Photocarcinogenesis – DNA Damage and Gene Mutations

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Ultraviolet Radiation, Skin Cancer, and Immunosuppressants

Ultraviolet (UV) radiation in sunlight is cytotoxic and, in overdosages, clearly detrimental to the skin, as becomes manifest in common sunburn reactions in which epidermal cells die in apoptosis ("sunburn cells") and strong inflammation occurs (vasodilation, extravasation, and infiltrates of leukocytes), turning the skin red (erythema) and swollen (edema). In excessive cases the skin ends up peeling, or it may even develop blisters. Fair-skinned people are clearly most susceptible to these sunburn reactions. Although these dramatic reactions may leave a different impression, it appears that the skin is quite well adapted to the persistent UV challenge in its natural environment – even the excessive reactions may be considered part of a formidable adaptation.

The skin is well equipped to repair the damage inflicted by solar UV irradiation, even damage from excessive exposures. Nevertheless, the repair mechanisms are not perfect, and in the course of time errors may creep in, most specifically, in the repair of the genome of skin cells. An accumulation of such errors in the genetic code may eventually give rise to skin cancer.

Squamous cell carcinomas (SCCs) have been associated with cumulative sun (UV) exposure, but the risk of basal cell carcinomas and cutaneous malignant melanomas shows a most significant increase with intermittent overexposures (i.e., episodes of severe sunburn). Interestingly, it is the risk of SCC that is most strongly increased in organ transplant recipients (OTRs). This risk has been considered an inevitable collateral effect of the immunosuppressive treatment, because animal experiments had shown that skin tumors induced by (chronic) UV exposure were highly antigenic and subject to immunosurveillance and elimination [1]. However, in the late 1980s it became clear that conventional immunosuppressive drugs also adversely affected DNA repair in the skin, which could contribute to the enhanced UV carcinogenesis observed with these drugs [2]. This latter finding did not seem to receive much attention at first, but it now comes into the limelight with the arrival of

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a new generation of immunosuppressants that differ in the mode of action from the earlier generation. If local adverse side effects of immunosuppressants in the skin can be minimized while maintaining adequate immunosuppression, the risk of SCC in OTRs may substantially decrease.

In the following sections, we discuss the UV-related steps in carcinogenesis in skin cells and the potential impact of immunosuppressants on these steps, as schematically represented in Fig. 1.

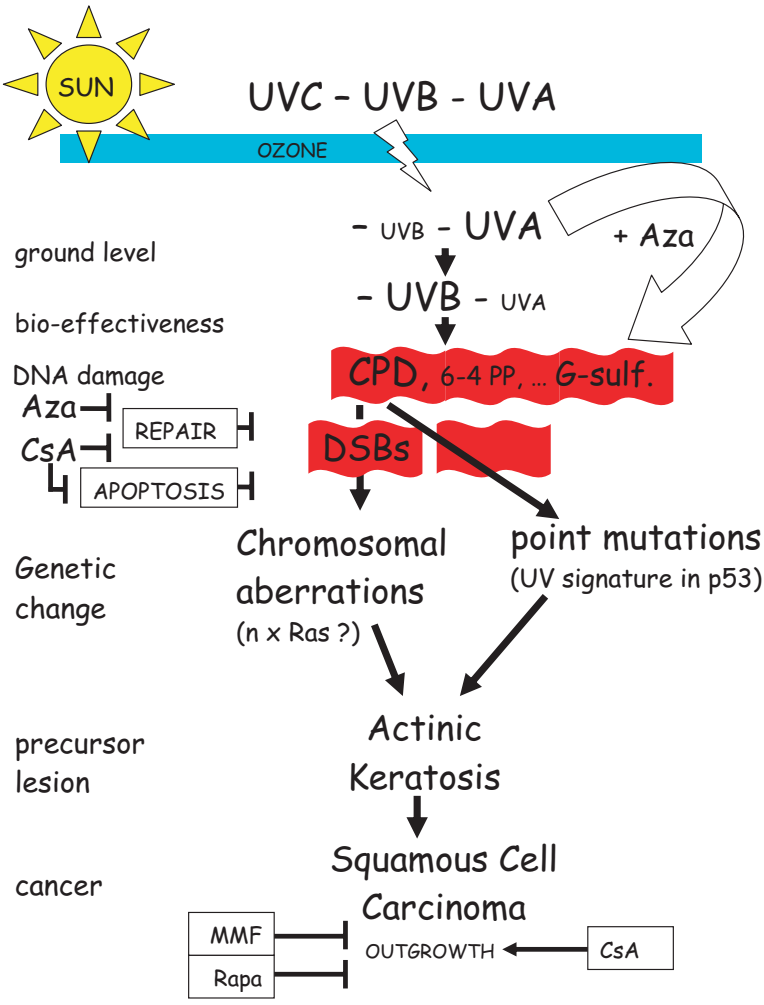

Fig. 1 Scheme of steps in ultraviolet (UV) carcinogenesis, and effects of azathioprine (*Aza*), cyclosporin A (*CsA*), mycophenolate mofetil (*MMF*), and rapamycin (*Rapa*)

UV Radiation and DNA Damage

The shorter the wavelength of UV radiation, the more energy each photon carries. The shortest wavelengths emitted by the sun, in the UV-C band (wavelengths <280 nm), are generally most damaging to organic molecules (specifically those with conjugated bonds and aromatic rings), but this radiation does not reach the Earth's surface. UV-C radiation and a part of the UV-B (wavelengths 280–315 nm) radiation are absorbed in the stratospheric ozone layer. Most of the UV-A radiation (wavelengths 315–400 nm; bordering the visible spectrum, 400–780 nm) passes through the atmosphere on a clear day and comprises more than 95% of the solar radiant UV energy at ground level. The minor fraction of UV-B radiation is, however, largely responsible for the sunburn reaction of the skin and, most likely, also for skin carcinogenicity (i.e., >80% of the effective UV dose stems from the UV-B wavelength band), as both effects appear to be related to the UV-induced DNA damage in the skin [3].

UV-B radiation is absorbed by DNA (owing to an abundance of aromatic rings in its bases), and dimeric lesions are formed at neighboring pyrimidine bases, mostly cyclobutane pyrimidine dimers (CPDs) and, to a lesser extent, 6–4 photoproducts (6–4PPs) in a ratio of about 4:1. The effectiveness of inducing CPDs appears to extend into the UV-A band (especially at wavelengths bordering UV-B) [3, 4], but that of inducing 6–4PPs does not [5]. Although indirect DNA base damage (e.g., 8-oxo-guanosine) from reactive oxygen species becomes relatively more important going from short UV-B to longer UV-A wavelengths [4], the dominant DNA lesion induced by broadband UV-A radiation in the skin is the CPD [5]. CPDs in turn can give rise to frank double-strand breaks (DSBs) in the DNA during replication in S phase [6].

UV-A radiation is far less effective in causing DNA damage than UV-B radiation, but the conventional immunosuppressant azathioprine was found to sensitize the DNA for UV-A radiation [7, 8]. Through the purine synthesis pathway, azathioprine is incorporated in the DNA as a 6-thioguanine pseudo-base, which causes UV-A sensitization and subsequent oxidation to form a guanine sulfonate. Thus, azathioprine enhances DNA damage and increases skin sensitivity to sunburn from UV-A radiation [8].

DNA Repair, Cell-Cycle Arrest, and Apoptosis

CPDs and 6–4PPs (and guanine sulfonates) block transcription and replication of DNA. Hence, the cell needs to repair these lesions to remain functional and viable and to be able to replicate its DNA without errors. Nucleotide excision repair (NER) is primarily responsible for the repair of these lesions. NER entails a cut-and-paste type of mechanism that involves a multitude of enzymes to recognize and cut out an oligomer containing the lesion and to fill in the gap in the DNA strand. There are two distinct pathways of NER: global genome (GG-) NER, operating

on helix-distorting lesions throughout the genome, and transcription-coupled (TC-) NER, that efficiently removes lesions that stall RNA polymerase in transcription. Defects in TC-NER greatly enhance the acute (sunburn) sensitivity to UV radiation, whereas a defect in GG-NER does not. The latter does, however, greatly increase mutagenesis and carcinogenesis, as seen in xeroderma pigmentosum (XP) patients who lack GG-NER [9]. If CPDs and 6–4PPs are not adequately removed from transcribed DNA strands, the cell is more sensitive to cell-cycle arrest and apoptosis. The arrest allows for more time to repair, and apoptosis will kill a cell that may otherwise replicate with an overly damaged genome that may give rise to errors in replication, that is, mutations in genes. If only GG-NER is defective (as in XP-C patients), the alarm signals for cell-cycle arrest and apoptosis will not be enhanced because of the still proficient TC-NER, and the cell may therefore enter replication with damage remaining in its nontranscribed DNA. Replication of this damaged DNA is bound to raise mutagenesis. If not strictly avoiding UV (solar) exposure, XP patients with severe impairment of GG-NER contract multiple skin cancers in childhood and succumb to these cancers before reaching the age of 20 years.

As the conventional immunosuppressants cyclosporin A and azathioprine were found to adversely affect NER [2,10], they can increase UV mutagenesis in the skin, and thus increase the risk of skin carcinomas, SCC in particular [11]. Cyclosporin A also appears to hamper apoptotic responses, which may further enhance UV mutagenesis and carcinogenesis [10]. As discussed earlier, azathioprine leads to thioguanine pseudo-bases in DNA. These pseudo-bases are substrates of mismatch repair (MMR; correcting mismatches between two complementary DNA strands). Through survival and growth advantages, thioguanine bases can introduce a selection pressure for loss of MMR [12]. If this mechanism occurs in the skin of OTRs on azathioprine, the skin carcinoma risk may further increase because MMR also operates on UV-induced DNA damage.

Certain variants (polymorphisms) of genes coding for proteins (XRCC2, XRCC3, ligase IV) involved in the repair of DSBs were found to increase the risk of skin carcinomas [13]. These results agree nicely with the recent finding that people who had had skin carcinomas removed showed an increased susceptibility to DSB induction in their UV-irradiated leukocytes [14].

No data exist on whether novel immunosuppressants, such as rapamycin (Rapa) or mycophenolate Mofetil (MMF), affect DNA repair or apoptosis. Although MMF does not lead to pseudo-bases, its inhibitory effect on the purine synthesis pathway could perhaps have repercussions on filling the DNA gap in NER. The inhibitory effect of Rapa on mTOR in the Akt ("survival") pathway may deregulate cell-cycle control and enhance apoptosis, as found in p53-null cells [15].

UV Radiation and Genetic Alterations

The UV-induced DNA damage may lead to changes in the genome, ranging from subtle point mutations (single base changes) to gross chromosomal aberrations, and even the formation of "micronuclei" (small satellites to the main nuclei). As

mentioned in the previous section, the S phase in the cell cycle appears to be most critical for acquiring these genomic alterations: Damage encountered in template DNA generally poses an obstacle to the replication machinery, which can then mobilize alternative pathways to sidestep the problem, either by error-prone replication over a noninstructive damaged base (lesional bypass) or by pulling in the DNA strands from the other parental allele of the gene to be copied and using these undamaged strands as templates (homologous recombination). The lesional bypass may give rise to point mutations, and the recombination repair may enhance sister chromatid exchange, or may even lead to loss or duplication of chromosome fragments. If the damage is not adequately circumvented, DSBs may occur at stalled replication forks with "less than perfect" patch-ups (e.g., nonhomologous end-joining) which further enhance the risk of gross chromosomal aberrations. Coding errors can be detected and repaired post hoc by comparing complementary strands in MMR.

The UV-induced pyrimidine dimers (CPDs and 6–4PPs) characteristically lead to cytosine (C) to thymine (T) single base changes at dipyrimidine sites (about 70% or more of mutations), and even to CC-to-TT tandem mutations (about 10%) [16]; these are considered UV-signature mutations. Other genetic changes, such as caused by oxidative damage or DSBs, can be induced by many genotoxic agents and are therefore not UV specific.

Genetic Defects in Skin Carcinomas and Precursor Lesions

Skin carcinomas carry UV-signature mutations in the gene that codes for p53 tumor suppressor protein [17]. The benign precursor lesions, actinic keratoses (AKs), of SCCs already show these mutations, and even before any macroscopic lesion is visible, microscopic clusters of cells (clones) appear that overexpress mutant-p53. A close and most likely causal relationship between these early mutant p53 foci and eventual SCCs has been established in experiments with hairless mice [18]. In contrast to SCCs, the mutant p53 foci do not appear to be subject to immunological surveillance and elimination [19], but they do occur in higher frequency in OTRs than in immunocompetent individuals (ICIs) in normal skin neighboring SCCs [De Graaf et al., manuscript in preparation]. Hence, the increase in mutant p53 foci in OTRs is likely to result from local adverse effects of immunosuppressants (viz. azathioprine and cyclosporin A) on the skin cells, and this in turn is likely to contribute to the SCC risk independently of immunosuppression per se.

Similar to the *p53* mutations found in skin carcinomas from ICIs, those in carcinomas from OTRs show the UV signature [20]. In contrast to this earlier study in which no CC-to-TT mutations were found, a recent study on 25 skin carcinomas from 20 OTRs found a rather high percentage (35%) of these tandem mutations among the *p53* mutations [21], reminiscent of what is observed in XPC patients who specifically lack GG-NER. The early study may well have included more OTRs with long-term azathioprine treatment, whereas the OTRs in the more recent study

may have been treated more with cyclosporin A. The authors of the recent study speculate that the increased percentage in tandem mutations that they observed is attributable to a slow repair in combination with a repressed apoptosis, as is caused by cyclosporin A. In the *p53* mutation spectrum they found no deviations that may have arisen from azathioprine-derived photosensitization. However, the total number of mutations studied ($n = 24$) may have been too low to establish this firmly. No apparent effort was made to correlate *p53* mutations to specific immunosuppressive treatments in either study, but the numbers of OTRs were rather small for such analyses.

SCCs and AKs in ICIs carry many chromosomal aberrations [22]. An amplification of the *H-RAS* oncogene has been reported for SCCs from OTRs [23]; that is, multiple copies of the gene are present in the cells of SCCs. This is a chromosomal aberration (repeats of chromosomal fragments). Considering the effects of azathioprine and calcineurin inhibitors such as cyclosporin A, the risk of UV-induced DSBs and chromosomal aberrations may well also be elevated in OTRs.

Tumor Outgrowth

Cyclosporin A has been found to enhance tumor growth and metastases by cellular changes related to increased expression of TGF-β, independent of its immunosuppressive effect [24]. In contrast to the conventional immunosuppressants, the novel drugs mycophenolate mofetil and rapamycin have antitumor effects [25], and rapamycin has been experimentally proven to inhibit angiogenesis and tumor growth while providing adequate immunosuppression to maintain an allograft [26]. Results from our group and the group of Dr. A. Vanbuskirk [manuscripts in preparation] show net inhibitory effects of rapamycin on UV carcinogenesis, specifically on the development of larger skin tumors.

Conclusions

With the successful long-term retention of organ transplantations, adverse side effects, such as an enhanced rate of skin carcinoma development, become more and more pronounced. The traditional notion that the immunosuppressive regimen per se necessarily results in the collateral formation of skin carcinomas is in need of revision: The conventional immunosuppressants, azathioprine and cyclosporine, exert adverse effects on DNA repair and apoptosis in skin cells, which may importantly contribute to the risk of skin carcinomas. A novel generation of immunosuppressants, most notably rapamycin, differs in mode of action from the conventional drugs, and exerts antitumor effects while maintaining adequate immunosuppression for organ transplantation. Although the effects of these novel drugs on UV-induced skin carcinogenesis are not fully studied, the first experimental results hold great

promise for lowering substantially the risk of skin carcinomas in OTRs. Some recent clinical reports appear to point in the same direction [27, 28].

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