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A review of DNA repair and possible DNA-repair adjuvants and selected natural anti-oxidants

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Abstract

Few other organs have the environmental exposure-neoplasia relationship that has been observed between epithelial cutaneous malignancy and UVB exposure. Important insights into the molecular processes that affect the response of cells to UVB have been provided by the study of rare inherited diseases characterized by DNA repair defects. Nucleotide excision repair is the best characterized of these and its importance is illustrated by the disease, xeroderma pigmentosum. More recently, other DNA mechanisms have been shown to have some role in skin cancer, such as DNA-mismatch repair and double-stranded DNA breaks. Herein, we discuss the DNA-repair adjuvants a aqueous extract of Urcaria tomentosa (AC-11, Optigenex, Inc.), and T4 endonuclease V that is prepared in a liposome lotion (Dimericine, Applied Genetics Inc. Dermatics). The positive effects on the integrity DNA of other substances (from nature, heat shock proteins and cytokines) including IL-12, Polypodium leucotomos, and ubiquitin are also reviewed. Understanding DNA repair mechanisms is far from complete; further understanding will provide insight into the pathogenesis of cancer and pave the way for efficacious therapeutic agents.

Introduction

Damage to DNA that results in defective DNA can result in cancer. The interaction of endogenous and exogenous factors is a complex process in the pathogenesis of skin cancer. Few other organs have the exposure-neoplasia relationship that has been observed between epithelial cutaneous malignancy and UVB exposure. Thus, the skin has proven to be an excellent model for investigating an exogenous factor's influence on the cell's genetic machinery to result in cancer.

Despite our increase in understanding of the pathogenesis, skin cancer is still a major public health problem. The Centers for Disease Control and Prevention believes 1.5 million new cases of basal or squamous cell carcinoma are diagnosed each year [1]. Billions of dollars are spent annually on the treatment of pre-malignant and malignant cutaneous lesions, as well as the cosmetic procedures used to treat photoaging [2, 3].

Although the treatment of skin cancer is very effective, the best treatment remains prevention. Preventative measures include sun exposure avoidance, protective clothing, and full-spectrum sunscreen. Poor compliance and improper use are the primary reasons for failure of these methods [2, 4]. Recent controversy surrounding reports of significantly reduced Vitamin D levels with sun avoidance could mean a greater demand for an orally administered agent.

Recent discoveries regarding the cell's innate ability to repair damaged genetic machinery has led to a greater understanding of the pathogenesis of skin cancer. Anti-oxidants have long been getting a great deal of attention as prophylactics of and panaceas for cancer, but enhancing cellular DNA repair has received less attention. New understanding of repair mechanisms and an awareness that some substances enhance DNA repair will pave the way for rational and efficacious dermatologic therapies.

Aside from the well characterized mutation in epithelial DNA caused by UVB, UVB also has deleterious effects on the skin's DNA repair mechanisms, cutaneous cell-mediated immune system, cell death (apoptotic) pathway, and structural integrity.

UV-induced cellular injury

A significant DNA type of defective linking of DNA nucleotides involves pyrimidine dimers. Cyclobutane pyrimidine dimers (CPDs) make up much of the damage (perhaps 75 %, depending on the sequence context), and pyrimidine (6-4)

pyrimidinone dimers (known as 6-4 photoproducts) are among the photo-products that can result from DNA being exposed to ultraviolet light. The fingerprint or signature of UVB on DNA is the distinctive pattern of mutations that they cause [5, 6].

Indirect forms of DNA damage induced by exposure to UVB include oxidized or hydrated pyrimidines (cytosine photohydrates and thymine glycols), oxidized purines (8-hydroxy-2-deoxyguanosine, 8-OHdg), single-strand breaks and DNA protein cross-links [7, 8, 9]. The UV fingerprint mutations are not caused by free radicals—free radicals cause random damage.

The transcription factor p53 functions to slow DNA replication when DNA is damaged by UV radiation. Following DNA damage, there is a rapid increase in p53 levels. Transcription is stimulated by p53 on several genes that mediate cell-cycle arrest and apoptosis. Such a pause in cell cycling is welcome because it allows the cells enough time to repair the DNA damage inflicted by the mutagenic agent. This p53 also helps in the repair process directly by inducing the transcription of GADD45 (growth arrest and DNA damage), which encodes a protein involved in DNA repair. If during the pause in cell division the DNA damage cannot be successfully repaired, normal p53, induces apoptosis [10].

Ultraviolet induced distinctive mutations are not identified in systemic malignancies and have been isolated in a variety of tumor oncogenes within skin cancers. For example, 90 percent of squamous cell carcinomas and 50 percent of basal cell carcinomas from a cohort of New England patients contain UV-like mutations in the p53 tumor suppressor gene [11].

Ultraviolet exposure is responsible for abnormalities in cell-mediated immunity. These include diminished circulating T-cells, reduced T-helper/suppressor ratio and impaired delayed hypersensitivity reactions [12]. In addition, native cutaneous Langerhans cells are replaced by a population of histiocytes with a distinct antigen profile, with an inherent ability to activate T-suppressor cells. Patients with systemic immunosuppression (secondary to medication or from a primary immune disease) have a significantly increased rate of squamous cell carcinoma, thus highlighting the immune system role in suppressing cutaneous neoplastic proliferations [13].

Fas (CD95) induced apoptosis is a mediating mechanism in human epidermal cell cycling. Fas is downregulated in severely actinically damaged skin and in invasive squamous cell carcinoma. Fas elicits apoptosis through both the intrinsic and extrinsic apoptotic pathways; downregulation corresponds to promoted cell survival. Because of its protective function, alterations in UVB-induced apoptosis may have a profound impact in the induction of

skin cancer [14].

UV exposure and DNA repair mechanisms

Nucleotide excision repair

Nucleotide excision repair (NER) is an essential repair process for sun-exposed skin. In normal individuals, a repair process ensures that UV induced pyrimidine dimers are excised and replaced, and a subsequent restoration of correct DNA structure. Nucleotide excision repair proceeds via two alternative pathways: The global genome repair (GGR) is involved in the repair of any sequence in the genome regardless of its transcriptional status; the transcription-coupled repair (TCR) is only involved in the repair of actively transcribed DNA strands. Transcription-coupled repair occurs at a higher rate than GGR, but the reason for this difference is not fully understood. Most of the events of the two pathways are identical; in both cases, DNA unwinding is followed by excision of a 27-30-nucleotide oligonucleotide fragment containing the photoproduct of the damaged DNA strand and its replacement by de novo synthesis using the opposite, untouched, DNA strand as the template [15]. Thus, the major difference between GGR and TCR occurs at the level of recognition of the DNA damage. In the GGR pathway, the damage is initially recognized via a direct interaction of NER proteins XP (Xeroderma Pigmentosum) E and XPC with damaged DNA. In contrast, in TCR, damages appear to be signaled via the stalling of RNA polymerase II (Pol II) [16]. The release of RNA polymerase involves two proteins, CSA and CSB (mutations in these are responsible for Cockayne syndrome A and B, respectively) [17]. Persistence of UV-induced dimers blocks transcription of messenger RNA and inhibits the development of DNA replication forks, therefore preventing replication.

The importance of NER is best illustrated in xeroderma pigmentosum (XP), an autosomal recessively inherited disease affecting nucleotide excision repair. Xeroderma pigmentosum is a heterogenous disorder clinically characterized by premature skin aging and malignant tumor development. The tumors include squamous cell carcinoma, basal cell carcinoma, and, rarely, fibrosarcoma. In about 3 percent of patients, malignant melanoma arises. As a result, the rates of malignant disorders of the skin (basal-cell and squamous-cell carcinomas, and the melanocytic disease, melanoma) are 1000 times higher than in the general population; rates of actinic keratosis are also increased [18].

The last decade has seen the cloning of the key elements of NER, and the process has been reconstituted in vitro. Seven XP

repair genes (XPA-XPG) have been identified (see Table 1) and correlate with 7 complementation groups (XPA-XPG). These entities occur with different frequencies and differ with respect to disease severity. In vitro testing of skin fibroblasts from different patients with xeroderma pigmentosum shows considerable variation in the NER defect, the extent of repair replication varying between 0 and 90 percent of normal. There are at least five patients known in whom excision repair is normal but the cells are defective in a DNA repair mode referred to as post-replication repair. This represents the so-called XP variant form [19, 20, 21, 22, 23].

Mismatch repair (MMR)

Another mechanism involved in DNA repair is DNA mismatch repair (MMR) and defects in this mechanism have been found in skin cancer. Mismatch repair of DNA is detected through investigation of DNA microsatellites. Microsatellites are repetitive mono-, di-, tri-, or tetra-nucleotide sequences, scattered throughout the human genome, which frequently show length polymorphisms. These repetitive sequences are often located between genes and have been classified as 'junk' DNA. Microsatellite abnormalities have been recently implicated in the genesis of various human disorders, both hereditary and non-hereditary, including cancers. In particular, microsatellite instability (MSI), that is, variations in the number of repetitive unit sequences in each microsatellite, has been shown to be a key feature of the neoplastic cells compared with normal tissue in hereditary non-polyposis colorectal cancer (HNPCC) syndrome, in a subset of apparently sporadic colorectal tumors [24].

Muir-Torre syndrome is caused by germline mutations in mismatch repair genes and is characterized by internal malignancy (most often gastrointestinal) and sebaceous tumors and keratoacanthomas in the skin. The process of induction of cancer involves a widespread MSI resulting in the expansion or deletion of many repeat elements in the tumor DNA. Mismatch repair proteins have been shown to be recruited to sites of UV-induced DNA damage where they interact with nucleotide excision repair proteins [25]. In addition, MSH2/MSH6 (products of mismatch repair genes) heterodimers bind oligoduplexes engineered to contain the types of lesions formed by UV radiation [26].

Young et al. showed that impairment of the elimination of UVB-damaged cells via apoptosis promotes UVB-induced tumorigenesis in MMR-deficient mammals instead of promotion spontaneous tumorigenesis (which would be expected if MMR was the primary mechanism). This suggested that dysregulation other than genomic instability, (reflected by MSI), contributes to the

increased susceptibility to UVB-induced tumorigenesis when there is dysfunctional MMR. [27]

Double-strand beaks (DSBs)

Double-strand breaks (DSBs) is a distinct form of DNA damage which results from two nicks in opposite strands of the DNA helix. A requirement for DSB formation is that the two nicks are in sufficient close proximity to one another (<12 bp) that base pairing and chromatin structure are unable to maintain the broken DNA ends juxtaposed. DSBs can result from exposure to ionizing radiation, radiomimetic drugs and topoisomerase inhibitors. The main cellular response to DSBs include cell cycle regulation, DSB repair, transcriptional and post-transcriptional activation of relevant genes (including those associated with repair), increases in cellular DNA and induction of apoptosis. Double-strand breaks are considered particularly dangerous lesions because they are considerably more difficult to treat than other types of DNA damage. While also thought to be important in the pathogenesis of cancer, this form of DNA damage is effective in allowing physiologic chromosomal rearrangements (such as V(D)J recombination for immunoglobulin genes). The complex interplay between DSB, DSB repair, and cellular outcomes is an area of intense investigation and its role in UV-induced damage and cutaneous malignancy is not clear at this time. Further understanding of this mechanism is likely to further unravel the complex damage-repair mechanism in keratinocytes which cannot be solely explained by NER [28].

Although the above mentioned DNA damage and repair modalities give us some insight into the DNA repair mechanism as a whole, there remain many unanswered questions. Experimental studies have clearly shown that UV(B) radiation can cause deletions and chromosomal aberrations, such as detected by micro-nuclei formation, i.e., UV radiation is clastogenic, which have an unknown primary DNA lesion [29, 30, 31]. As DNA damage other than the herein mentioned mechanisms may cause such genetic alterations—and some may be even more carcinogenic—an optimum repair mechanism will likely require more than repair of pyrimidine dimers.

Recent discoveries in DNA repair

The mechanisms that underlie DNA repair have been extensively explicated in recent years. A few recent discoveries in the field DNA repair enzymes follow:

Modrich found that a protein called PCNA is clamped onto the DNA at the strand break. This PCNA, together with the protein that clamps PCNA onto the DNA double helix, regulate the enzyme whose job it is to snip out the segment containing the mismatch, by "aiming" the enzyme—known as *exonuclease I*—in the right direction to work itself along the strand, stripping out the segment containing the mismatch. A notable aspect of this PCNA repair system is that it can evaluate the placement of the strand signal to one side or the other of the mismatch and work from there. Placement of the strand break that directs repair to one side or the other of the mismatch might be the result of a mechanism by which DNA is copied by the replication machinery [32].

Ronai found that the protein ATF2 (Activating Transcription Factor-2) is activated by a protein kinase called ATM (Ataxia-Telangiectasia Mutated), which stimulates DNA repair. ATF2's role in regulating expression of proteins that control cell cycle and programmed cell death is well established. Ronai demonstrated ATF2's role in DNA repair, an intracellular process that prevents formation of genetic mutations, including those that lead to cancer. This ATF2 is regulated by ATM and this regulation is central to the cell's ability to initiate DNA repair processes following ionizing irradiation or other exposures that cause breaks in DNA. Activating Transcription Factor-2 likely works by halting the cell's cycle to allow repair of damaged DNA before such damage becomes permanent [33].

Powell discovered that MDC1, a protein previously recognized only for its function in sensing DNA damage and signaling its presence, also transports DNA-repair proteins to the site of DNA strand breaks. Without MDC1 to pave the way, repair happens slowly because the fix-it proteins have a hard time reaching damaged areas, which are buried in the tightly packed chromosomal material of the cell's nucleus. The MDC1 can bind to chromatin, the complex mixture of DNA and proteins that holds the genetic material. Because of chromatin's properties, getting into it to reach the DNA strand requires the right 'passwords'. The MDC1 provides the DNA-repair proteins with this privileged access, and efficiently transports them to the site of damage so they can effect repair [34].

Although the relationship between sun exposure, DNA damage, and the development of malignant melanoma remains controversial, a growing body of evidence supports a role for MSI and defective MMR protein expression in melanoma tumorigenesis. Absent hMLH1 and hMSH2 expression has been demonstrated in melanomas and correlates with tumor progression [35, 36, 37].

What is clear from our current understanding of DNA repair is that rather than being a process where a few protein complexes

detect and repair damaged DNA, the mechanisms of DNA repair are complex, intricate and involve a number of dynamic systems. Our understanding of genetic diseases in DNA repair and the profound clinical manifestations (e.g., XP) has shown that a clearer understanding of DNA mechanisms is likely to pave the way for understanding of carcinogenesis.

DNA repair adjuvants and selected anti-oxidants

A number of substances seem to have the ability to enhance DNA repair (Table 2) [38-89]. DNA repair adjuvants (from nature, enzymes and cytokines) and selected natural anti-oxidants discussed here include the following: selenium, *Urcaria tomentosa*, T4 endonuclease V, ubiquitin, and interleukin-12. Polypodium leucotomos will be discussed as well because it has been included in discussions of the aforementioned substances although it is primarily an antioxidant.

Selenium

Clark supervised a randomized trial of 1312 patients for the prevention of skin cancers supplementing diets with baker's yeast rich in selenium and found that the selenium rich yeast reduced the overall risk of developing cancer by 40 percent and reduced their risk of dying from cancer by nearly half, compared with the placebo group. Selenium intake did not protect against the malignant degeneration of skin cell into basal or squamous cell carcinomas of the skin [38]. This report of Clark, carried forward a study that Clark had published in 1991 showing that selenium supplementation had cancer chemopreventive effects in humans [40].

Studies have confirmed the cancer chemopreventive activity of selenium and have suggested that this effect may be related to selenium-induced apoptosis of cancer cells [39]. Seo et al. found that cells whose DNA was damaged by ultraviolet radiation, when treated with selenomethionine, elicit activation of Ref-1—a protein which is able to switch on p53 activity and double the rate of DNA repair. The presence of selenomethionine allows cells to tolerate greater levels of ultraviolet radiation, because of the higher level of competent p53 [41].

Rafferty concluded that selenite and selenomethionine protect keratinocytes from UVR-induced oxidative damage, but not through creation or formation of UVR-induced excision repair sites. So although selenium might prevent cancer, it might not be

acting to do this by promoting DNA repair [42].

More recently, however, Fischer et al. have shown that selenomethionine preferentially induced the DNA repair branch of the p53 pathway. Accordingly, pretreatment with selenomethionine protected normal fibroblasts from subsequent DNA damage. Interestingly, Brca1 (breast carcinoma gene 1) was required for SeMet-mediated DNA damage protection, as brca1 -/- mouse fibroblasts were not protected from UV-radiation by SeMet treatment. This indicates that besides p53 and Ref1, Brca1 is required for selenium protection from DNA damage. [43] The controversy surrounding the use of selenium as an antineoplastic agent can partially be explained by its dose related effects: Mutagenic, carcinogenic and probably teratogenic effects have been reported following administration of toxic doses [44, 45, 46].

The mechanism for the toxic effects of Se has been suggested to result from its high affinity for non-specific substitution for sulfur in SH-containing DNA repair proteins. Although the recommended daily allowance (RDA) of Se by the U.S. Food and Drug Administration is 50 µg/day, cancer preventive use of Se is typically 200 µg daily intake, exceeding the RDA by four-fold with no harmful effects. Human dietary intakes of Se vary according to ecological abundance, being as low as 20 µg/day in parts of New Zealand and as high as 5000 microgram/day in parts of China. [47]

Aqueous extract of *Uncaria tomentosa*

The aqueous extract of *Uncaria tomentosa* (previously named *C-Med-100* and now renamed *AC-11*), an extract of cat's claw, appears to enhance the normal repair of cyclobutyl pyrimidine dimers following UVB exposure. The observed reduction in oxidative DNA damage (8-hydroxyguanine and strand breaks) is possibly the result of enhanced base excision repair or an inherent antioxidant effect, or both.

Reduced non-melanoma skin cancer following topical application of AC-11 in hairless mice (an unpublished study) is likely from a reduced dimer burden. Decreased dimers - decreased p53 mutations - decreased actinic ketatosis - decreased malignancies.

The DNA data in humans has been supplemented with two animal studies in which the effects of known DNA damaging agents were compared in AC-11-treated and control animals.

In the first study of AC-11, 8 daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered to 20 rats (an additional 10 rats served as controls) by gavage for 8 weeks. Half the animals from

each group were exposed to 12 Gy whole body radiation (137Cs source) and allowed 3 hours to repair in vivo before DNA damage was assessed. AC-11-treated animals almost completely repaired single-strand DNA breaks ($p < 0.05$) for both AC-11 doses compared to untreated animals. Double-strand DNA breaks were substantially fewer in animals treated with 40 mg/kg/day of AC-11 and significantly ($p < 0.05$) fewer in animals treated with 80 mg/kg/day of AC-11 compared to untreated animals [48, 49].

In the second study, 9 daily doses of 40 mg/kg or 80 mg/kg of AC-11 were administered orally to 8 rats (4 at each dose) 24 hours after the last of three 2 mg/kg intraperitoneal doses of doxorubicin. Four animals received doxorubicin only. Animals treated with 80 mg/kg of AC-11 had significantly ($p < 0.05$) reduced DNA damage in the form of single-strand DNA breaks [48, 49].

More recently Pero reported on the combination of a cat's claw water extract (AC-11, carboxy alkyl esters = active ingredients) plus medicinal mushroom extracts (*Cordyceps sinensis, Grifola blazei, Grifolafrondosa, Trametes versicolor* and *Ganoderma lucidum*, polysaccharides = active ingredients) plus nicotinamide plus zinc into a formulation designed to optimize different modes of immunostimulatory action in 14 subjects treated for 4 weeks and found patient experienced reduced pain, reduced fatigue, weight loss and a reduced presence of DNA damage in peripheral blood assessed by (8-OH) guanine DNA adducts and elevation in serum protein thiols [50].

The mechanism for AC-11 activity has yet to be fully defined; however, research in humans and in human living skin equivalents shows that AC-11 reduces erythema and blistering after ultraviolet exposure. AC-11 significantly enhanced the repair, but not the formation, of cyclobutyl pyrimidine dimers (TT-dimers) in human living skin equivalents exposed to UV-B light [51, 52].

In a study of 5 healthy volunteers aged 35 to 55 year old taking 350 mg/day of AC-11 orally for 4 weeks, 8-hydroxyguanine levels were significantly ($p < 0.05$) decreased. The beneficial effect was noted to persist 2 weeks after therapy was discontinued. [53]

Another study reported a significant ($p < 0.05$) decrease in DNA single-strand breaks following peroxide-induced DNA damage in monocytes of healthy volunteers who received 8 weeks of AC-11 at 350 mg/day [54].

Pero et al. assessed oxidative DNA damage in 14 volunteers, most of whom (more than 75 %) had chronic diseases, and reported that 9 of the 14 volunteers had decreased 8-hydroxyguanine DNA adducts after 400 mg of AC-11 per day for 4 weeks. Finally, in a in a single-blind, right side-left side, beach sun exposure pilot study that included 42 healthy volunteers there were dramatic and

significant ($p < 0.0001$) reductions in erythema and blistering in volunteers who applied 0.5 percent topical AC-11 with an SPF-15 sunscreen when compared to the group who just applied an SPF-15 sunscreen [55].

In 2001 Sheung[78] did a study involving 12 men and women. He divided them into 3 groups (one placebo, one 250 mg of AC-11 daily and one of AC-11 350 mg daily) for 8 consecutive weeks. DNA damage was induced by a standard dose of H_2O_2 was measured 3 times before supplementation and 3 times after the supplementation during the last 3 weeks of the 8 week-supplement period. Supplement groups (250 and 350 mg/day) experienced statistically significant decreases of DNA damage and simultaneous increases of DNA repair versus men and women taking placebo.

In 2006 Mammone treated skin cultures with 5 mg/mL C-Med-100 or without 5 mg/mL C-Med-100 [80]. The cultures where then irradiated with 0-100 mJ/cm2 UVB, and microscopically analyzed for necrosis and the level of pyrimidine dimers using immunofluorescent TT-dimer antibody staining. It was found that co-incubation of keratinocytes with C-Med-100 reduced skin cell death from UV exposure likely related to an increase of DNA repair.

T4 endonuclease V

T4 endonuclease V (Dimericine) is a DNA repair enzyme produced in bacteria that is delivered in liposomes in the form of a topical cream [56]. The liposome utilized in T4 endonuclease V is a microsphere called a T4N5 liposome made from lipid lecithin, from the egg. It is thought to act via two mechanisms [57]. Immediately, T4 endonuclease V removes DNA dimers, primarily cyclobutane pyrimidine type. In the long term, it may restore p53 gene function and exert a lasting chemopreventative effect. T4 endonuclease V has been studied as a topical cream to decrease the development of skin cancer in patients with xeroderma pigmentosum [58, 59, 60] and renal transplant patients on immunosuppressive therapy. T4 endonuclease V received orphan drug designation for this indication in 1989 [61].

One study found the T4 endonuclease V lotion reduced the incidence of basal cell carcinomas by 30 percent and actinic keratoses by 68 percent. Furthermore, the effects on actinic keratoses were observed within the first 3 months of treatment, so the improved repair of DNA damage seems to affect tumour promotion or progression. In the same study, no adverse effects were observed among the patients during treatment, and no antibodies against the enzyme were detected in the patients' serum.

This absence of toxicity confirms early safety studies and may be explained by immunohistological observations that T4 endonuclease V delivered by liposomes is localized in the epidermis and does not readily penetrate into dermis [59, 62].

In another study, in vivo testing involving T4N5 liposome lotion has yielded intriguing results. In a test conducted with twelve xeroderma pigmentosum (XP) patients and 15 patients without this condition who had a history of skin cancer, researchers applied the cream at various intervals after controlled UV exposure. Biopsies conducted 6 hours after UV exposure revealed that patients with XP had achieved approximately 15 percent fewer CPDs (improved DNA repair) while patients with a history of skin cancer achieved less than 10 percent fewer CPDs. the results of this study demonstrate that liposomal delivery represents an effective way of introducing proteins into the cells of human skin, including keratinocytes and Langerhans cells. The results showed that a DNA repair enzyme delivered in this manner can reverse some of the deleterious effects of UV irradiation that seem to be caused by DNA damage, such as the upregulation of the immunosuppressive cytokines, IL-10 and TNF-α. Topical DNA repair enzyme application therefore may be a clinically useful approach of photo-protection in humans. In contrast to conventional sunscreens, which are effective owing to their content of chemical or physical UV filters, liposomes containing DNA repair enzymes may be able to protect against UV-induced damage to the skin, even when they are applied after UV exposure and initiation of the sunburn reaction. Thus, the immunoprotective effects of topical DNA repair enzyme application may open new avenues for photoprotection, particularly by protecting efficiently against the effects of UV radiation on the immune system, which are not always prevented by sunscreening agents [63].

T4N5 liposomes overcome the drug block in DNA repair seen in immunosupression in organ transplant patients. This indicates that the inhibition is an early incision step of DNA repair [64].

Phases I and II trials of T4 endonuclease V for prevention of skin cancer in xeroderma pigmentosum patients were completed. T4 endonuclease V, however, is not commercially available. The company states, "The XP trial was registered with the FDA as a Phase III trial because it had a clinical endpoint: reduction of actinic keratoses and skin cancer and that its application is open. The FDA has undergone reorganization twice in the last 3 years and our application has been moved. We are discussing the number of XP patients required for market approval."

New studies of T4 endonuclease V are ongoing. Craig A. Elmets, M.D., Chair of the University of Alabama at Birmingham's Department of Dermatology and Senior Scientist at the UAB Comprehensive Cancer Center is leading a 3-year, multicenter,

Phase II, randomized, double-blind controlled study of T4N5 liposome lotion to determine its success in preventing recurrence of non-melanoma skin cancer in 100 renal transplant patients. Enrollment is ongoing [65].

It would seem that the most useful role for T4 endonuclease V would be its inclusion in sun-block. With no reported side effects, T4 endonuclease V is promising. Whether it will change clinical outcomes will become clearer as Phase III trials are completed.

Interleukin-12 (IL12)

IL-12 is one of the major players involved in orchestrating both innate and acquired immune responses. It has been shown to prevent UV-induced immunosupression, though the mechanism behind this remains elusive [66, 67].

In addition to its immunomodulatory activities, IL-12 exhibits the capacity to remove UV-induced DNA damage. This effect was not noted in XPA knock out mice (lacking functional NER) indicating that IL12 may reduce CPDs via induction of NER to remove UV-induced DNA damage [68, 69].

The mechanisms leading to the induction of NER by IL-12 are still not clear. Although it was shown that IL-12 induces certain components of NER at the RNA level [70], this has not been confirmed on the protein level. It is also not known which signaling mechanisms IL-12 utilizes to induce DNA repair. Impairment of the immune system is mediated in large part by the immunosuppressive cytokine IL-10, the release of which is induced by UVB. There is compelling evidence that DNA damage is the primary trigger for IL-10 release upon UVB exposure. IL-12 inhibits UVB induced IL-10 release, which is in good agreement with this finding. It is tempting to speculate about the therapeutic and preventative potential of IL-12 for UV induced cancer—in contrast to the external application of DNA-repair enzymes, IL-12 affects the cell's own NER system [71, 72]. Clinical trials into the use of IL-12 in cancers have mainly focused on IL-12's ability to stimulate interferon and the immune system as well as inhibiting angiogenesis [73].

Ubiquitin

The ubiquitin-proteasome pathway (UPP) is the major eukaryotic mechanism for regulated intracellular proteolysis. In order to maintain homeostasis cells must have a mechanism by which they can, in a precise, regulated fashion, degrade unwanted

target proteins. The vast majority of this turnover occurs through the coordinated action of the ubiquitin-proteasome pathway (UPP). Targeting this pathway with proteasome inhibitors has been validated as a rational strategy against hematologic malignancies, and, more recently has advocated for use in solid tumor populations, particularly breast cancer, Ubiquitin also plays a role in the NER mechanism for UV induced dimers [74, 75, 76].

The Rad23/Rad4 nucleotide excision repair (NER) protein complex functions at an early stage of the NER reaction, possibly promoting the recognition of damaged DNA. The ubiquitin-proteasome pathway (UPP) regulates NER via two distinct mechanisms. The first occurs independently of de novo protein synthesis, and requires Rad23 and a nonproteolytic function of the 19S regulatory complex of the 26S proteasome. The second requires de novo protein synthesis, and relies on the activity of the newly identified E3 ubiquitin ligase. Following UV radiation, NER is mediated by nonproteolytic activities of the UPP, via the ubiquitin-like domain of Rad23 and UV radiation-induced ubiquitination of Rad4 [77].

Polypodium leucotomos extract (PL) [81-87]

Polypodium leucotomos extract (PL) is derived from a fern plant grown in Central America. The Spanish first described PL in 1788. Extracts have been available in Spain for the last 30 years in the form of oral supplements and have been used principally as anti-inflammatory agents in patients with arthritis and other inflammatory skin diseases such as psoriasis. It is sold as a supplement under the name Heliocare®.

The mechanisms of PL are complex. In vitro studies have shown that PL acts as an effective antioxidant by quenching superoxide anion, singlet oxygen, lipid peroxides and the hydroxyl radical [84]. During a trial for its use for vitiligo, it was noted to offer protection against phototoxic reactions following PUVA therapy [82].

Previous studies have shown that topical and oral PL decreases acute sunburn response. After exposure to sunlight, solar simulator, or psoralen-UVA there is decreased erythema, cyclobutane pyrimidine dimer formation, UV-induced epidermal hyperproliferation, and sunburn cell formation. There is also preservation of Langerhans cells [83].

It is thought that the decreased CPDs are not the result of oxidative damage. DNA repair enzymes are susceptible to damage through oxidative stress, and it is possible that the antioxidant properties of PL reduced this damage, allowing a better DNA

repair and subsequently leading to lower numbers of CPDs [85].

Others have found reduced amounts of CPDs in mice following UVR exposure with topical antioxidant use, with a similar rate of CPD reduction afterward in treated and control groups. The authors concluded that this was not the result of enhanced repair [86]. However, DNA repair is known to be a cellular process that takes place continuously and, therefore, it is possible that enhanced repair might be one of the factors involved. Additional research is warranted to investigate this issue [87].

Conclusion

We now know that anti-oxidants and anti-inflammatory adjuvants are not the only ways to enhance the health of patients and retard the development of skin cancer. The mechanisms of the body itself can be augmented and enhanced. T4N5 liposome lotion which contains endonuclease V removes DNA dimers, primarily cyclobutane pyrimidine type. The key question that must be answered is if the mere increased presence of this enzyme will enhance DNA repair. That is, it remains to be determined whether it is the quantity of enzyme that matters most or is it the timing of the expression of the enzyme in relation to other factors that matters most. If it is the former T4N5 lotion will benefit patients, if it is the later, T4N5 might join the ranks of products whose science is promising but whose practical importance is limited.

The mechanisms behind the other substances that might enhance DNA repair remains to be fully defined if they indeed exist. The main mechanism for the effect of DNA repair adjuvants seems to involved repair of DNA dimers, primarily cyclobutane pyrimidine type. Possible other mechanisms for these substances or for future DNA adjuvants to be developed include the following:

- enhancing of stability of DNA while it is being repaired
- making the clamps of the DNA repair mechanism hold things in place better
- making DNA more apparent to the repair mechanisms
- improving enzyme sensing of DNA damage

The process of DNA replication and repair seem so precise, how a substance could enter the nucleus and interact with replicating DNA remain unclear. Moreover, its seems at least in the case of selenium as it pertains to human health that too little or too much is deleterious to human health. Why that is and what the optimum form and quantity of selenium remains to be defined.

In summary, DNA repair adjuvants appear to be promising means for enhancing health. AC-11 and Dimericine appear to be

best used in conjunction with other agents to optimize their health promoting utility. While these agents appear promising, the ultimate clinical effectiveness and modes of administration and utilization of these DNA repair agents require further explication.

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