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REVIEW ARTICLE

Xeroderma pigmentosum: clues to understanding cancer initiation

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ABSTRACT

Xeroderma pigmentosum (XP) type C is a rare autosomal recessive disorder that occurs because of inactivation of the xeroderma pigmentosum group C (XPC) protein, which is an important DNA damage recognition protein involved in DNA nucleotide excision repair (NER). This defect, which prevents removal of a wide array of direct and indirect DNA lesions, is associated with a decrease in catalase activity. As a novel photoprotective approach, lentivirus-mediated catalase overexpression in XPC human keratinocytes results in a marked decrease in sunburn cell formation, caspase-3 activation, and p53 accumulation following UVB irradiation. While not correcting the gene defect, indirect gene therapy using antioxidant enzymes may be helpful in limiting photosensitivity in XP type C, as well as in other monogenic/polygenic photosensitive disorders characterized by reactive oxygen species (ROS) accumulation. Hypoxia-inducible factor-1 (HIF-1), a major transcription factor sensitive to oxygen levels, responds to various stress factors. As a common stressor of skin, UVB induces a biphasic HIF-1α variation through ROS generation in keratinocytes. HIF-1α has an important regulator effect on the expression of XPC protein and other NER genes, indicating indirect regulation of NER by ROS. The intrinsic genomic instability arising in XP type C provides a good opportunity to investigate the complex molecular mechanisms underlying the Warburg effect (the shift of mitochondrial metabolism towards glycolysis). Overall, the monogenic disorder XP type C is a powerful tool for studying photoprotection and cancer.

Introduction

Different causes of monogenic disease, such as albinisms and nucleotide excision repair (NER) diseases, contribute to photosensitivity and cancer. The most important contributors to UV adaptive responses include (1) DNA lesions, which can induce a pigmentary response[1] and DNA repair machinary; (2) apoptosis, which deletes damaged cells; (3) enzymatic and non enzymatic antioxidant defenses; (4) melanogenesis; (5) stratum corneum, which acts as a physical barrier and a sensor for UV danger responses;[2] and (6) the skin immune system, both innate and adaptive.[3] Interestingly, most of these responses include reactive oxygen species (ROS) mediated effects. Two major effects of ROS have been identified, namely oxidative lesions on large molecules (complex glucids, lipids, proteins and nucleic acids), and physiologic signaling as second messengers to modulate transcription factors such as upstream stimulatory factor, activator protein-1, and hypoxia-inducible factor-1α (HIF-1α).[4–8]

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From clinical observations to antioxidant photoprotective therapy

Heterogeneity for skin cancer proneness in two DNA repair disorders, trichothiodystrophy and xeroderma pigmentosum

UV irradiation causes two major photoproducts in DNA: cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts (6-4PPs). Measurement of

post-UV unscheduled DNA synthesis (UDS) testing indicates that similar impairment of DNA repair could lead to disorders with different phenotypes and different proneness to skin cancer. In fact, the main system responsible for correcting UV-induced damage is the NER, which includes two distinct subpathways: global genome repair (GGR), which repairs DNA damage throughout the genome; and transcription-coupled repair (TCR), which repairs DNA lesions in the transcribed strand of active genes (Figure 1). Absence or dysfunction of NER results in three distinct disorders: xeroderma pigmentosum (XP), trichothiodystrophy (TTD), and Cockayne syndrome. Among them, XP and TTD are associated with reduced UDS levels.[9,10]

XP is an autosomal recessive NER disease with catalase deficiency and cancer[11] which manifests with delayed clinical photosensitivity and a highly increased predisposition

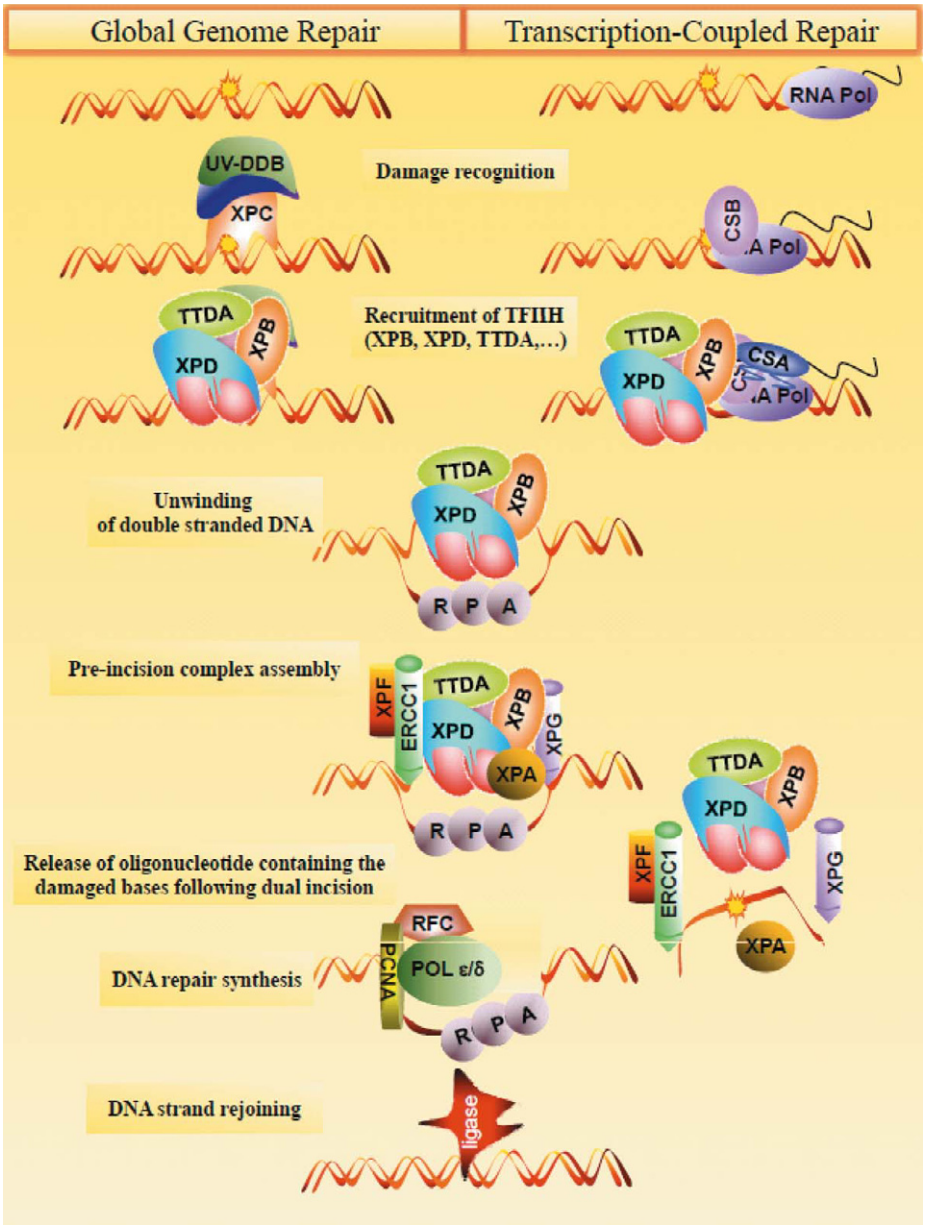

Figure 1 Molecular mechanisms of global genome repair (GGR) and transcription-coupled repair (TCR). Nucleotide excision repair (NER) is a highly versatile system capable of removing a wide variety of helix-distorting lesions from genomic DNA. This system proceeds through two distinct yet overlapping pathways, GGR and TCR, where the main difference is in the recognition step. In GGR, DNA damage throughout the genome is recognized by xeroderma pigmentosum type C (XPC) and type E (XPE) proteins (or UV-DDB), while in TCR, damage that blocks transcription is detected by the chondroitin sulfate A and B proteins. In the unwinding step, transcription factor II H (including XPB, XPD, p8/TTDA and several other subunits), most likely together with XP type G (XPG), type A (XPA) proteins and replication protein A, unwinds the DNA helix through its DNA helicase activity. After incisions have been made on both sides of the lesion by XPF-ERCC1 and XPG, the oligonucleotide containing the damaged base(s) is released as part of a piece of 25–30 bases. Finally, the gapped DNA region is restored by a DNA polymerase (δ or ε) and DNA ligase. The synthesis of this repaired DNA requires proliferating cell nuclear antigen, which forms a homotrimeric clamp on template strands, and replication factor C, which is a heteropentameric, DNA-dependent ATPase complex.

to UV-induced skin cancers. The phenotype includes xerosis and hyperpigmentation. Extracutaneous specific lesions may involve the eye and central nervous system. Internal cancers can also occur, especially leukemia and various solid cancers. This phenotype is genetically heterogeneous (8 genes, XP-A to XP-G and the variant XP-V). Apart from xeroderma pigmentosum group C (XPC) protein, which serves to recognize the damaged bases at the beginning of GGR, other factors participate in both GGR and TCR. The variant form is milder and the age of onset of cancers is 40 years old. This disorder corresponds to a different pathomechanism targeting the bypassing of CPDs due to a defect in DNA polymerase η.[9,10]

Cockayne syndrome is a rare autosomal recessive disease with features of photosensetivity, retinal pigmentation, and progressive neurological degeneration. This disease is caused by a defect in chondroitin sulfate A or B proteins, which are important in the recognition of damaged bases at the beginning of TCR. Unlike XP, Cockayne syndrome has normal levels of UDS and is not associated with skin cancer.[10]

The so called "photosensitive" TTD corresponds by UDS testing to an XP-type NER deficiency in vitro without catalase activity deficiency[11] or cancer,[12] but is associated with premature aging. The early phenotype is dominated by abnormal terminal differentiation of the epidermis and hairs, which causes ichthyosis (collodion baby is common) and hair brittleness. The molecular basis of TTD with NER deficiency has been partially identified and corresponds to mutations of subunits (XPB, XPD, and p8/TFB5 also called TTDA, with XPD mutants being the most common) of transcription factor II H (TFIIH) that acts downstream of XPC in the GGR chain.[13] Interestingly, the TTD ichthyotic phenotype is strongly associated with in vitro photosensitivity, suggesting a link of TFIIH with the regulation of epidermal and hair terminal differentiation. Figure 2[11] shows the respective clinical features of XP and TTD.

Recently, Chigançaş et al[14] examined why TTD/XPD (TTD patients with a mutated XPD gene) patients are more severely affected in the NER of CPDs than of 6-4PPs. They showed that some TTD/XPD mutations affect the recruitment of TFIIH specifically to CPDs, but not to 6-4PPs. For 6-4PPs, they found that TFIIH complexes carrying an NH_2-terminal XPD mutated protein are also deficient in recruitment of NER proteins downstream of TFIIH. Chigançaş et al[14] also demonstrated that a defect in the NER of CPDs in some TTD/XPD patients can be partially associated with the accessibility of DNA damage in closed chromatin regions.[14] Nishiwaki et al[15] showed that when comparing XPD mutants

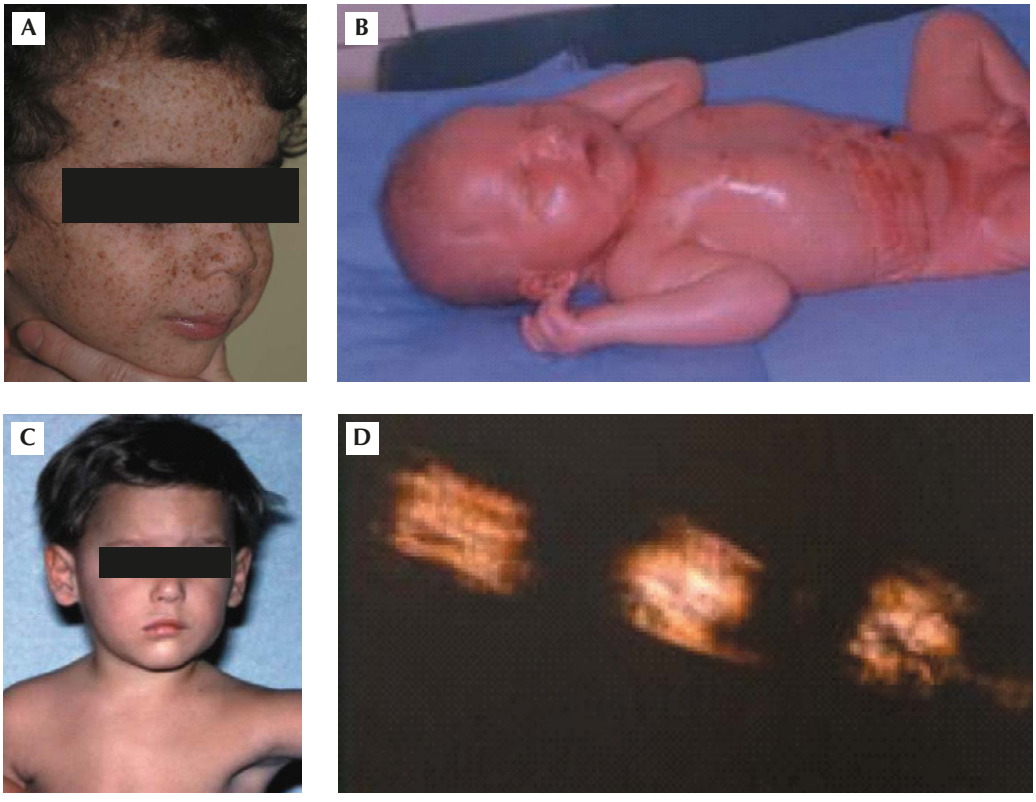

Figure 2 Clinical phenotype of xeroderma pigmentosum (XP) type C, and trichothiodystrophy (TTD). (A) XP type C is characterized by a marked photosensitivity, skin freckling typical of photoaging, and early development of skin cancer. (B) Photosensitive TTD is characterized by ichthyosis, an early marker in the case of collodion baby syndrome, and hair brittleness; patient at birth (collodion baby). (C) The same patient featured in (B) at age 4. Compared with the skin aging of an XP type C patient at age 3 shown in (A). The TTD patient depicted here is XP type B and has minimally associated involvement but limited DNA repair proficiency in vitro (see reference 11 for details). (D) Typical TTD tiger tail pattern of hairs under a polarizing microscope.

giving rise to either XP or TTD phenotypes, TTD cell strains had an approximately 50% decrease in cellular TFIIH content and defective recruitment of TFIIH to DNA damage sites. These studies indicate that molecular mechanisms are related to differences in DNA repair capacity in TTD/XPD versus XP/XPD, but fail to explain the lack of increased incidence of skin cancers in TTD patients. The decrease in catalase activity and its cofactor NADPH observed in XP cells versus TTD cells,[16] which can affect cellular redox status, may prove to be important for defining new therapeutic strategies.

Catalase as a physiological enhancer of photoprotection in keratinocytes of black individuals

In vitro studies have suggested that modulation of the epidermal redox status is a good target for improving natural photoprotection. In vitro pigmented reconstructed epidermis, which was developed in our laboratory in the 1990s, is a good tool for assessing the various components of photoprotection. We demonstrated that phototype affects not only constitutive and reactive melanogenesis, but also antioxidant cell status. In particular, catalase activity was found to be higher in keratinocytes of black individuals.[17] Furthermore, recent evidence suggests that melanosomes could be a physical link for catalase transfer to keratinocytes.[18]

Catalase overexpression limits UVB-induced apoptosis in normal human keratinocytes

UV radiation is a well-known generator of ROS in different types of cells. To neutralize ROS, living cells have acquired several lines of defense systems including non-enzymatic (α–tocopherol and vitamin C) and enzymatic antioxidants at the forefront. When these systems are overwhelmed, degradation systems such as proteasomes and autophagy intervene. Finally, cell death (apoptosis) may occur. Apoptosis is a highly complex process involving extrinsic and intrinsic pathways through which caspase activation is triggered (Figure 3).[19–21] Recent data have shown that in addition to death receptor activation and DNA damage, UVB-induced ROS generation contributes to induction of apoptosis.[22] Reduction of the deleterious effects of UV-induced ROS through an increase in antioxidant defense systems supports this notion.[23–25] However, it is unclear how ROS generation is interrelated to other apoptotic events (Figure 3).

Using UVB irradiation of normal human keratinocytes sustainably overexpressing catalase, MnSOD, and/or CuZnSOD, we investigated the role of UVB-induced ROS production and antioxidant enzymes in cell death and its relationships with other apoptotic pathways.[26] While investigating the kinetics of ROS generation following UVB irradiation, we first showed that UVB irradiation induces an increase in ROS levels at two different time intervals, and

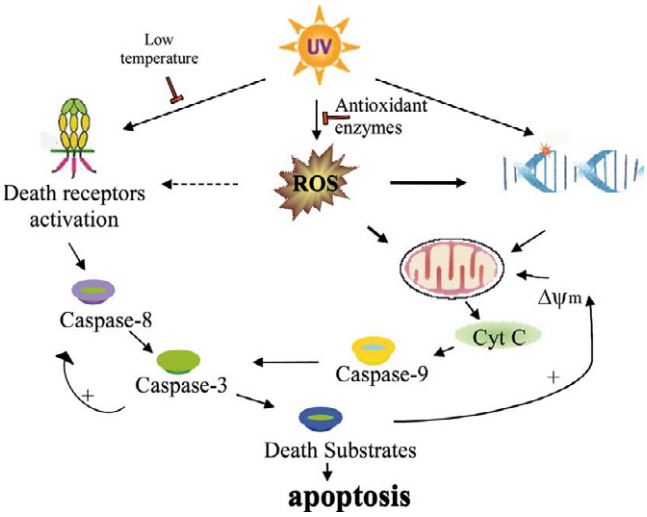

Figure 3 UV-induced apoptosis in the epidermis. UVB-induced death receptor activation, DNA damage, and reactive oxygen species (ROS) generation all contribute to induction of apoptosis. Overexpression of antioxidant enzymes, especially catalase, limits apoptosis mainly through the reduction of a UV-induced intrinsic apoptotic pathway.

that the second (but not the first) increase in ROS production has a major role in UVB-induced apoptosis. We found that catalase overexpression inhibits only the late increase in ROS levels and that this inhibition decreases apoptosis with a reduction of caspase-9 activation accompanied by a decrease in p53. Irradiation at a low temperature (Figure 3) also reduces UV-induced apoptosis in normal keratinocytes independently of any changes in p53, with a decrease in caspase-8 activation. Maintaining cells at a low temperature and catalase overexpression has additive effects on apoptosis reduction, indicating that catalase overexpression mainly reduces the activation of the intrinsic pathway. Our data further suggested that catalase (but not CuZnSOD) overexpression has a protective role against UVB irradiation by preventing DNA damage mediated by a late ROS increase, supporting the therapeutic concept of the possible reinforcement of natural antioxidant photoprotective defenses against sunburn and possibly skin cancer.[26]

Stable genetic modification of human skin equivalents to examine the concept of antioxidant gene therapy and application to XP

To confirm our observations in vitro in a more physiological system, we investigated the effects of UVA and UVB irradiation on reconstructed epidermis made with human keratinocytes transduced by CuZnSOD, MnSOD or catalase. We found that following UVB irradiation, there is a marked decrease in apoptotic keratinocytes (also know as sunburn cells), caspase-3 activation and p53 accumulation in human reconstructed epidermis overexpressing catalase. Moreover, UVA-induced hypertrophy and DNA oxidation (8-oxodeoxyguanosine) are decreased by catalase overexpression.

These effects were not achieved by overexpression of CuZnSOD or MnSOD.[27] To test the hypothesis that reinforcement of antioxidant enzymes affects the phenotypic expression of severe photosensitive disorders, we extended our studies to epidermis reconstructed with XPC keratinocytes, based on previous findings of reduced catalase activity.[11] We found that catalase overexpression can reduce UVB-induced apoptosis in epidermis reconstructed with XPC keratinocytes, and that reduced apoptosis is accompanied by a decrease in p53 accumulation.[28] Direct gene therapy for XPC has been successfully attempted in vitro, and has shown complete correction of repair-defective cellular phenotypes.[29–32] However, in vivo attempts will probably be problematic because of the complete absence of the protein in affected patients, which means that an immune reaction is likely to occur in case of synthesis recovery. While not correcting the gene defect, our study[28] indicates that indirect gene therapy using antioxidant enzymes may be helpful in limiting photosensitivity in XPC. Furthermore, this novel type of therapy is theoretically applicable to other disorders of photoprotection, downstream of a primary gene defect, that are characterized by the accumulation of ROS.

ROS as second messengers: HIF-1α as a major regulator of UV responses including NER

HIF-1α has exquisite sensitivity to oxygen levels in the cellular environment and participates in the regulation of numerous genes involved in angiogenesis, glycolysis, apoptosis, migration and metastasis.[33–36] HIF-1 is a heterodimeric factor consisting of two α and β subunits.[37] In normoxia, HIF-1α is rapidly targeted for ubiquitination and proteasomal degradation. The hydroxylation of HIF-1α mediated by prolyl-hydroxylases is a prerequisite for this degradation.[33,38,39] Reduction in prolyl-hydroxylase activity under hypoxic conditions results in the stabilization and the accumulation of HIF-1α. Hypoxia-mediated ROS modulation and posttranscriptional modification (mainly phosphorylation) of HIF-1α is important in its stabilization and/or transcriptional activation process.[40,41]

We decided to investigate HIF-1α expression and its relationship with ROS production in keratinocytes in response to UVB irradiation, because ROS are known to influence HIF-1α regulation and hypoxia-induced apoptosis.[42] Moreover, both the involvement of HIF in the modulation of cell responses to growth factors under normoxia in a ROS-dependent manner[42,43] and the UVB-mediated induction of vascular endothelial growth factor (one of the major HIF-1α target genes),[44–46] lends further support to our hypothesis.

We demonstrated, for the first time, the mechanisms contributing to the modulation of HIF-1α in response to UVB-induced ROS production and the effect of HIF-1α on UVB-induced apoptosis. We found that UVB induces a biphasic HIF-1α variation through ROS generation with a rapid down-regulation of HIF-1α followed by a gradual increase. Our data revealed the following: (1) that the early increase in ROS levels is mostly dependent on the activity of a cytoplasmic NADPH oxidase, while the late increase in ROS levels originates from the mitochondria, and (2) that HIF-1α down-regulation immediately after irradiation is dependent on ROS produced by NADPH oxidase, whereas its late increase in a phosphorylated form is induced by ROS produced in mitochondria through c-Jun N-terminal kinase and p38 mitogen-activated protein kinase activation (Figure 4).

Our results indicate that HIF-1α exerts a proapoptotic effect through both the extrinsic (caspase-8 activation) and the intrinsic (caspase-9 activation) apoptotic pathways. Furthermore, we found that p53 activation following UV irradiation was affected by HIF-1α expression levels, suggesting a functional link between UVB-induced ROS production, HIF-1α variation, and DNA repair. This finding raised an important question: how does modulation of HIF-1α regulate DNA repair? This could possibly be answered by considering the following points: first, UV irradiation can induce transcriptional expression of NER factors in cells;[47,48] second, UV irradiation modulates the expression of HIF-1α, a transcription factor;[49] and third, software analysis has shown multiple potential hypoxia response elements (HRE) in the promoter regions of two important NER enzymes, XPC and XPD. Therefore, we tested the hypothesis that HIF-1α plays a critical role in the transcriptional regulation of XPC and XPD expression. The two NER enzymes (XPC and XPD) selected for functional studies in human keratinocytes were found to be regulated by HIF-1α in a biphasic manner. Two HREs in the XPC promoter (hereafter named XPC-HRE1 and XPC-HRE2) were found to be critical for basal and UVB-induced expression of XPC. A region of seven overlapping HREs in the XPD promoter has a crucial role in XPD expression. Our study demonstrated that the XPC-HRE2 region includes a HIF-1α-binding site and a Sp-1 binding site with overlapping bases. We found that binding of HIF-1α to XPC-HRE2 in non-irradiated cells inhibits the attachment of Sp-1 to the XPC promoter. The immediate down-regulation of HIF-1α after irradiation permits Sp-1 to bind to the XPC promoter, leading to the initial increase in XPC mRNA expression. Finally, there is late accumulation of phosphorylated HIF-1α protein following irradiation up-regulated XPC mRNA expression by direct binding to XPC-HRE1. Analysis of the repair kinetics of 6-4PPs and CPD revealed that HIF-1α down-regulation leads to an increased rate of immediate removal of both photolesions but attenuates their late removal following UVB irradiation. Quantitative ChIP assays further revealed putative HREs in the genes encoding other DNA repair proteins (XPB, XPG, chondroitin sulfate A and B[48]), suggesting that an additional role of HIF-1α in the epidermal system could be that of an important regulator of the UV-dependent DNA repair machinery.[50]

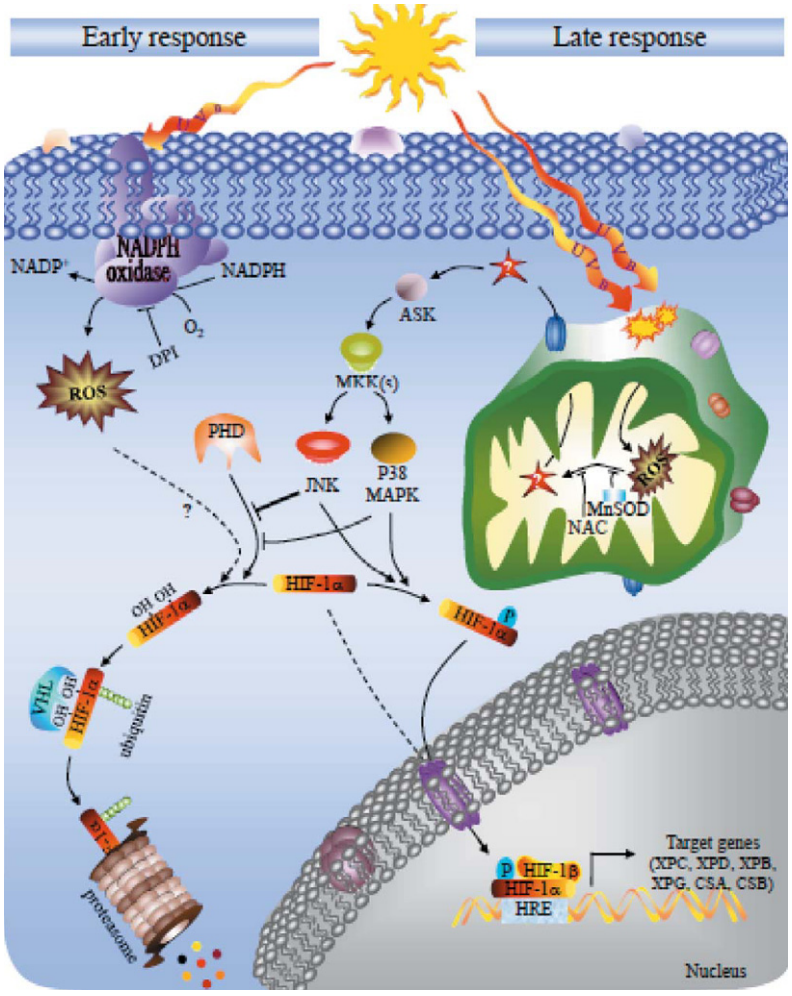

Figure 4 Hypoxia-inducible factors 1 (HIF-1), UV and DNA repair. Nucleotide excision repair genes have recently been discovered as HIF-1α target genes (see text for explanations).

ROS, XPC, HIF-1 and the Warburg effect: deciphering the molecular aspects

Whatever causes underlie cancer (e.g. virus, mutation and DNA damage), tumor metabolism appears to be similar across a broad range of cancer types. Otto Warburg[51] first showed the propensity for cancer cells to convert glucose to lactate even in the presence of oxygen, a phenomenon he had discovered in his work on tissue slices, at the beginning of the last century. He proposed that increased glycolysis is a paradigmatic feature of cancer cells. He showed that malignant cells are more dependent on the glycolytic pathway for ATP generation compared with normal cells, even in the presence of sufficient oxygen concentration. He proposed that a respiratory deficiency might result in neoplastic transformation,[51] prompting many investigators to analyze the metabolism of tumor cells.[52–54] These analyses revealed that cancer cells have a higher rate of glycolysis, an increased rate of glucose transport, increased pentose phosphate pathway activity, decreased numbers of mitochondria, and a reduction in mitochondrial oxidative phosphorylation proteins and activity compared with normal cells.[52–54] The cause of the "Warburg Effect" has been much debated, and is still not well understood (reviewed by Stubbs and Griffiths[55]). However, the Warburg effect has been useful in clinical practice: positron emission tomography scans use the increased uptake of glucose as a diagnostic tool in oncology (Figure 5). Recent studies indicate that this metabolism variation in cancer cells could be related to somatic mutations in mitochondrial DNA, increased oxidative stress, and/or adaptation to environmental hypoxia.[53,56,57] Investigation of pathways underlying the metabolic alteration has revealed that TP53, HIF-1α, c-MYC and PI3K could be involved in the balance between glycolysis flux and mitochondrial respiration through regulation of different factors, and that changes in the expression of these factors could influence the metabolic shift.[58–60]

However, the causal relationship between genomic mutations, the Warburg effect, and increased ROS levels in tumor induction remains unclear.[53,56,61] Furthermore, there is no clear mechanism(s) linking genomic mutations and modified cellular bioenergetics. To understand the relationships between these factors, we speculated that cells with an increased predisposition to becoming cancerous, or cells with the capacity to accumulate mutations, could be helpful in

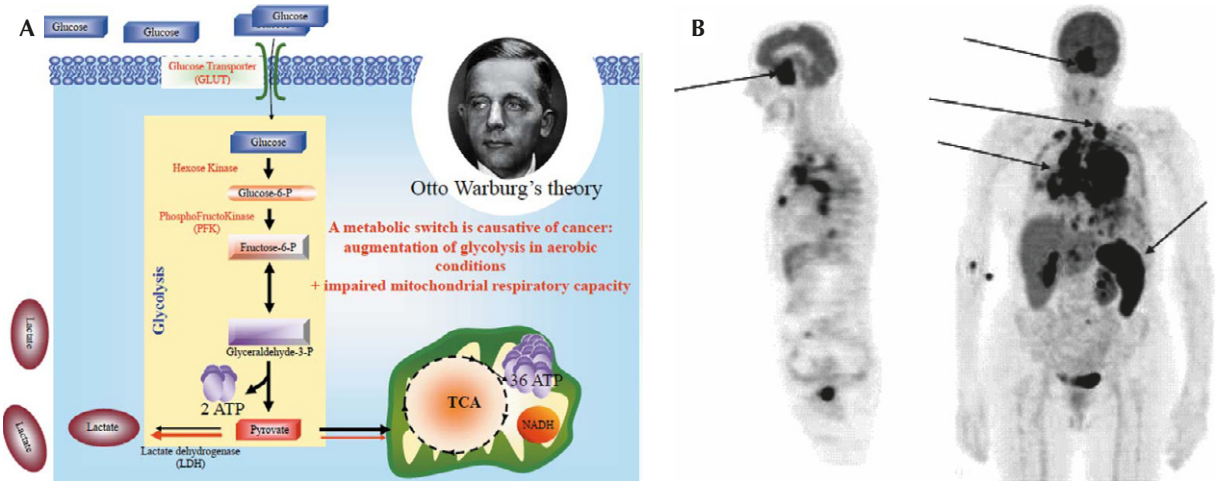

Figure 5 (A) The basic biochemical defect of the so-called "Warburg effect". Insert, Otto Warburg, Nobel Prize, 1931. (B) Positron emission tomography scan. Arrows indicate increased uptake of fluorodeoxyglucose, a glucose analogue used as the radionuclide tracer, in metastases (see text for details).

elucidating this mechanism. XP cells are a useful tool since they manifest varying decreases in NER, rendering them more susceptible to neoplastic transformation and cancer induction. NER is the major pathway for repairing numerous types of DNA damage including helix-distorting lesions produced mostly by UV radiation and bulky lesions created by carcinogenic chemicals and chemotherapeutic drugs. Among NER monogenic diseases, only XPC patients have proficient TCR and defective GGR. Therefore, in these patients, the cells accumulate mutations in nontranscribed parts of the genome, leading to neoplastic transformation.[9] Interestingly, studies with XPC knock-out mice have shown a predisposition to many types of UV-induced cancers, as well as the occurrence of spontaneous ones,[62,63] indicating that XPC also plays a role in the removal of non-UV-related DNA lesions. Moreover, bladder cancer progression has been shown to strongly correlate with attenuated XPC protein expression;[64] lymphocytes from XPC$^{-/-}$ mice accumulate spontaneous lesions in the hypoxanthine guanine phosphoribosyltransferase gene.[65] Interestingly, the most frequent mutations are G to T transversions, which also result from oxidative processes,[65] suggesting a causative role for ROS. Thus, XPC appears to be a good candidate to analyze the relative contribution of three major components of carcinogenesis: genomic instability, increased ROS production, and the Warburg effect. Preliminary results of our group indicate that XPC invalidation induces the Warburg effect without UV (Rezvani et al, unpublished data)

Conclusion

Based on the work developed in the last decades on patients and cells of rare disorders of photoprotection in our department, those appear as very useful models to understand complex diseases. Monogenic disorders of innate photoprotection are especially good cancer models, because they are triggered by a standardizable environmental hazard (UV irradiation). Based on the biochemical differences noted between two clinically distinct but molecularly related NER diseases, XP and TTD, we have been able to provide evidence that antioxidant enzyme therapy is effective for photoprotection, at least during the acute stage following UV irradiation. Further studies are necessary to determine if this therapy is effective following long term exposure to UV. We have shown the critical role of HIF-1 as a sensor not only of hypoxia but of other stresses in the epidermis, and as a major regulator of NER responses. Because the Warburg effect, a constant feature in cancer cells, can be detected downstream of XPC silencing, understanding the mechanisms leading to increased ROS in these cells is now a priority. This may ultimately lead to the implementation of new strategies for the prevention of skin as well as other cancers.

Acknowledgments

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References

1. Eller MS, Ostrom K, Gilchrest BA. DNA damage enhances melanogenesis. *Proc Natl Acad Sci USA* 1996;93:1087–92.
2. Holleran WM, Uchida Y, Halkier-Sorensen L, et al. Structural and biochemical basis for the UVB-induced alterations in epidermal

barrier function. *Photodermatol Photoimmunol Photomed* 1997; 13:117–28.
3. Gläser R, Navid F, Schuller W, et al. UV-B radiation induces the expression of antimicrobial peptides in human keratinocytes in vitro and in vivo. *J Allergy Clin Immunol* 2009;123:1117–23.
4. Heck DE, Gerecke DR, Vetrano AM, Laskin JD. Solar ultraviolet radiation as a trigger of cell signal transduction. *Toxicol Appl Pharmacol* 2004;195:288–97.
5. McArdle F, Rhodes LE, Parslew R, et al. UVR-induced oxidative stress in human skin in vivo: effects of oral vitamin C supplementation. *Free Radic Biol Med* 2002;33:1355–62.
6. Punnonen K, Puntala A, Ahotupa M. Effects of ultraviolet A and B irradiation on lipid peroxidation and activity of the antioxidant enzymes in keratinocytes in culture. *Photodermatol Photoimmunol Photomed* 1991;8:3–6.
7. Shindo Y, Witt E, Han D, et al. Recovery of antioxidants and reduction in lipid hydroperoxides in murine epidermis and dermis after acute ultraviolet radiation exposure. *Photodermatol Photoimmunol Photomed* 1994;10:183–91.
8. Tyrrell RM. Ultraviolet radiation and free radical damage to skin. *Biochem Soc Symp* 1995;61:47–53.
9. Cleaver JE. Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat Rev Cancer* 2005;5:564–73.
10. Moriwaki S, Kraemer KH. Xeroderma pigmentosum-bridging a gap between clinic and laboratory. *Photodermatol Photoimmunol Photomed* 2001;17:47–54.
11. Vuillaume M, Daya-Grosjean L, Vincens P, et al. Striking differences in cellular catalase activity between two DNA repair-deficient diseases: xeroderma pigmentosum and trichothiodystrophy. *Carcinogenesis* 1992;13:321–8.
12. Morice-Picard F, Cario-André M, Rezvani H, et al. New clinicogenetic classification of trichothiodystrophy. *Am J Med Genet A* 2009;149A:2020–30.
13. Hashimoto S, Egly JM. Trichothiodystrophy view from the molecular basis of DNA repair/transcription factor TFIIH. *Hum Mol Genet* 2009;18:R224–30.
14. Chigançaş V, Lima-Bessa KM, Stary A, et al. Defective transcription/repair factor IIH recruitment to specific UV lesions in trichothiodystrophy syndrome. *Cancer Res* 2008;68:6074–83.
15. Nishiwaki T, Kobayashi N, Iwamoto T, et al. Comparative study of nucleotide excision repair defects between XPD-mutated fibroblasts derived from trichothiodystrophy and xeroderma pigmentosum patients. *DNA Repair (Amst)* 2008;7:1990–8.
16. Hoffschir F, Daya-Grosjean L, Petit PX, et al. Low catalase activity in xeroderma pigmentosum fibroblasts and SV40-transformed human cell lines is directly related to decreased intracellular levels of the cofactor, NADPH. *Free Radic Biol Med* 1998;24: 809–16.
17. Bessou-Touya S, Picardo M, Maresca V, et al. Chimeric human epidermal reconstructs to study the role of melanocytes and keratinocytes in pigmentation and photoprotection. *J Invest Dermatol* 1998;111:1103–8.
18. Maresca V, Flori E, Briganti S, et al. Correlation between melanogenic and catalase activity in in vitro human melanocytes: a synergic strategy against oxidative stress. *Pigment Cell Melanoma Res* 2008;21:200–5.
19. Okada H, Mak TW. Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat Rev Cancer* 2004;4:592–603.
20. Budihardjo I, Oliver H, Lutter M, et al. Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 1999; 15:269–90.
21. Denning MF, Wang Y, Tibudan S, et al. Caspase activation and disruption of mitochondrial membrane potential during UV radiation-induced apoptosis of human keratinocytes requires activation of protein kinase C. *Cell Death Differ* 2002;9:40–52.
22. Kulms D, Zeise E, Poppelmann B, Schwarz T. DNA damage, death receptor activation and reactive oxygen species contribute to ultraviolet radiation-induced apoptosis in an essential and independent way. *Oncogene* 2002;21:5844–51.
23. Maalouf S, El-Sabban M, Darwiche N, Gali-Muhtasib H. Protective effect of vitamin E on ultraviolet B light-induced damage in keratinocytes. *Mol Carcinog* 2002;34:121–30.
24. Morley N, Curnow A, Salter L, et al. N-acetyl-L-cysteine prevents DNA damage induced by UVA, UVB and visible radiation in human fibroblasts. *J Photochem Photobiol B* 2003;72:55–60.
25. Pelle E, Huang X, Mammone T, et al. Ultraviolet-B-induced oxidative DNA base damage in primary normal human epidermal keratinocytes and inhibition by a hydroxyl radical scavenger. *J Invest Dermatol* 2003;121:177–83.
26. Rezvani HR, Mazurier F, Cario-André M, et al. Protective effects of catalase overexpression on UVB-induced apoptosis in normal human keratinocytes. *J Biol Chem* 2006;281:17999–8007.
27. Rezvani HR, Cario-André M, Pain C, et al. Protection of normal human reconstructed epidermis from UV by catalase overexpression. *Cancer Gene Ther* 2007;14:174–86.
28. Rezvani HR, Ged C, Bouadjar B, et al. Catalase overexpression reduces UVB-induced apoptosis in a human xeroderma pigmentosum reconstructed epidermis. *Cancer Gene Ther* 2008;15:241–51.
29. Zhou NY, Bates SE, Bouziane M, et al. Efficient repair of cyclobutane pyrimidine dimers at mutational hot spots is restored in complemented Xeroderma pigmentosum group C and trichothiodystrophy/xeroderma pigmentosum group D cells. *J Mol Biol* 2003;332:337–51.
30. Bernerd F, Asselineau D, Frechet M, et al. Reconstruction of DNA repairdeficient xeroderma pigmentosum skin in vitro: a model to study hypersensitivity to UV light. *Photochem Photobiol* 2005; 81:19–24.
31. Marchetto MC, Correa RG, Menck CF, Muotri AR. Functional lentiviral vectors for xerodermapigmentosum gene therapy. *J Biotechnol* 2006;126:424–30.
32. Zeng L, Quilliet X, Chevallier-Lagente O, et al. Retrovirus mediated gene transfer corrects DNA repair defect of xeroderma pigmentosum cells of complementation groups A, B and C. *Gene Ther* 1997;4:1077–84.
33. Bardos JI, Ashcroft M. Negative and positive regulation of HIF-1: a complex network. *Biochim Biophys Acta* 2005;1755:107–20.
34. Harris AL. Hypoxia-1-a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002;2:38–47.
35. Maxwell PH, Dachs GU, Gleadle JM, et al. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. *Proc Natl Acad Sci USA* 1997;94:8104–09.
36. Semenza GL. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 2001;107:1–3.
37. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loophelix- PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci USA* 1995;92:5510–4.
38. Bardos JI, Chau NM, Ashcroft M. Growth factor-mediated induction of HDM2 positively regulates hypoxia-inducible factor 1alpha expression. *Mol Cell Biol* 2004;24:2905–14.
39. Lee JW, Bae SH, Jeong JW, et al. Hypoxia-inducible factor (HIF-1) alpha: its protein stability and biological functions. *Exp Mol Med* 2004;36:1–12.
40. Bell EL, Emerling BM, Chandel NS. Mitochondrial regulation of oxygen sensing. *Mitochondrion* 2005;5:322–32.
41. Brahimi-Horn C, Mazure N, Pouyssegur J. Signalling via the hypoxia-inducible factor-1alpha requires multiple posttranslational modifications. *Cell Signal* 2005;17:1–9.
42. Kietzmann T, Gorlach A. Reactive oxygen species in the control of hypoxia-inducible factormediated gene expression. *Semin Cell Dev Biol* 2005;16:474–86.
43. Haddad JJ, Land SC. A non-hypoxic, ROS-sensitive pathway mediates TNF-alpha-dependent regulation of HIF-1alpha. *FEBS Lett* 2001;505:269–74.

44. Blaudschun R, Brenneisen P, Wlaschek M, et al. The first peak of the UVB irradiation-dependent biphasic induction of vascular endothelial growth factor (VEGF) is due to phosphorylation of the epidermal growth factor receptor and independent of autocrine transforming growth factor alpha. *FEBS Lett* 2000;474: 195–200.
45. Kim MS, Kim YK, Eun HC, et al. All-trans retinoic acid antagonizes UV induced VEGF production and angiogenesis via the inhibition of ERK activation in human skin keratinocytes. *J Invest Dermatol* 2006;126:2697–706.
46. Mildner M, Weninger W, Trautinger F, et al. UVA and UVB radiation differentially regulate vascular endothelial growth factor expression in keratinocyte-derived cell lines and in human keratinocytes. *Photochem Photobiol* 1999;70:674–9.
47. Sugasawa K, Okuda Y, Saijo M, et al. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* 2005;121:387–400.
48. Wang QE, Praetorius-Ibba M, Zhu Q, et al. Ubiquitylation-independent degradation of Xeroderma pigmentosum group C protein is required for efficient nucleotide excision repair. *Nucleic Acids Res* 32007;5:5338–50.
49. Rezvani HR, Dedieu S, North S, et al. Hypoxia-inducible factor-1alpha, a key factor in the keratinocyte response to UVB exposure. *J Biol Chem* 2007;282:16413–22.
50. Rezvani HR, Mahfouf W, Ali N, et al. Hypoxia-inducible factor-1{alpha} regulates the expression of nucleotide excision repair proteins in keratinocytes. *Nucleic Acids Res* 2010;38:797–809.
51. Warburg O. On the origin of cancer cells. *Science* 1956;123: 309–14.
52. Modica-Napolitano JS, Singh KK. Mitochondria as targets for detection and treatment of cancer. *Expert Rev Mol Med* 2002;4: 1–19.
53. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* 2004;4:891–9.
54. Brandon M, Baldi P, Wallace DC. Mitochondrial mutations in cancer. *Oncogene* 2006;25:4647–62.
55. Stubbs M, Griffiths JR. The altered metabolism of tumors: HIF-1 and its role in the Warburg effect. *Adv Enzyme Regul* 2009;Nov 6. [Published online]
56. Clerkin JS, Naughton R, Quiney C, Cotter TG. Mechanisms of ROS modulated cell survival during carcinogenesis. *Cancer Lett* 266:30–6.
57. Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer* 2005;5:857–66.
58. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. *Genes Dev* 2009;23:537–48.
59. Bensaad K, Vousden KH. p53: new roles in metabolism. *Trends Cell Biol* 2007;17:286–91.
60. Yeung SJ, Pan J, Lee MH. Roles of p53, MYC and HIF-1 in regulating glycolysis—the seventh hallmark of cancer. *Cell Mol Life Sci* 2008;65:3981–99.
61. Pelicano H, Martin DS, Xu RH, Huang P. Glycolysis inhibition for anticancer treatment. *Oncogene* 2006;25:4633–46.
62. Hollander MC, Philburn RT, Patterson AD, et al. Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc Natl Acad Sci USA* 102: 13200–5.
63. Miccoli L, Burr KL, Hickenbotham P, et al. The combined effects of xeroderma pigmentosum C deficiency and mutagens on mutation rates in the mouse germ line. *Cancer Res* 2007;67: 4695–9.
64. Chen Z, Yang J, Wang G, et al. Attenuated expression of xeroderma pigmentosum group C is associated with critical events in human bladder cancer carcinogenesis and progression. *Cancer Res* 2007;67:4578–85.
65. Wijnhoven SW, Kool HJ, Mullenders LH, et al. Age-dependent spontaneous mutagenesis in Xpc mice defective in nucleotide excision repair. *Oncogene* 2000;19:5034–7.