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REPAIR, ABORT, IGNORE? STRATEGIES FOR DEALING WITH UV DAMAGE

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ABSTRACT

DNA repair is a prominent member of the nuclear transactions triad (replication, transcription, and repair). Sophisticated mechanisms govern the cellular process of decision-making (to repair or not to repair, to proceed with cell cycle or not and, eventually, to let the cell survive or die) and the temporal and spatial distribution of the DNA repair activities. UV radiation is a very common and virtually unavoidable mutagen whose carcinogenic potential seems to accumulate over time. Various strategies have been developed to avoid or decrease UV damage to cellular DNA, based on prevention of exposure as well as on post-irradiation measures. It is, however, important to acknowledge that the individual capacity for DNA repair varies during the life of the individual and must, therefore, be assessed so as to determine whether the individual is coping with environmental UV damage. Assessment of individual repair capacity might greatly modify the existing therapeutic strategies for common cancers and ought to become a routine part of health prophylaxis.

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UV damage basics

Ultraviolet radiation (UV) is electromagnetic energy with wavelength between 10 and 400 nm, generally invisible to the human eye. It is a very common and virtually unavoidable environmental mutagen which is also produced by a number of machines, appliances and artificial light sources, even by LEDs in some devices. The individual received UV dose may greatly increase through light therapy for a number of human diseases and conditions, such as psoriasis, acne vulgaris, cutaneous T-cell leukemia, some skin cancers, etc. The ozone layer of the atmosphere blocks about 97-99% of the sun radiation, predominantly the short-wavelength UV, that is, practically all of the very short wavelength (<280 nm, UV-C) and the major part of the short-wavelength UV (280-320 nm, UV-B). The prevailing amount (about 95%) of solar UV radiation that reaches Earth surface is long wavelength (UV-A, 320-400 nm).

The depth of penetration of UV into human skin is dependent on the wavelength of the UV. Generally, UV-B radiation penetrates more deeply than UV-A as the latter is easily absorbed by the uppermost keratinized skin layers (23, 27). The most common reaction to chronic UV exposure is darkening of the skin, commonly known as tanning. UV-A produces immediate tanning that fades within days, while delayed tanning occurs 2-3 days after exposure to UV-A or UV-B and may last for several weeks to months. It is believed that UVA-induced tanning results from photooxidation of melanin coupled with redistribution of pigment granules, while UVB actually causes up-regulation of the melanin synthesis. UV is also infamously known to accelerate skin aging and promote neoplastic transformation of skin cells, causing basal skin cancers, squamous cancers and melanoma. This is directly dependent on the mutagenic capacity of UV irradiation but is also enhanced by the immunosuppressive properties of UV, and especially of UV-B (26, 38). UV wavelength is also related to the type of skin cancer, at least in animal models, with two peaks for squamous cancer around 293 and 354 nm, respectively, while melanoma is usually related to exposure to UV-B wavelengths of 290 to 320 nm (17). This may explain the relative increased prevalence of the squamous cancer occurrence when compared to melanoma.

UV causes various types of damage to living cells affecting both cellular DNA and proteins. Acute damage to the cell by prolonged or intensive UV exposure may cause irreversible damage to key cell components and may, therefore, bring about quick keratinocyte necrosis manifesting as inflammation, blistering and subsequent desquamation of the damaged skin layers. Repeated UV exposure may result in accumulation of unrepaired DNA damage which, ultimately, triggers the development of skin cancer. The incidence of skin cancers has increased dramatically in the past decades, raising the lifetime risk to be diagnosed with melanoma to about 2% for fair-skinned individuals and to 0.08% for individuals with more deeply pigmented skin (15, 20).

DNA damage caused by UV usually are single-base alterations, crosslinking between the two strands of DNA or between nucleotides of the same strands (dimerization) and creation of reactive oxygen species (ROS). The latter may, in turn, cause strand breaks and DNA-protein crosslinks, expanding the damage further.

The type of DNA damage caused by UV is generally dependent on its energetic content. Dimers in DNA are usually produced by UV-B (28, 45). The two major photoproducts are cyclobutane pyrimidine dimers (CPDs, about 75% of all photoproducts) and (6-4) pyrimidine dimers ((6-4) PDs). The

latter can further undergo a UVB -dependent conversion to Dewar pyrimidinones, their valence isomers (40). It is believed that CPDs play a major role in UV-induced mutagenesis (25, 30). Mutations known to be caused by UV (UV-signatures) have been found in the *P53* gene of about 80% of precancerous skin lesions and in about 90% of squamous skin cancers (32).

Contrary to the popular belief that UV-A is relatively harmless to the skin, it has been repeatedly shown that it has a strong potential for inducing mutagenic transformation, primarily by means of increasing oxidative stress via generation of ROS, but also including direct generation of dimers (21, 22, 36).

It is believed that UV-induced lesions are randomly distributed throughout DNA. Cyclobutane pyrimidine dimers caused by UVB, however, have a preference to pyrimidine-rich sequences, especially thymines in DNA (29). Cytosines methylated at pos. 5 are also common hotspots for formation of CPDs (42).

UV-induced lesions in DNA, depending on the nature of the lesion, are efficiently recognized and repaired by the base excision repair (BER) or the nucleotide excision repair (NER) mechanism of DNA repair. Presence of unrepaired DNA damage is a powerful trigger for halting the cell cycle until damage is repaired or, if it is deemed irreparable – for activating the cellular program for apoptosis. Generally, in order to avoid replication of damaged DNA, its structural and sequence integrity is assessed in several checkpoints, each governing a transition point in the cell cycle, and damage is repaired before the cell cycle could proceed further (13, 37, 48). It has been proposed that for some cells that are not expected to divide further, such as terminally differentiated cells, damage assessment and repair is limited to actively transcribed genes only (7, 8, 9, 31).

Bases of modern UV prevention and post-irradiation options

At present, the stable approach of prevention of UV-associated skin trouble is prevention, that is, avoidance of UV exposure and/or wearing protective sunscreen when expecting to be exposed to UV sources. For practical purposes, many specialists still use the scale of Fitzpatrick (16) to obtain crude but surprisingly accurate assessment for the amount of UV (solar radiation) that the particular patient may tolerate without significantly elevating the risk of UV photodermatitis and/or skin cancer. According to Fitzpatrick's scale, the skin phototype consisting of fair skin, red hair and any amount of skin freckling (type I) is the type most sensitive to UV, followed by type II (fair skin that does not tan, light hair colour, no freckling). Persons with types I and II skin are generally advised not to expose themselves to any but minute amounts of solar radiation, to wear sunscreen and light-absorbing clothing on a daily basis and to screen regularly for precancerous skin lesions and skin cancer.

This approach, however, will work only if the individual is aware of the dangers associated with UV exposure and is prepared to comply with the specialists' recommendations in order to avoid UV-induced damage. This may require a considerable amount of attention and perseverance, and, no less important, unwillingness to bend to fashion and/or peer pressure. It has been repeatedly proven that use of tanning beds is directly linked to an increase of the risk for melanoma and non-melanoma skin cancers (12, 19).

While use of indoor tanning appliances, however, is perfectly avoidable, exposure to solar radiation is not always preventable, especially with young children and adolescents. It has been shown that sunburns before age 18 are associated with at least 2-fold increase of the risk for skin cancer (1, 3). Recently, an opportunity has emerged which may allow intervention after UV irradiation has already happened, namely, use of T4 endonuclease V (T4eV) – containing preparations. T4eV is a phage protein recognizing CPDs in DNA and catalyzing the excision of the 5'-base in the dimer and the subsequent introduction of a single-strand break at the newly created apyrimidine site (46), the number of the breaks approximately proportional to the number of pyrimidine dimers present in the DNA. DNA strand breaks are a very powerful recruiting signal for the repair machinery of the cell. Therefore, T4 endonuclease V is not, strictly speaking, a repair enzyme but, rather, a signal and amplification protein which translates the relatively weak sign of structural DNA damage into imperative summons for action because of physical damage. The opportunity to decrease the risk of enduring UV-damage after the irradiation has already happened constitutes a significant difference, as all classical anti-UV approaches are based on prevention of skin damage. After the damage has been done, the routine short-term treatment options are purely symptomatic and, in the long run, the management strategy is based entirely on watchful waiting.

Packaged in nanoparticles (e.g. liposomes), T4 endonuclease V is able to penetrate through the keratinized skin layers and reach the living cells in the skin, where it tags the sites of cyclobutane dimers with strand breaks, allowing for more effective recognition of damage. Application of topical preparations containing T4eV in order to ensure its efficient delivery into target cells has been shown to aid in management of short-term as well as the long-term consequences of UV irradiation. It has been shown that the application of T4eV on irradiated skin inhibits the up-regulation of the proinflammatory factors IL-10 and TNF-alpha (44) and may ameliorate the short-term as well as long-term adverse consequences of UV-induced damage in healthy individuals as well as in patients defective in DNA repair capacity, such as xeroderma pigmentosum (14, 47). Relatively recently, a rapid and efficient method for production of high-grade T4 endonuclease V by heterologous expression in prokaryotes was developed (6).

Damage assessment – how to tell whether there is a problem

Usually, the damage in cellular DNA inflicted by UV irradiation is repaired effectively enough so as not to cause

significant trouble. There are, however, variations in the rate with which different lesions are repaired. For example, CPDs are usually repaired more effectively in the actively transcribed regions of the genome than in nontranscribed chromatin. This may vary among different species. Rodents are the absolute champion of DNA repair among mammals, as they can withstand doses of UV irradiation significantly higher than any other taxa, but this is related mainly to the fact that they strongly prioritize the transcription-coupled repair over global repair, targeting all the effectiveness of DNA repair at the transcribed genes and basically ignoring the DNA damage in the heterochromatin, a phenomenon termed as 'rodent repairadox' (18). This makes sense, as rodents are usually short-lived, therefore, it is not likely that their genome, proportional to the size of the genomes of long-lived mammals such as humans ($\approx 10^9$ bp), would accumulate enough mutations so as to launch neoplastic growth. (6-4) photoproducts are usually repaired more slowly than CPDs and their removal is usually entrusted to global genome repair.

Provided that UV radiation is so abundant, and that the mechanisms that control the resulting damage are not 100% leakproof, is it possible that when these and the related defense mechanisms fail – albeit temporarily – that malignant skin disease is imminent? Considering that human skin cells labour under constant barrage of UV since birth, the average healthy individual seems to cope very well with UV irradiation, with the rare exceptions of inherited defects of DNA repair proteins which produce disease phenotype. In fact, it was the research of human disease (11) that brought about the importance of DNA repair as a fundamental process in living cells and added the DNA repair as a member in the 'nuclear transactions' triad (replication, transcription, repair). For a long time it was believed that defects in DNA repair are so severe that affected foetuses are seldom born alive. It turned out, however, that the readiness for repair of lesions in DNA constitutes a major factor in human health status and was not a constant throughout the life of the individual and that it may fluctuate depending on the age and general health of the individual and environmental factors. In the last decade it became clear that inter-individual variations in the capacity for repair of DNA damage in clinically healthy individuals may significantly alter the risk for development of various tumours, influence the outcome after various treatments and even modify the success rate of HLA-matched transplantations (2, 9, 22, 24, 33, 35). Also, the capacity to repair the DNA damage inflicted onto tumour cells by anticancer therapy may be used to assess the risk for acute toxicity in some patients (10, 39).

All this immediately brought about the question about how to measure individual repair capacity. The latter proved to be difficult, as the types of DNA damage are legion and, consequently, the living cells have developed similarly diverse mechanisms for recognizing and repairing them. Finally, the key to this problem was found in the basic assumption that since all types of DNA repair – eventually – end up in DNA synthesis, therefore, the rate of repair could be measured by the rate of DNA synthesis (5, 34, 43). This solution was far from ideal, however, as the relative amount of DNA synthesis due to repair is about 1-2 orders of magnitude lower than the replicative DNA synthesis. Moreover, large amounts of DNA damage could effectively block the progress through the cell cycle and allow the non-replicative component of DNA synthesis to come forward, but they could also cause rapid cell death without any DNA repair. On the other hand, small amounts of certain types of damage stimulated both types of DNA synthesis, which complicated things even further. The answer to the dosage dilemma was found in an agent whose replication-inhibiting properties were discovered long ago, namely, hydroxyurea (41). Model systems based on hydroxyurea blockage have been set up and tested (4, 5) but it was not until recently that these models have been integrated into the context for practical use in routine assessment and monitoring individual repair capacity (8). It could be expected that the differential synthesis blockage may become a method of choice for measurement of individual repair capacity, as it is independent of the particular type of the damage and the genomic region in which the damage occurs. Also, unlike other methods, it works *in vitro* (that is, with cultured cells) as well as *in vivo*, which may be a significant advantage when assessing repair capacity in individuals with known or suspected DNA repair defects, e.g. sun sensitivity (xeroderma pigmentosum), general sensitivity to ionizing and/or UV radiation (Ataxia telangiectasia, Nijmegen syndrome), cancer susceptibility (familial melanoma, familial breast cancer, etc.).

Conclusions

As UV is a very common environmental agent, accidental overexposure is virtually inevitable throughout the life of the individual. Usually, UV-caused damage to DNA is repaired effectively, and a very small proportion of UV-related defects result in mutations with oncogenic potential inherited in a dividing cell's progeny. Various strategies have been developed to avoid or decrease UV damage to cellular DNA, based on prevention of exposure as well as on post-irradiation measures. The capacity for DNA repair varies during the life of the individual and must, therefore, be periodically assessed so as to determine whether the individual is coping with environmental UV damage. Assessment of individual repair capacity might modify the existing therapeutic strategies for common cancers. Also, it is possible that the assessment of DNA repair capacity may become an important component for prognostication in human disease and, therefore, ought to become a routine part of health prophylaxis.

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