytes are located in the basal layer of skin and are protected by the natural pigments present in the tissue, especially in dark skin (46-48). The extent of this natural photoprotection is similar to that observed in the surrounding keratinocytes (48, 49).

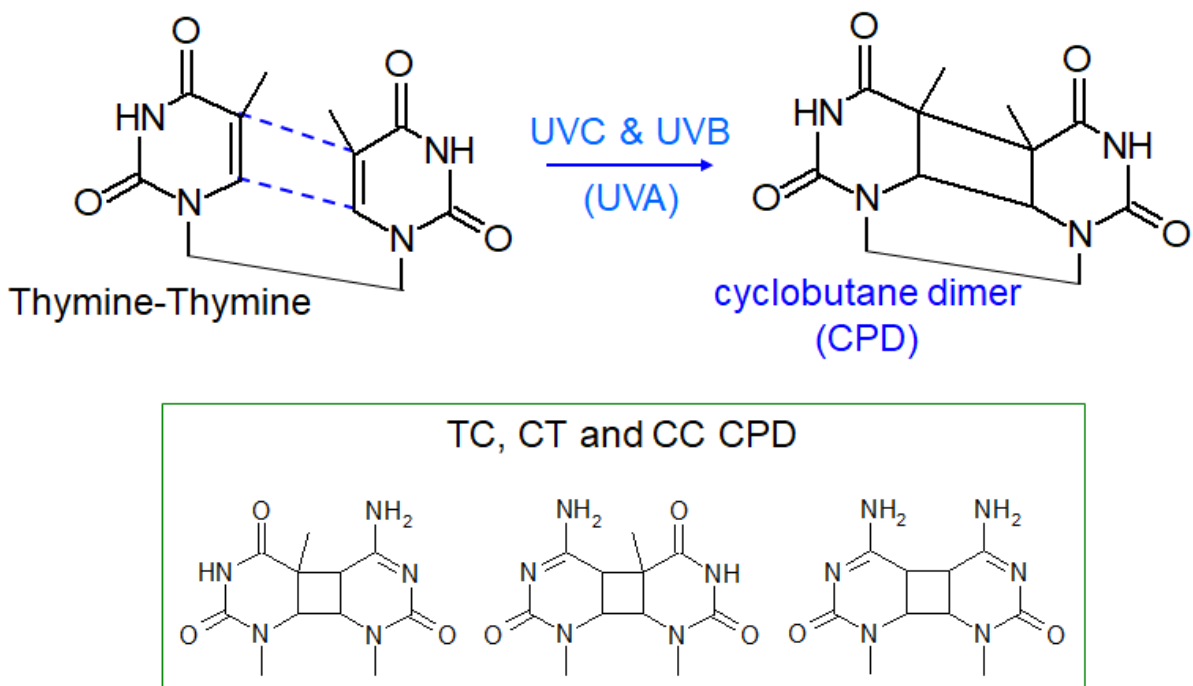


Figure 2. Formation of thymine-thymine cyclobutane pyrimidine dimer (TT-CPD) by cycloaddition of the two C5-C6 double. Only the *cis*,-*syn* diastereoisomer is formed in double-stranded DNA. The structure of the corresponding TC, CT and CC CPD is also shown.

The nature of DNA damage is more diverse upon exposure to UVA. It should first be stressed that the overall amount of damage produced in this wavelength range at a given dose is approximately two orders of magnitude lower than with UVB (33, 32, 37, 35). Considering that the fluence of UVA is 20 times larger than that of UVB in sunlight, it may be estimated to be at the origin of a few percent of the overall load of DNA damage. It has been unambiguously shown by different assays that CPD, but not 64PP or Dewar, were efficiently produced by UVA in human cells and skin (37, 50-52). CPD is even now known to be the most frequent photodamage in the UVA range (53, 32, 37, 54). However, oxidatively generated lesions are also produced in significant amounts in this wavelength range (33, 55, 56). Oxidation reactions result from photosensitization processes mediated by endogenous chromophores, including porphyrins, flavins, pterins and NAD(P)H, present in most cell types

(57). Pure DNA was also found, in cell free systems, to induce oxidative processes (58-61), Different types of damage (Fig. 3) are produced by the different reactive oxygen species produced in cells (62, 30). Singlet oxygen specifically reacts in DNA by addition to guanine (30, 63, 64), which leads to the formation of a C8 hydroperoxide. The latter species is readily reduced into 8-hydroguanine, which evolves into its more stable 8-oxo-7,8-dihydroguanine (8-oxoGua) tautomer. No other products are produced in significant amounts by reaction of singlet oxygen with DNA. Strand breaks were observed at high dose in cell free systems but these breaks actually result from the formation of unstable secondary oxidation products of 8-oxoGua (65) such as guanidinohydantoin and spiroiminodihydantoin derivatives (64). Guanine is also the main target of those type I photosensitization processes that involve electron abstraction from DNA (30, 63, 66). Again, 8-oxoGua is the major product together with the ring open 2,6-diamino-4-hydroxy-5-formamidopyrimidine derivative (FapyGua). This process is yet a minor one in UVA-irradiated cells (64). Another oxidative damaging pathway involves the release of superoxide anions upon UVA irradiation. This species is the least reactive ROS and can diffuse in the cell. It may dismutate into hydrogen peroxide that is also moderately reactive with DNA. In contrast, metal-catalyzed Haber-Weiss chemistry produces hydroxyl radicals ($\cdot\text{OH}$) from H_2O_2 and these are highly reactive. It should be stressed that UVA was shown to induce release of iron ions within cells (67, 68), thereby facilitating this conversion of $\text{O}_2^{\cdot-}$ into $\cdot\text{OH}$. The $\cdot\text{OH}$ radical reacts with all four bases (30, 63, 69, 70). For example, it adds to the C8 position of purines, leading to the formation of 8-oxoGua, 8-oxo-7,8-hydroadenine (8-oxoAde), FapyGua and 4,6-diamino-5-formamidopyrimidine (FapyAde). $\cdot\text{OH}$ also adds to the C5-C6 double bond of pyrimidines, leading thus to lesions such as thymine glycols (ThyGly) and 5-hydroxycytosine (5-OHCyt). $\cdot\text{OH}$ abstracts hydrogen atoms

from the methyl group of thymine yielding 5-hydroxymethyl- and 5-formyluracil. Lastly, $\cdot\text{OH}$ is the only ROS that can damage deoxyribose moieties (71, 72). Hydrogen abstraction triggers degradation of the sugar units of DNA backbone and induces single strand breaks and abasic sites. Biochemical assays using purified glycosylases as probes have shown that oxidized purines, mostly 8-oxoGua, or other purine oxidation products are the most frequent oxidatively generated lesions in UVA-irradiated cells. The ratio between the yield of Fpg-sensitive sites and strand breaks was reported to be 7.2 in AS52 cells (33), 2.2 in CHO cells (32) and 2.1 in THP1 monocytes (56). The respective values for the ratios between the yields of Fpg- EndoIII-sensitive sites, which are mostly oxidized pyrimidines, were 4.8, 2.5, 6.3. In primary culture of normal human melanocytes, the ratio between Fpg-sensitive sites and strand breaks was found to be 1.7 (73). Ionizing radiations induce SSB in much larger yield than Fpg- and EndoIII-sensitive sites. Comparison of the distribution of oxidatively generated DNA lesions in cells exposed to either γ -radiation or UVA led to the conclusion of a major contribution of singlet oxygen to UVA-mediated oxidation of DNA, with a minor additional effect (20 %) of hydroxyl radicals produced from superoxide anions by Fenton chemistry (56). Using these pieces of information, it could be calculated that pyrimidine dimers, 8-oxoGua and single strand breaks are produced in a 100/0.23/0.08 ratio upon exposure to sunlight with 5% UVB and 95% UVA (74).

These data on oxidatively generated lesions, collected in isolated DNA and in various cell types, make it possible to understand the results reported in UVA-irradiated melanocytes (Fig. 3). First, the presence of melanin explains the larger yield of oxidatively generated lesions in UVA-irradiated melanocytes when compared to fibroblasts (75) and keratinocytes (73). It

should be stressed, though, that the yield of CPD remained larger than that of 8-oxoGua in UVA-irradiated melanocytes like in keratinocytes (73). As already mentioned, both singlet oxygen and superoxide anion are produced upon UVA irradiation of pheomelanin (25, 23, 24). This observation accounts for some results reported in melanocytes exposed to UVA. Formation of 8-oxoGua, which can be produced by these two types of ROS, was found to increase when the concentration of melanin increased in melanocytes (76). The same study showed a positive correlation between the ratio between pheomelanin and eumelanin contents, and the yield in 8-oxoGua. Free radicals produced from $O_2^{\cdot-}$, and in particular $\cdot OH$, were also shown to be involved in the photo-oxidative stress induced by melanin in melanocytes. The formation of single strand breaks, like that of 8-oxoGua, was found to increase when the melanin concentration increased and when the ratio between the content in pheomelanin and eumelanin increased (21, 22). It may be added that the basal level of oxidatively generated lesions in unexposed cells was also found to be higher in melanocytes than in keratinocytes, with 2.6 times more strand breaks and 2.5 times more Fpg-sensitive sites (73). This observation is in line with evidence of the induction of a significant oxidative stress associated with melanogenesis and tyrosinase activity (77-79).

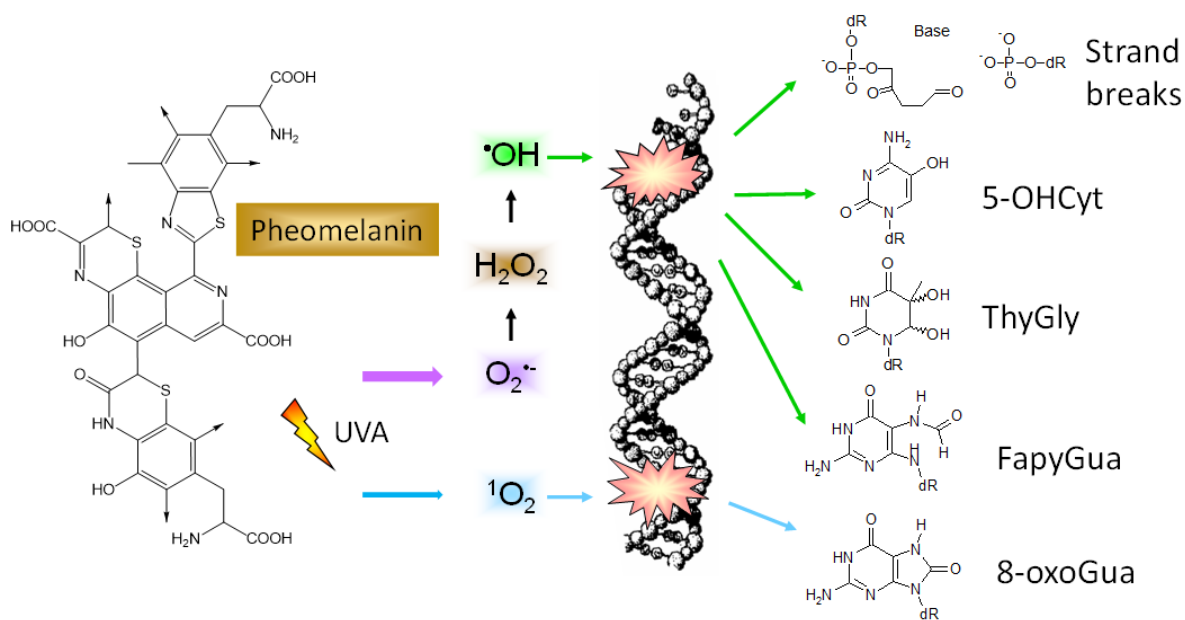


Figure 3. Oxidatively generated DNA lesions that may be produced by melanin-photosensitization. Singlet oxygen (1O_2) leads to the specific formation of 8-oxoGua. Superoxide anion are precursors of hydroxyl radicals that may damage all four DNA bases (2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 5-hydroxycytosine (5-OHCyt), thymine glycols (ThyGly), etc.) and induce strand breaks by degradation of the 2-deoxyribose moieties.

Another mechanism of UVA-induced DNA damage is related to CPD produced after the end of the irradiation and commonly referred to as "dark CPD". This unexpected phenomenon was first observed in cultured melanocytes, as well as in epidermal keratinocytes and melanocytes in pigmented mouse skin (80). Intriguingly, the process does take place neither in melanocytes from albino mice nor in murine fibroblasts. The relevance of this process to humans is suggested by the recent observation of a significant formation of these dark CPDs in the skin of volunteers exposed to low energy UVA1 at 385 nm (81). The underlying mechanism is associated with oxidative stress as shown by the inhibition of the formation of dark CPDs upon addition of antioxidant (80, 82). Although formation of dark CPD has been reported for cultured keratinocytes (82), the major role of melanin in the formation of dark CPD in

melanocytes was shown by their different yields in melanocytes from pigmented and albino mice (80). A tentative explanation is that UV-induced reactive oxygen and nitrogen species lead to the formation of some endoperoxides of melanin or melanin precursors, which may then decompose into excited carbonyls and induce CPDs by triplet-triplet energy transfer (80). It is likely that additional mechanisms are involved. They could explain the formation of dark CPD in cultured keratinocytes that do not contain melanin (82). It should be stressed that the presence of these dark CPDs is transient. Available data in cultured cells and skin shows that the peak of dark CPD is only observed during 4 or 5 hours at the most (80, 82). They are thus unlikely to contribute significantly to mutagenesis that requires DNA replication, and thus a longer persistence in the genome.

MUTAGENIC SIGNATURE IN MELANOMA

This short review on UV-induced DNA damage leads to the conclusion that no persistent difference is expected in the formation of pyrimidine dimers between melanocytes and other cell types. However, the presence of melanin, and in particular of pheomelanin, is unambiguously associated with the larger yield of oxidatively generated lesions in melanocytes. It may thus be proposed that the latter class of damage is involved in the initiation phase of the development of melanoma. The observation that production of free radicals in the skin of fish exposed to UVA exhibits the same wavelength-dependence as melanoma (83) made this hypothesis very attractive. A major role played by oxidatively generated DNA lesions in the onset of melanoma should be reflected by the mutational signature. The mutational properties of pyrimidine dimers are well known. It was shown, using

cells and animals transfected with damage-specific photolyases, that most mutations are induced by CPD (84, 85). 64PP were actually found to be more mutagenic than CPD in *in vitro* assays because they induce a larger distortion of the DNA structure (86-88). However, the latter property makes them more easily detected by DNA repair proteins (36, 89, 90), thereby limiting their contribution to mutagenesis. The hallmark of CPD in terms of mutation are TC to TT transitions. This mutational event results most likely from the deamination of cytosine residues into uracil in TC CPD (91-94). U in TU CPD codes like T and leads upon replication to the incorporation of A and mutation of the initial C:G into a T:A base pair. A similar molecular mechanism is at the origin of the induction of CC to TT tandem mutations (95). An additional mechanism, but not exclusive of deamination, could involve translesion polymerases. Cells possess a series of such DNA-polymerases that can bypass damage in an error-free manner. *In vitro* experiments, using mostly for TT photoproducts, have shown that pol η is responsible for this tolerance mechanism towards pyrimidine dimers, (96, 97). When this enzyme is lacking like in XP-variant cells patients (98), other polymerases such as pol ι are used but are error-prone when replicating CPD and induce mutations. Information is also available on the mutagenic properties of oxidatively generated lesions. It was reported that oxidation of guanine at the C8 position induces a rotation of the base along the N-glycosidic bond (99, 100). Consequently, the coding properties are modified and adenine rather than cytosine is incorporated opposite 8-oxoGua. The mutational signature of this damage is thus a G \rightarrow T transversion (101). FapyGua exhibits similar mutational properties (102). As far as pyrimidine oxidation products are concerned, thymine glycols were reported to be poorly mutagenic (103) while cytosine derivatives often induce C \rightarrow T transitions, mostly as the result of deamination (104).

The major role played by CPD in the mutational events leading to skin tumors was well documented for skin carcinomas (105). It was shown that most of the mutational hotspots in specific genes sequenced in tumors from patients exhibited the UV signature with C→T transitions at TC sites and CC→TT tandem mutations (106, 107, 105, 108, 109). This trend was found to be increased in xeroderma pigmentosum patients who lack proper repair of pyrimidine dimers (110, 111). The development of next generation sequencing techniques has made possible a much broader characterization of the mutational landscape in human cancers, and in particular of melanoma. A first observation, based on the complete sequencing of thousands of mutations, is that melanoma are among the human cancers with the largest number of mutations per tumor, in the range of 100 per Mbp (112). Other types of cancers like leukemia or pediatric cancers exhibit a 10-time lower load of mutations (113, 114, 64). This could be partly explained by the fact that sun exposed skin is constantly exposed to mutagenic UV and accumulates mutations. The same modern sequencing technologies made possible a more precise definition of mutational signatures. Sequencing of thousands of tumors corresponding to most types of cancers allowed the identification of 80 million somatic mutations (114, 115, 113). Statistical analysis led to the definitions of more than 50 specific signatures based on single base substitutions within all the possible triplets (available at <https://cancer.sanger.ac.uk/cosmic/signatures/>). The same consortium applied a similar approach to tandem mutations and small insertion and deletions. In addition to a few percent of signatures common to most cancers or of unknown origin, the most frequently detected signature in skin melanoma is signature 7 that corresponds to C→T transition at bipyrimidine sites. CC→TT tandem mutations are also unambiguously associated with melanomas by this approach. This signature is significantly detected in 85% of melanoma. The same mutational

signature is also present in all the skin carcinomas present in the database. These observations are a sign that the formation of pyrimidine dimers upon UVB absorption by DNA is a main cause of mutation in melanoma. The role of solar light in skin melanoma is confirmed by this type of approach since UV signature was found in cutaneous melanoma but not in acral and mucosal melanomas (116, 117). In that respect, mutations observed in skin melanoma are much more similar to those found in skin carcinomas (108, 109) than in acral melanomas (Fig. 4). The relevance of the UV signature to the melanoma disease was further suggested by the recent report that UVR-induced mutations in a set of ten genes can predict patient survival and melanomagenesis in mice (118). Another evidence that the UV-induced mutations are key to the onset of melanoma is that it is not only associated with passenger but also with driver mutations (117).

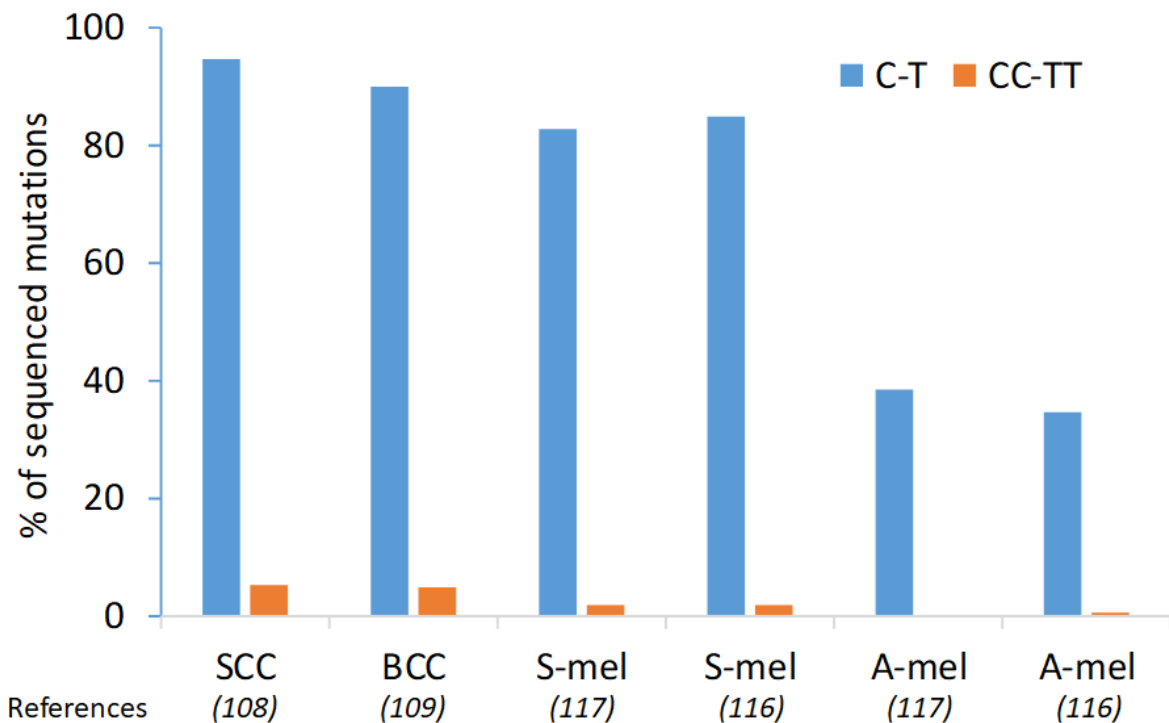


Figure 4. Comparison of the proportion of $C \rightarrow T$ and $CC \rightarrow TT$ mutation in the induction of substitution in different types of skin cancer (SCC: squamous cell carcinoma, BCC: basal cell

carcinoma, S-mel: skin melanoma, A-mel: acral melanoma). Data were obtained from whole-exome or whole-genome sequencing using next generation sequencing techniques. Data were taken from references (109), (108), (117) and (116), respectively.

The bulk of these data unambiguously show that pyrimidine dimers are the most deleterious form of DNA damage in terms of mutational consequences in the induction of melanoma. However, this overwhelming involvement of the UV mutation in melanoma is not enough to rule out a possible role for oxidatively generated lesions. The COSMIC database actually harbors signature 18 that is believed to be specific to oxidative stress (<https://cancer.sanger.ac.uk/cosmic/signatures/>). This signature is in particular dominated by G→T transversions associated with 8-oxoGua. It is thus interesting as a probe of the genotoxicity induced by ROS since, as discussed above, 8-oxoGua is the most frequent oxidatively generated DNA damage in UVA-irradiated cells. None of the reported mutational analyses based on the use of next generation sequencing approach was able to detect a significant association of skin melanoma with this signature 18 (114, 116). Oxidative mutagenesis is thus not significantly associated with melanoma. However, it should be kept in mind that the mutational events in the latter type of skin cancer are yet not limited to pyrimidine dimers. In contrast to skin carcinomas, melanomas also often harbor mutations in the *BRAF* gene. The most frequent one is the *BRAF*^{V600E} mutation, which correspond to an A→T transversion (6). This mutation does not take place at a bipyrimidine site and does not correspond to known oxidatively generated lesions. Other less frequent point mutations were identified in *BRAF* and their origin remain to be identified (119). The *BRAF* mutation, in particular *BRAF*^{V600E}, is actually quite common in internal cancers (120, 121). It seems thus likely that the frequent point mutations observed in *BRAF* in melanoma do not result from a

photo-induced process. However, determining the nature of a mutagen on the basis of mutation analysis is always challenging since even rare mutational events in very important genes like oncogenes may have drastic effects on tumor development (105). Interestingly, BRAF mutations are found in benign melanocytic nevi. It was thus proposed that BRAF mutation is necessary but not sufficient to induce melanoma and that UV-induced mutations actually trigger the tumorigenesis process (6).

DNA REPAIR AS A TARGET OF UVA-INDUCED OXIDATIVE STRESS IN MELANOCYTES?

We have so far seen that the presence of melanin makes the melanocytes more sensitive to photo-oxidative stress, especially in the UVA range. As a result, formation of oxidatively generated DNA damage is more efficient than in other skin cell types like keratinocytes. Yet, the presence of oxidatively generated lesions does not explain the mutations encountered in the tumors. In fact, the most frequently encountered mutational signature in melanoma is associated with UVB-induced pyrimidine dimers, like in skin carcinomas. Several explanations can be provided. First, the difference in the yield of UVA-induced oxidatively generated lesions between melanocytes and keratinocytes was only a factor of five (73). Exposure of melanocytes to actual sunlight, which contains UVB and UVA, would therefore lead to the same predominance of pyrimidine dimers observed in other cell types (32, 33). Further explanations of the predominance of the classical UV signature in melanoma are related to DNA repair. Oxidized bases like 8-oxoGua have a half-lifetime of a few hours (122) and single-strand breaks are repaired even faster (123). These features are quite different from the

rate of removal of CPD. More than 24 h are necessary to repair 50% of CPD in cultured cells (89, 90). The repair-time is even longer in skin (50). Another feature of DNA repair that could be related to the overwhelming contribution of pyrimidine dimers to the mutagenesis in melanocytes is a lower basal nucleotide excision repair activity than in keratinocytes, as inferred from a host cell reactivation assay (75). As discussed below, these results could be explained by a degradation of repair protein by the oxidizing species produced during melanogenesis (77, 78, 73, 79).

In addition to these specific features, increased photo-oxidative stress in melanocytes could link DNA repair, mutagenesis and melanoma. Indeed, growing evidence has been obtained for a decrease in repair capacities as the result of UVA-induced oxidative stress. It was first observed that the repair rate was significantly slower in primary cultures of human keratinocytes exposed UVA than UVB at respective doses leading to similar amounts of CDP (124). Similar observations were then reported for human skin explants (37). In addition, keratinocytes exposed to UVA prior to being irradiated with UVB removed pyrimidine dimers more slowly than cells exposed only to UVB (124). The same observation was recently made with cultured melanocytes (125). A possible explanation can be found in the fact that proteins, like DNA, are targets of oxidative stress. Proteins were found to be highly reactive with singlet oxygen (126, 127). Aromatic amino acids like tryptophan, tyrosine and histidine undergo by ring opening and rearrangement reactions. Cysteine and methionine are converted into sulfoxide derivatives. Similar oxidation reactions also take place with other reactive oxygen species like hydrogen peroxide and hydroxyl radical (128). Induction of oxidatively generated damage in protein results in fragmentation, cross-links, precipitation and enzymatic

inactivation. This efficient photochemistry explains why DNA repair proteins were shown to be the target of these photodegradation phenomena. For example, RPA (129, 130) or PCNA (131) are degraded by the ROS produced upon photo-oxidative stress, as observed in cultured cells exposed to UVA in the presence or the absence of added sensitizer. It should be mentioned that one study actually reported a faster repair after exposure to UVA than UVB (132). One explanation to the discrepancies with other works is that the level of CPD induced with UVB much larger than with UVA. This may hamper a quantitative comparison since the rate of repair was shown to depend on the initial level of CPD (133). The major impact of photo-oxidative stress on the DNA repair capacities (134) may be highly relevant to melanoma induction. Other proteins involved in NER were reported to be targets of other types of oxidative conditions (135-139). As already pointed out in a recent review (2), the presence of melanin would make the DNA repair machinery more sensitive to UVA-induced ROS in melanocytes than in other cell types and increase the lifetime of pyrimidine dimers in DNA, thereby increasing their risk of giving rise to mutations.

CONCLUSION

Melanocytes are more prone than other cutaneous cells to oxidative stress upon exposure to sunlight, in particular to the UVA portion of the spectrum. However, although the yield of oxidatively generated DNA damage is larger than in other cell types, the contribution of these lesions is not seen at the mutation level. It may yet be proposed that UVA indirectly increases the number of mutations induced by pyrimidine dimers by decreasing their repair rate. The scheme where UVB induces damage, and UVA-induces ROS that hampers their repair could

partly explain the role of UVA in the initiation step of the onset of melanoma (Fig. 5). Other important physiological responses, not related to genotoxicity, are obviously also involved in the UV-induction of melanoma (1-6). In particular, it has been proposed that UVA was involved in the promotion rather than in the initiation phase of melanoma (140). Furthermore, it should be reminded that oxidative stress could play other roles in the progression of cancer (141). For instance, its ability to promote cell survival through pathways like PTEN activation or to increase proliferation by the ERK pathway are major processes in the promotion step. UVA-induced oxidative stress could also be involved in the metastases of melanoma through the induction of metabolic changes (142). Altogether, it appears too restrictive to limit the role of oxidative stress in melanoma to the induction of DNA lesions that could be at the origin of mutations appears therefore highly restrictive. This conclusion is not limited to melanoma but is also true for various pathological conditions and exposure to carcinogens leading to oxidative stress.

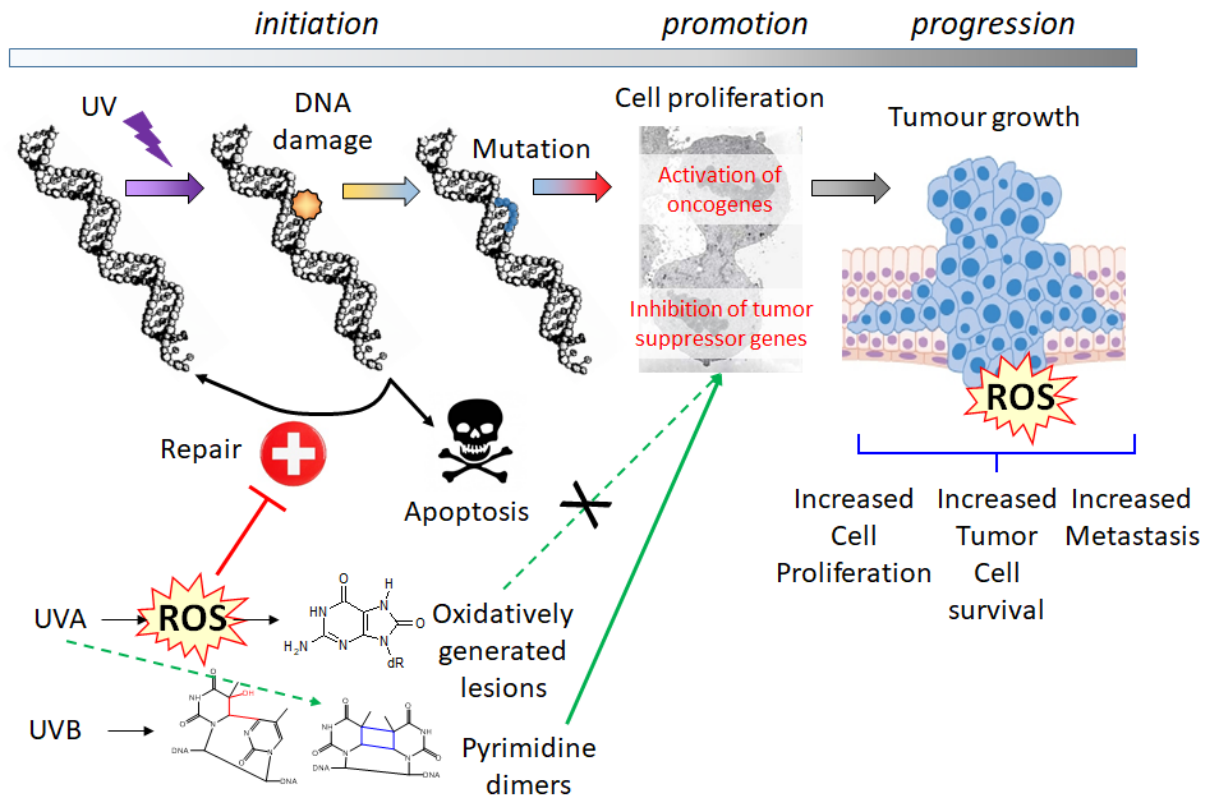


Figure 5: Role of formation and repair of DNA damage in melanoma. Like in other skin cells, pyrimidine dimers are the overwhelming DNA damage in melanocytes exposed to solar UV. UVA-induced reactive oxygen species induce oxidatively generated lesions in larger yield than in other cell types because of the oxidative stress mediated by pheomelanin. The mutations involved in the development of the tumor are due to pyrimidine dimers rather than oxidatively generated lesions. However, ROS may play an indirect role in melanoma-associated genotoxicity by decreasing the repair capacities of cells. In addition, oxidative stress play significant roles in the promotion and progression steps of cancer.

REFERENCES

1. Sample, A. and Y. Y. He (2018) Mechanisms and prevention of UV-induced melanoma. *Photodermatol. Photoimmunol. Photomed.* **34**, 13-24.
2. Khan, A. Q., J. B. Travers and M. G. Kemp (2018) Roles of UVA radiation and DNA damage responses in melanoma pathogenesis. *Environ. Molec. Mutagen.* **59**, 438-460.
3. Emri, G., G. Paragh, A. Tosaki, E. Janka, S. Kollar, C. Hegedus, E. Gellen, I. Horkay, G. Koncz and E. Remenyik (2018) Ultraviolet radiation-mediated development of cutaneous melanoma: An update. *J. Photochem. Photobiol. B-Biol.* **185**, 169-175.
4. Craig, S., C. H. Earnshaw and A. Viros (2018) Ultraviolet light and melanoma. *J. Pathol.* **244**, 578-585.
5. Arisi, M., C. Zane, S. Caravello, C. Rovati, A. Zanca, M. Venturini and P. Calzavara-Pinton (2018) Sun Exposure and Melanoma, Certainties and Weaknesses of the Present Knowledge. *Front Cell. Neurosci.* **12**.
6. Shain, A. H. and B. C. Bastian (2016) From melanocytes to melanomas. *Nature Rev. Cancer* **16**, 345-358.
7. Setlow, R. B., E. Grist, K. Thompson and A. D. Woodhead (1993) Wavelengths effective in induction of malignant melanoma. *Proc. Natl. Acad. Sci. USA* **90**, 6666-6670.
8. Ley, R. D. (2001) Dose response for ultraviolet radiation A-induced focal melanocytic hyperplasia and nonmelanoma skin tumors in *Monodelphis domestica*. *Photochem. Photobiol.* **73**, 20-23.
9. Mitchell, D. L., A. A. Fernandez, R. S. Nairn, R. Garcia, L. Paniker, D. Trono, H. D. Thames and I. Gimenez-Conti (2010) Ultraviolet A does not induce melanomas in a *Xiphophorus* hybrid fish model. *Proc. Natl. Acad. Sci. USA* **107**, 9329-9334.
10. Patton, E. E., D. L. Mitchell and R. S. Nairn (2010) Genetic and environmental melanoma models in fish. *Pigment Cell Melanoma Res.* **23**, 314-337.
11. De Fabo, E. C., F. P. Noonan, T. Fears and G. Merlino (2004) Ultraviolet B but not ultraviolet A radiation initiates melanoma. *Cancer Res.* **64**, 6372-6376.
12. Noonan, F. P., M. R. Zaidi, A. Wolnicka-Glubisz, M. R. Anver, J. Bahn, A. Wielgus, J. Cadet, T. Douki, S. Mouret, M. A. Tucker, A. Popratiloff, G. Merlino and E. C. De Fabo (2012) Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nature Comm.* **3**, 884.
13. Hore, T., E. Robinson and R. C. W. Martin (2010) Malignant Melanoma Amongst Maori and New Zealand Europeans, 2000-2004. *World Journal of Surgery* **34**, 1788-1792.
14. Norval, M., P. Kellett and C. Y. Wright (2014) The incidence and body site of skin cancers in the population groups of South Africa. *Photodermatol. Photoimmunol. Photomed.* **30**, 262-265.
15. Solano, F. (2016) Photoprotection versus photodamage: updating an old but still unsolved controversy about melanin. *Polym. Int.* **65**, 1276-1287.

16. Micillo, R., L. Panzella, K. Koike, G. Monfrecola, A. Napolitano and M. d'Ischia (2016) "Fifty Shades" of Black and Red or How Carboxyl Groups Fine Tune Eumelanin and Pheomelanin Properties. *Int. J. Molec. Sci.* **17**.
17. Simon, J. D., D. Peles, K. Wakamatsu and S. Ito (2009) Current challenges in understanding melanogenesis: bridging chemistry, biological control, morphology, and function. *Pigment Cell Melanoma Res.* **22**, 563-579.
18. Simon, J. D. and D. N. Peles (2010) The Red and the Black. *Accounts Chem. Res.* **43**, 1452-1460.
19. Meredith, P. and T. Sarna (2006) The physical and chemical properties of eumelanin. *Pigment Cell Res.* **19**, 572-594.
20. Panzella, L., L. Leone, G. Greco, G. Vitiello, G. D'Errico, A. Napolitano and M. d'Ischia (2014) Red human hair pheomelanin is a potent pro-oxidant mediating UV-independent contributory mechanisms of melanomagenesis. *Pigment Cell Melanoma Res* **27**, 244-252.
21. Wenczl, E., G. P. van der Schans, L. Roza, R. Kolb, N. Smit and A. A. Schothorst (1998) UVA-induced oxidative DNA damage in human melanocytes is related to their (pheo)melanin content. *J. Invest. Dermatol.* **110**, 693-693.
22. Wenczl, E., G. P. Van der Schans, L. Roza, R. M. Kolb, A. J. Timmerman, N. P. Smit, S. Pavel and A. A. Schothorst (1998) (Pheo)melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes. *J. Invest. Dermatol.* **111**, 678-682.
23. Chiarelli-Neto, O., A. S. Ferreira, W. K. Martins, C. Pavani, D. Severino, F. Faiao-Flores, S. S. Maria-Engler, E. Aliprandini, G. R. Martinez, P. Di Mascio, M. H. Medeiros and M. S. Baptista (2014) Melanin photosensitization and the effect of visible light on epithelial cells. *PLoS One* **9**, e113266.
24. Szewczyk, G., A. Zadlo, M. Sarna, S. Ito, K. Wakamatsu and T. Sarna (2016) Aerobic photoreactivity of synthetic eumelanins and pheomelanins: generation of singlet oxygen and superoxide anion. *Pigment Cell Melanoma Res.* **29**, 669-678.
25. Chedekel, M. R., P. P. Agin and R. M. Sayre (1980) Photochemistry of pheomelanin - Action spectrum for superoxide production. *Photochem. Photobiol.* **31**, 553-555.
26. Bastiaens, M. T., J. A. C. ter Huurne, C. Kielich, N. A. Gruis, R. G. J. Westendorp, B. J. Vermeer, N. J. B. Bavinck and L. S. C. S. Team (2001) Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair. *Am. J. Hum. Genet.* **68**, 884-894.
27. Swope, V. B. and Z. A. Abdel-Malek (2018) MC1R: Front and Center in the Bright Side of Dark Eumelanin and DNA Repair. *Int. J. Molec. Sci.* **19**.
28. Nguyen, N. T. and D. E. Fisher (2019) MITF and UV responses in skin: From pigmentation to addiction. *Pigment Cell Melanoma Res* **32**, 224-236.
29. Nasti, T. H. and L. Timares (2015) MC1R, Eumelanin and Pheomelanin: Their Role in Determining the Susceptibility to Skin Cancer. *Photochem. Photobiol.* **91**, 188-200.
30. Cadet, J. and T. Douki (2018) Formation of UV-induced DNA damage contributing to skin cancer development. *Photochem. Photobiol. Sci.* **17**, 1816-1841.
31. Markovitsi, D. (2016) UV-induced DNA damage: The role of electronic excited states. *Photochem. Photobiol.* **92**, 45-51.

32. Douki, T., A. Reynaud-Angelin, J. Cadet and E. Sage (2003) Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry* **42**, 9221-9226.
33. Kielbassa, C., L. Roza and B. Epe (1997) Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis* **18**, 811-816.
34. Kuluncsics, Z., D. Perdiz, E. Brulay, B. Muel and E. Sage (1999) Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct or indirect mechanisms and possible artefacts. *J. Photochem. Photobiol. B: Biol.* **49**, 71-80.
35. Perdiz, D., P. Grof, M. Mezzina, O. Nikaido, E. Moustacchi and E. Sage (2000) Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. Possible role of Dewar photoproducts in solar mutagenesis. *J. Biol. Chem.* **275**, 26732-26742.
36. Courdavault, S., C. Baudouin, S. Sauvaigo, S. Mouret, S. Candéias, M. Charveron, A. Favier, J. Cadet and T. Douki (2004) Unrepaired cyclobutane pyrimidine dimers do not prevent proliferation of UVB-irradiated cultured human fibroblasts. *Photochem. Photobiol.* **79**, 145-151.
37. Mouret, S., C. Baudouin, M. Charveron, A. Favier, J. Cadet and T. Douki (2006) Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc. Natl. Acad. Sci. USA* **103**, 13765-13770.
38. Pfeifer, G. P., R. Drouin and G. P. Holmquist (1993) Detection of DNA adducts at the DNA sequence level by ligation-mediated PCR. *Mutat. Res.* **288**, 39-46.
39. Bastien, N., J. P. Therrien and R. Drouin (2013) Cytosine containing dipyrimidine sites can be hotspots of cyclobutane pyrimidine dimer formation after UVB exposure. *Photochem. Photobiol. Sci.* **12**, 1544-1554.
40. Mao, P., M. J. Smerdon, S. A. Roberts and J. J. Wyrick (2016) Chromosomal landscape of UV damage formation and repair at single-nucleotide resolution. *Proc. Natl. Acad. Sci. USA* **113**, 9057-9062.
41. Mitchell, D. L., J. P. Allison and R. S. Nairn (1990) Immunoprecipitation of pyrimidine(6-4)pyrimidone photoproducts and cyclobutane pyrimidine dimers in UV-irradiated DNA. *Radiat. Res.* **123**, 299-303.
42. Douki, T. and E. Sage (2015) Dewar valence isomers, the third type of environmentally relevant DNA photoproducts induced by solar radiation. *Photochem. Photobiol. Sci.* **15**, 24-30.
43. Taylor, J.-S. and M. P. Cohrs (1987) DNA, light, and Dewar pyrimidones: the structure and biological significance of TpT3. *J. Am. Chem. Soc.* **109**, 2834-2835.
44. Chadwick, C. A., C. S. Potten, O. Nikaido, T. Matsunaga, C. Proby and A. R. Young (1995) The detection of cyclobutane thymine dimers, (6-4) photolesions and the Dewar valence photoisomers in sections of UV-irradiated human skin using specific antibodies, and the demonstration of depth penetration effects. *J. Photochem. Photobiol. B: Biol.* **28**, 163-170.
45. Young, A. R., C. S. Potten, O. Nikaido, P. G. Parsons, J. Boenders, J. M. Ramsden and C. A. Chadwick (1998) Human melanocytes and keratinocytes exposed to UVB or UVA *in vivo* show comparable levels of thymine dimers. *J. Invest. Dermatol.* **111**, 936-940.
46. Del Bino, S. and F. Bernerd (2013) Variations in skin colour and the biological consequences of ultraviolet radiation exposure. *Br. J. Dermatol.* **169**, 33-40.

47. Del Bino, S., J. Sok and F. Bernerd (2013) Assessment of ultraviolet-radiation-induced DNA damage within melanocytes in skin of different constitutive pigmentation. *Br. J. Dermatol.* **168**, 1120-1123.
48. Fajuyigbe, D., S. M. Lwin, B. L. Diffey, R. Baker, D. J. Tobin, R. P. E. Sarkany and A. R. Young (2018) Melanin distribution in human epidermis affords localized protection against DNA photodamage and concurs with skin cancer incidence difference in extreme phototypes. *FASEB J.*, fj201701472R.
49. Shih, B. B., M. D. Farrar, M. S. Cooke, J. Osman, A. K. Langton, R. Kift, A. R. Webb, J. L. Berry, R. E. B. Watson, A. Vail, F. R. de Gruijl and L. E. Rhodes (2018) Fractional Sunburn Threshold UVR Doses Generate Equivalent Vitamin D and DNA Damage in Skin Types I-VI but with Epidermal DNA Damage Gradient Correlated to Skin Darkness. *J. Invest. Dermatol.* **138**, 2244-2252.
50. Mouret, S., M. Charveron, A. Favier, J. Cadet and T. Douki (2008) Differential repair of UVB-induced cyclobutane pyrimidine dimers in cultured human skin cells and whole human skin. *DNA Repair* **7**, 704-712.
51. Bacqueville, D., T. Douki, L. Duprat, S. Rebelo-Moreira, B. Guiraud, H. Dromigny, V. Perier, S. Bessou-Touya and H. Duplan (2015) A new hair follicle-derived human epidermal model for the evaluation of sunscreen genoprotection. *J. Photochem. Photobiol. B: Biol.* **151**, 31-38.
52. Josse, G., T. Douki, J. Le Digabel and E. Gravier (2018) The use of suction blisters to measure sunscreen protection against UVR-induced DNA damage. *J. Photochem. Photobiol. B: Biol.* **179**, 1-6.
53. Courdavault, S., C. Baudouin, M. Charveron, A. Favier, J. Cadet and T. Douki (2004) Larger yield of cyclobutane dimers than 8 oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. *Mutat. Res.* **556**, 135-142.
54. Rochette, P. J., J.-P. Therrien, R. Drouin, D. Perdiz, N. Bastien, E. A. Drobetsky and E. Sage (2003) UVA-induced cyclobutane pyrimidine dimers form predominantly at thymine-thymine dipyrimidines and correlate with the mutation spectrum in rodent cells. *Nucleic Acids Res.* **31**, 2786-2794.
55. Cortat, B., C. C. M. Garcia, A. Quinet, A. P. Schuch, K. M. de Lima-Bessa and C. F. M. Menck (2013) The relative roles of DNA damage induced by UVA irradiation in human cells. *Photochem. Photobiol. Sci.* **12**, 1483-1495.
56. Pouget, J.-P., T. Douki, M.-J. Richard and J. Cadet (2000) DNA damage induced in cells by gamma and UVA radiations as measured by HPLC/GC-MS, HPLC-EC and comet assay. *Chem. Res. Toxicol.* **13**, 541-549.
57. Wondrak, G. T., M. K. Jacobson and E. L. Jacobson (2006) Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochem. Photobiol. Sci.* **5**, 215-237.
58. Mohammad, T. and H. Morrison (1996) Evidence for the photosensitized formation of singlet oxygen by UVB irradiation of 2'-deoxyguanosine 5'-monophosphate. *J. Am. Chem. Soc.* **118**, 1221-1222.
59. Yagura, T., A. P. Schuch, C. C. M. Garcia, C. R. R. Rocha, N. C. Moreno, J. P. F. Angeli, D. Mendes, D. Severino, A. B. Sanchez, P. Di Mascio, M. H. G. de Medeiros and C. F. M. Menck (2017) Direct participation of DNA in the formation of singlet oxygen and base damage under UVA irradiation. *Free Radic. Biol. Med.* **108**, 86-93.

60. Gomez-Mendoza, M., A. Banyasz, T. Douki, D. Markovitsi and J. L. Ravanat (2016) Direct oxidative damage of naked DNA generated upon absorption of UV radiation by nucleobases. *J. Phys. Chem. Lett.* **7**, 3945-3948.
61. Banyasz, A., L. Martinez-Fernandez, C. Balty, M. Perron, T. Douki, R. Improta and D. Markovitsi (2017) Absorption of low-energy uv radiation by human telomere G-quadruplexes generates long-lived guanine radical cations. *J. Am. Chem. Soc.* **139**, 10561-10568.
62. Cadet, J., T. Douki and J. L. Ravanat (2015) Oxidatively generated damage to cellular DNA by UVB and UVA radiation. *Photochem. Photobiol.* **91**, 140-155.
63. Cadet, J., A. Grand and T. Douki (2015) Solar UV radiation-induced DNA bipyrimidine photoproducts: Formation and mechanistic insights. In *Photoinduced Phenomena in Nucleic Acids II: DNA Fragments and Phenomenological Aspects*, Vol. 356. (Edited by M. Barbatti, A. C. Borin and S. Ullrich), pp. 249-275. Springer-Verlag Berlin, Berlin.
64. Di Mascio, P., G. R. Martinez, S. Miyamoto, G. E. Ronsein, M. H. G. Medeiros and J. Cadet (2019) Singlet Molecular Oxygen Reactions with Nucleic Acids, Lipids, and Proteins. *Chem. Rev.* **119**, 2043-2086.
65. Di Mascio, P., H. Wefers, H.-P. Do-Thy, M. V. Lafleur and H. Sies (1989) Singlet oxygen causes loss of biological activity in plasmid and bacteriophage DNA and induces single-strand breaks. *Biochim. Biophys. Acta* **1007**, 151-157.
66. Cadet, J., T. Douki and J. L. Ravanat (2008) Oxidatively generated damage to the guanine moiety of DNA: Mechanistic aspects and formation in cells. *Accounts Chem. Res.* **41**, 1075-1083.
67. Aroun, A., J. L. Zhong, R. M. Tyrrell and C. Pourzand (2012) Iron, oxidative stress and the example of solar ultraviolet A radiation. *Photochem. Photobiol. Sci.* **11**, 118-134.
68. Pourzand, C., R. D. Watkin, J. E. Brown and R. M. Tyrrell (1999) Ultraviolet A radiation induces immediate release of iron in human primary skin fibroblasts: The role of ferritin. *Proc. Natl. Acad. Sci. USA* **96**, 6751-6756.
69. Cadet, J., T. Douki and J. L. Ravanat (2010) Oxidatively generated base damage to cellular DNA. *Free Radic. Biol. Med.* **49**, 9-21.
70. Wagner, J. R. and J. Cadet (2010) Oxidation reactions of cytosine DNA components by hydroxyl radical and one-electron oxidants in aerated aqueous solutions. *Accounts Chem. Res.* **43**, 564-571.
71. Dedon, P. C. (2008) The chemical toxicology of 2-deoxyribose oxidation in DNA. *Chem. Res. Toxicol.* **21**, 206-219.
72. von Sonntag, C. (2006) *Free-radical-induced DNA damage and its repair*. Springer-Verlag, Berlin, Heidelberg.
73. Mouret, S., A. Forestier and T. Douki (2012) The specificity of UVA-induced DNA damage in human melanocytes. *Photochem. Photobiol. Sci.* **11**, 155-162.
74. Douki, T. (2016) Sunlight-induced DNA damage: molecular mechanisms and photoprotection strategies. In *Skin Stress Response Pathways: Environmental Factors and Molecular Opportunities*. (Edited by G. T. Wondrak), pp. 49-77. Springer Int. Publish.
75. Wang, H. T., B. Choi and M. S. Tang (2010) Melanocytes are deficient in repair of oxidative DNA damage and UV-induced photoproducts. *Proc. Natl. Acad. Sci. USA* **107**, 12180-12185.

76. Kvam, E. and R. M. Tyrrell (1999) The role of melanin in the induction of oxidative DNA base damage by ultraviolet A irradiation of DNA or melanoma cells. *J. Invest. Dermatol.* **113**, 209-213.
77. Denat, L., A. L. Kadekaro, L. Marrot, S. A. Leachman and Z. A. Abdel-Malek (2014) Melanocytes as Instigators and Victims of Oxidative Stress. *J. Invest. Dermatol.* **134**, 1512-1518.
78. Maresca, V., E. Flori, S. Briganti, A. Mastrofrancesco, C. Fabbri, A. M. Mileo, M. G. Paggi and M. Picardo (2008) Correlation between melanogenic and catalase activity in in vitro human melanocytes: a synergic strategy against oxidative stress. *Pigment Cell Melanoma Res.* **21**, 200-205.
79. Smit, N. P. M., F. A. Nieuwpoort, L. Marrot, C. Out, B. Poorthuis, H. van Pelt, J. R. Meunier and S. Pavel (2008) Increased melanogenesis is a risk factor for oxidative DNA damage - Study on cultured melanocytes and atypical nevus cells. *Photochem. Photobiol.* **84**, 550-555.
80. Premi, S., S. Wallisch, C. M. Mano, A. B. Weiner, A. Bacchiocchi, K. Wakamatsu, E. J. H. Bechara, R. Halaban, T. Douki and D. E. Brash (2015) Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science* **347**, 842-847.
81. Lawrence, K. P., T. Douki, R. P. E. Sarkany, S. Acker, B. Herzog and A. R. Young (2018) The UV/visible radiation boundary region (385 – 405 nm) damages skin cells and induces “dark” cyclobutane pyrimidine dimers in human skin *in vivo*. *Sci. Rep.* **8**, 12722.
82. Delinasios, G. J., M. Karbaschi, M. S. Cooke and A. R. Young (2018) Vitamin E inhibits the UVAI induction of "light" and "dark" cyclobutane pyrimidine dimers, and oxidatively generated DNA damage, in keratinocytes. *Sci. Rep.* **8**.
83. Wood, S. R., M. Berwick, R. D. Ley, R. B. Walter, R. B. Setlow and G. S. Timmins (2006) UV causation of melanoma in Xiphophorus is dominated by melanin photosensitized oxidant production. *Proc. Natl. Acad. Sci. USA* **103**, 4111-4115.
84. Jans, J., W. Schul, Y. G. Sert, Y. Rijksen, H. Rebel, A. P. Eker, S. Nakajima, H. van Steeg, F. R. de Gruijl, A. Yasui, J. H. Hoeijmakers and G. T. van der Horst (2005) Powerful skin cancer protection by a CPD-photolyase transgene. *Curr. Biol.* **15**, 105-115.
85. You, Y. H., D. H. Lee, J. H. Yoon, S. Nakajima, A. Yasui and G. P. Pfeifer (2001) Cyclobutane pyrimidine dimers are responsible for the vast majority of mutations induced by UVB irradiation in mammalian cells. *J. Biol. Chem.* **276**, 44688-44694.
86. Horsfall, M. J. and C. W. Lawrence (1993) Accuracy of replication past the T-C (6-4) adduct. *J. Mol. Biol.* **235**, 465-471.
87. LeClerc, J. E., A. Borden and C. W. Lawrence (1991) The thymine-thymine pyrimidine-pyrimidone (6-4) ultraviolet light photoproduct is highly mutagenic and specifically induces 3' thymine-to-cytosine transitions in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **88**, 9685-9689.
88. Smith, C. A., M. Wang, N. Jiang, L. Che, X. D. Zhao and J. S. Taylor (1996) Mutation spectra of M13 vectors containing site-specific cis-syn, trans-syn-I, (6-4), and dewar pyrimidone photoproducts of thymidylyl-(3'→5')-thymidine in *Escherichia coli* under SOS conditions. *Biochemistry* **35**, 4146-4154.

89. Mitchell, D. L., C. A. Haipek and J. M. Clarkson (1985) (6-4) Photoproducts are removed from the DNA of UV-irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. *Mutat. Res.* **143**, 109-112.
90. Young, A. R., C. A. Chadwick, G. I. Harrison, J. L. Hawk, O. Nikaido and C. S. Potten (1996) The in situ repair kinetics of epidermal thymine dimers and 6-4 photoproducts in human skin types I and II. *J. Invest. Dermatol.* **106**, 1307-1313.
91. Barak, Y., O. Cohen-fix and Z. Livneh (1995) Deamination of cytosine containing photodimers in UV-irradiated DNA. *J. Biol. Chem.* **270**, 24174-24179.
92. Horsfall, M. J., A. Borden and C. W. Lawrence (1997) Mutagenic properties of the T-C cyclobutane dimer. *J. Bacteriol.* **179**, 2835-2839.
93. Jiang, N. and J.-S. Taylor (1993) *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* **32**, 472-481.
94. Tessman, I., M. A. Kennedy and S. K. Liu (1994) Unusual kinetics of uracil formation in single and double -stranded DNA by deamination of cytosine in cyclobutane pyrimidine dimers. *J. Mol. Biol.* **235**, 807-812.
95. Gibbs, P. E. M. and C. W. Lawrence (1993) U-U and T-T cyclobutane dimers have different mutational properties. *Nucleic Acids Res.* **21**, 4059-4065.
96. Yoon, J. H., L. Prakash and S. Prakash (2009) Highly error-free role of DNA polymerase eta in the replicative bypass of UV-induced pyrimidine dimers in mouse and human cells. *Proc. Natl. Acad. Sci. USA* **106**, 18219-18224.
97. Yu, S. L., R. E. Johnson, S. Prakash and L. Prakash (2001) Requirement of DNA polymerase eta for error-free bypass of UV-induced CC and TC photoproducts. *Mol. Cell Biol.* **21**, 185-188.
98. Masutani, C., R. Kusumoto, A. Yamada, N. Dohmae, M. Yokoi, M. Yuasa, M. Araki, S. Iwai, K. Takio and F. Hanaoka (1999) The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* **399**, 700-704.
99. Kouchakdjian, M., V. Bodepudi, S. Shibutani, M. Eisenberg, F. Johnson, A. P. Grollman and D. J. Patel (1991) NMR structural studies of the ionizing-radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7h-dG) opposite deoxyadenosine in a DNA duplex - 8-oxo-7h-dG(syn).dA(anti) alignment at lesion site. *Biochemistry* **30**, 1403-1412.
100. McAuley-Hecht, K. E., G. A. Leonard, N. J. Gibson, J. B. Thomson, W. P. Watson, W. N. Hunter and T. Brown (1994) Crystal structure of a DNA duplex containing 8-hydroxydeoxyguanine-adenine base pairs. *Biochemistry* **33**, 10266-10270.
101. Shibutani, S., M. Takeshita and A. P. Grollman (1991) Insertion of specific bases during DNA synthesis past the oxidation- damaged base 8-oxodG. *Nature* **349**, 431-434.
102. Kalam, M. A., K. Haraguchi, S. Chandani, E. L. Loechler, M. Moriya, M. M. Greenberg and A. K. Basu (2006) Genetic effects of oxidative DNA damages: comparative mutagenesis of the imidazole ring-opened formamidopyrimidines (Fapy lesions) and 8-oxo-purines in simian kidney cells. *Nucleic Acids Res.* **34**, 2305-2315.
103. Basu, A. K., E. L. Loechler, S. E. Leadon and J. M. Essigman (1989) Genetic effects of thymine glycol: site specific mutagenesis and molecular modeling studies. *Proc. Natl. Acad. Sci. USA* **86**, 7677-7681.
104. Kreutzer, D. A. and J. M. Essigmann (1998) Oxidized, deaminated cytosines are a source of C -> T transitions in vivo. *Proc. Natl. Acad. Sci. USA* **95**, 3578-3582.

105. Brash, D. E. (2015) UV signature mutations. *Photochem. Photobiol.* **91**, 15-26.
106. Brash, D. E., J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna, H. P. Baden, A. J. Halperin and J. Ponten (1991) A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Natl. Acad. Sci. USA* **88**, 10124-10128.
107. Ziegler, A., D. J. Leffel, S. Kunala, H. W. Sharma, P. E. Shapiro, A. E. Bale and D. E. Brash (1993) Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. *Proc. Natl. Acad. Sci. USA* **90**, 4216-4220.
108. Durinck, S., C. Ho, N. J. Wang, W. Liao, L. R. Jakkula, E. A. Collisson, J. Pons, S. W. Chan, E. T. Lam, C. Chu, K. Park, S. W. Hong, J. S. Hur, N. Huh, I. M. Neuhaus, S. S. Yu, R. C. Grekin, T. M. Mauro, J. E. Cleaver, P. Y. Kwok, P. E. LeBoit, G. Getz, K. Cibulskis, J. C. Aster, H. Y. Huang, E. Purdom, J. Li, L. Bolund, S. T. Arron, J. W. Gray, P. T. Spellman and R. J. Cho (2011) Temporal Dissection of Tumorigenesis in Primary Cancers. *Cancer Discov.* **1**, 137-143.
109. Bonilla, X., L. Parmentier, B. King, F. Bezrukov, G. Kaya, V. Zoete, V. B. Seplyarskiy, H. J. Sharpe, T. McKee, A. Letourneau, P. G. Ribaux, K. Popadin, N. Basset-Seguin, R. Ben Chaabene, F. A. Santoni, M. A. Andrianova, M. Guipponi, M. Garieri, C. Verdan, K. Grosdemange, O. Sumara, M. Eilers, I. Aifantis, O. Michielin, F. J. de Sauvage, S. E. Antonarakis and S. I. Nikolaev (2016) Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nature Genet.* **48**, 398-+.
110. Dumaz, N., C. Drougard, A. Sarasin and A. L. Daya-Grosjean (1993) Specific UV-induced mutation spectrum in the p53 gene of skin tumors from DNA-repair-deficient xeroderma pigmentosum patients. *Proc. Natl. Acad. Sci. USA* **90**, 10529-10533.
111. Dumaz, N., H. J. van Kranen, A. de Vries, R. J. Berg, P. W. Wester, C. F. van Kreijl, A. Sarasin, L. Daya-Grosjean and F. R. de Gruijl (1997) The role of UV-B light in skin carcinogenesis through the analysis of p53 mutations in squamous cell carcinomas of hairless mice. *Carcinogenesis* **18**, 897-904.
112. Vogelstein, B., N. Papadopoulos, V. E. Velculescu, S. B. Zhou, L. A. Diaz and K. W. Kinzler (2013) Cancer Genome Landscapes. *Science* **339**, 1546-1558.
113. Alexandrov, L. B., J. Kim, N. J. Haradhvala, M. N. Huang, A. W. Tian Ng, Y. Wu, A. Boot, K. R. Covington, D. A. Gordenin, E. N. Bergstrom, S. M. A. Islam, N. Lopez-Bigas, L. J. Klimczak, J. R. McPherson, S. Morganella, R. Sabarinathan, D. A. Wheeler, V. Mustonen, P. M. S. W. Group, G. Getz, S. G. Rozen, M. R. Stratton and P. Consortium (2020) The repertoire of mutational signatures in human cancer. *Nature* **578**, 94-101.
114. Alexandrov, L. B., S. Nik-Zainal, D. C. Wedge, S. A. J. R. Aparicio, S. Behjati, A. V. Biankin, G. R. Bignell, N. Bolli, A. Borg, A. L. Borresen-Dale, S. Boyault, B. Burkhardt, A. P. Butler, C. Caldas, H. R. Davies, C. Desmedt, R. Eils, J. E. Eyfjord, J. A. Foekens, M. Greaves, F. Hosoda, B. Hutter, T. Ilicic, S. Imbeaud, M. Imielinski, N. Jager, D. T. W. Jones, D. Jones, S. Knappskog, M. Kool, S. R. Lakhani, C. Lopez-Otin, S. Martin, N. C. Munshi, H. Nakamura, P. A. Northcott, M. Pajic, E. Papaemmanuil, A. Paradiso, J. V. Pearson, X. S. Puente, K. Raine, M. Ramakrishna, A. L. Richardson, J. Richter, P. Rosenstiel, M. Schlesner, T. N. Schumacher, P. N. Span, J. W. Teague, Y. Totoki, A. N. J. Tutt, R. Valdes-Mas, M. M. van Buuren, L. van 't Veer, A. Vincent-Salomon, N. Waddell, L. R. Yates, J. Zucman-Rossi, P. A. Futreal, U. McDermott, P.

- Lichter, M. Meyerson, S. M. Grimmond, R. Siebert, E. Campo, T. Shibata, S. M. Pfister, P. J. Campbell, M. R. Stratton, A. P. C. Genome, I. B. C. Consortium, I. M.-S. Consortium and I. PedBrain (2013) Signatures of mutational processes in human cancer. *Nature* **500**, 415-421.
115. Alexandrov, L. B., S. Nik-Zainal, D. C. Wedge, P. J. Campbell and M. R. Stratton (2013) Deciphering Signatures of Mutational Processes Operative in Human Cancer. *Cell Rep.* **3**, 246-259.
116. Hayward, N. K., J. S. Wilmott, N. Waddell, P. A. Johansson, M. A. Field, K. Nones, A. M. Patch, H. Kakavand, L. B. Alexandrov, H. Burke, V. Jakrot, S. Kazakoff, O. Holmes, C. Leonard, R. Sabarinathan, L. Mularoni, S. Wood, Q. Y. Xu, N. Waddell, V. Tembe, G. M. Pupo, R. De Paoli-Iseppi, R. E. Vilain, P. Shang, L. M. S. Lau, R. A. Dagg, S. J. Schramm, A. Pritchard, K. Dutton-Regester, F. Newell, A. Fitzgerald, C. A. Shang, S. M. Grimmond, H. A. Pickett, J. Y. Yang, J. R. Stretch, A. Behren, R. F. Kefford, P. Hersey, G. V. Long, J. Cebon, M. Shackleton, A. J. Spillane, R. P. M. Saw, N. Lopez-Bigas, J. V. Pearson, J. F. Thompson, R. A. Scolyer and G. J. Mann (2017) Whole-genome landscapes of major melanoma subtypes. *Nature* **545**, 175-180.
117. Hodis, E., I. R. Watson, G. V. Kryukov, S. T. Arold, M. Imielinski, J. P. Theurillat, E. Nickerson, D. Auclair, L. R. Li, C. Place, D. DiCara, A. H. Ramos, M. S. Lawrence, K. Cibulskis, A. Sivachenko, D. Voet, G. Saksena, N. Stransky, R. C. Onofrio, W. Winckler, K. Ardlie, N. Wagle, J. Wargo, K. Chong, D. L. Morton, K. Stemke-Hale, G. Chen, M. Noble, M. Meyerson, J. E. Ladbury, M. A. Davies, J. E. Gershenwald, S. N. Wagner, D. S. B. Hoon, D. Schadendorf, E. S. Lander, S. B. Gabriel, G. Getz, L. A. Garraway and L. Chin (2012) A Landscape of Driver Mutations in Melanoma. *Cell* **150**, 251-263.
118. Trucco, L. D., P. A. Mundra, K. Hogan, P. Garcia-Martinez, A. Viros, A. K. Mandal, N. Macagno, C. Gaudy-Marqueste, D. Allan, F. Baenke, M. Cook, C. McManus, B. Sanchez-Laorden, N. Dhomen and R. Marais (2019) Ultraviolet radiation-induced DNA damage is prognostic for outcome in melanoma (vol 25, pg 221, 2018). *Nature Med.* **25**, 350-350.
119. Richtig, G., C. Hoeller, K. Kashofer, A. Aigelsreiter, A. Heinemann, L. N. Kwong, M. Pichler and E. Richtig (2017) Beyond the BRAF(V600E) hotspot: biology and clinical implications of rare BRAF gene mutations in melanoma patients. *Br. J. Dermatol.* **177**, 936-944.
120. Cantwell-Dorris, E. R., J. J. O'Leary and O. M. Sheils (2011) BRAFV600E: implications for carcinogenesis and molecular therapy. *Mol. Cancer Ther.* **10**, 385-394.
121. Davies, H., G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M. J. Garnett, W. Bottomley, N. Davis, N. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, R. Wilson, H. Jayatilake, B. A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G. J. Riggins, D. D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J. W. C. Ho, S. Y. Leung, S. T. Yuen, B. L. Weber, H. F. Siegler, T. L. Darrow, H. Paterson, R. Marais, C. J. Marshall, R. Wooster, M. R. Stratton and P. A. Futreal (2002) Mutations of the BRAF gene in human cancer. *Nature* **417**, 949-954.

122. Klungland, A., I. Rosewell, S. Hollenbach, E. Larsen, G. Daly, B. Epe, E. Seeberg, T. Lindahl and D. E. Barnes (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl. Acad. Sci. USA* **96**, 13300-13305.
123. Collins, A. R., A. G. Ma and S. J. Duthie (1995) The Kinetics of Repair of Oxidative DNA-Damage (Strand Breaks and Oxidized Pyrimidines) in Human-Cells. *Mutat Res-DNA Repair* **336**, 69-77.
124. Courdavault, S., C. Baudouin, M. Charveron, B. Canghilem, A. Favier, J. Cadet and T. Douki (2005) Repair of the three main types of bipyrimidine DNA photoproducts in human keratinocytes exposed to UVB and UVA radiations. *DNA Repair* **4**, 836-844.
125. Kimeswenger, S., R. Dingelmaier-Hovorka, D. Foedinger and C. Jantschitsch (2018) UVA1 impairs the repair of UVB-induced DNA damage in normal human melanocytes. *Exp. Dermatol.* **27**, 276-279.
126. Davies, M. J. (2003) Singlet oxygen-mediated damage to proteins and its consequences. *Biochem. Biophys. Res. Com.* **305**, 761-770.
127. Pattison, D. I., A. S. Rahmanto and M. J. Davies (2012) Photo-oxidation of proteins. *Photochem. Photobiol. Sci.* **11**, 38-53.
128. Davies, M. J. (2016) Protein oxidation and peroxidation. *Biochem. J.* **473**, 805-825.
129. Gueranger, Q., F. Li, M. Peacock, A. Larnicol-Fery, R. Brem, P. Macpherson, J. M. Egly and P. Karran (2014) Protein Oxidation and DNA Repair Inhibition by 6-Thioguanine and UVA Radiation. *J. Invest. Dermatol.* **134**, 1408-1417.
130. Guven, M., R. Brem, P. Macpherson, M. Peacock and P. Karran (2015) Oxidative Damage to RPA Limits the Nucleotide Excision Repair Capacity of Human Cells. *J. Invest. Dermatol.* **135**, 2834-2841.
131. Montaner, B., P. O'Donovan, O. Reelfs, C. M. Perrett, X. Zhang, Y. Z. Xu, X. Ren, P. Macpherson, D. Frith and P. Karran (2007) Reactive oxygen-mediated damage to a human DNA replication and repair protein. *EMBO Rep.* **8**, 1074-1079.
132. Karbaschi, M., S. Macip, V. Mistry, H. H. K. Abbas, G. J. Delinassios, M. D. Evans, A. R. Young and M. S. Cooke (2015) Rescue of cells from apoptosis increases DNA repair in UVB exposed cells: implications for the DNA damage response. *Toxicol. Res.* **4**, 725-738.
133. Greinert, R., O. Boguhn, D. Harder, E. W. Breitbart, D. L. Mitchell and B. Volkmer (2000) The dose dependence of cyclobutane dimer induction and repair in UVB-irradiated human keratinocytes. *Photochem. Photobiol.* **72**, 701-708.
134. Karran, P. and R. Brem (2016) Protein oxidation, UVA and human DNA repair. *DNA Repair* **44**, 178-185.
135. Grosskopf, C., T. Schwerdtle, L. H. F. Mullenders and A. Hartwig (2010) Antimony Impairs Nucleotide Excision Repair: XPA and XPE as Potential Molecular Targets. *Chem. Res. Toxicol.* **23**, 1175-1183.
136. Men, L. J., M. Roginskaya, Y. Zou and Y. S. Wang (2007) Redox-dependent formation of disulfide bonds in human replication protein A. *Rapid Commun. Mass Spectrom.* **21**, 2743-2749.
137. You, J. S., M. Wang and S. H. Lee (2000) Functional characterization of zinc-finger motif in redox regulation of RPA-ssDNA interaction. *Biochemistry* **39**, 12953-12958.

138. Zhou, X. X., X. Sun, K. L. Cooper, F. Wang, K. J. Liu and L. G. Hudson (2011) Arsenite Interacts Selectively with Zinc Finger Proteins Containing C3H1 or C4 Motifs. *J. Biol. Chem.* **286**, 22855-22863.
139. Zhou, X. X., K. L. Cooper, X. Sun, K. J. Liu and L. G. Hudson (2015) Selective Sensitization of Zinc Finger Protein Oxidation by Reactive Oxygen Species through Arsenic Binding. *J. Biol. Chem.* **290**, 18361-18369.
140. Mitchell, D. and A. Fernandez (2012) The photobiology of melanocytes modulates the impact of UVA on sunlight-induced melanoma. *Photochem. Photobiol. Sci.* **11**, 69-73.
141. Moloney, J. N. and T. G. Cotter (2018) ROS signalling in the biology of cancer. *Semin. Cell Dev. Biol.* **80**, 50-64.
142. Kamenisch, Y., I. Ivanova, K. Drexler and M. Berneburg (2018) UVA, metabolism and melanoma: UVA makes melanoma hungry for metastasis. *Exp. Dermatol.* **27**, 941-949.