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XERODERMA PIGMENTOSUM: LIVING IN THE DARK BUT WITH HOPE IN THERAPY

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ABSTRACT

Xeroderma pigmentosum patients suffer from extreme photosensitivity caused by a genetic defect in DNA repair pathways. This condition obliges them to live in darkness and avoid sunshine. Although the molecular basis of the defect has been known for more than 40 years now, the treatment possibilities are very limited, and to date all have been focused on the skin. Herein, we summarize the effects of sunlight and the molecular mechanisms implicated in the defects that lead to this syndrome, as well as the strategies that have been tested to alleviate skin manifestations, including cancer. Preclinical attempts to correct genetic defects by means of different gene therapy approaches are also described. All these efforts are now bringing hope and some light into the life of patients and their families.

INTRODUCTION

Sunlight is known to cause damage to genetic material in all living creatures, including human beings. In fact, we are constantly being advised, especially by dermatologists, to avoid overexposure to sunlight in order to protect the skin. This advice is very clear, as one of the long-term effects of sunburn is skin cancer in its various forms, melanoma being the most frightening of these due to the fact that it is very difficult to treat and often leads to death. By multiplying this fear of sunlight at least 1,000 times, we can get a slight idea of how xeroderma pigmentosum (XP) patients feel about venturing outside into daylight, and why there is almost no choice but living in the dark.

XP is a rare hereditary human syndrome characterized by extremely high sensitivity to sunlight, especially in those regions of the skin normally exposed to the sun. The cells of these patients are defective in repair processes related to certain DNA lesions, including those normally induced by sunlight (Fig. 1). As a consequence, the skin of most XP patients develops xerosis (a medical term for dry skin), with hyperpigmentation at an early age, thereby acquiring the appearance of a highly sun-exposed elderly person, although they may in fact be quite young. The main symptoms appear in very early childhood, although sometimes they appear only during the teenage years. In addition, actinic keratoses (scaly crusty bumps) and many types of tumors (squamous cell carcinomas, angiomas and fibromas) occur very early in the skin of XP patients. It should be mentioned that XP syndrome features are not only restricted to the skin, as some patients may also develop internal tumors and may present neurological complications, all characterized by progressive deterioration.

There is no cure for XP patients. The available treatments are extremely limited, living in the dark with strict avoidance of exposure to sunlight from a very early age on being the best alternative for preventing most skin manifestations, including premature cancer. Most families with affected children simply adopt habits that invert night and day, whenever possible. As a result, youngsters affected by XP syndrome are sometimes called "children of the moon". An improvement in quality of life requires appropriate and frequent medical advice from dermatologists, as well as surgical tumor resection as soon as detected. Strict solar protection from an early age on, including light-protective clothing and face visors, helps to reduce skin-related problems in these patients. However, even the strict avoidance of exposure to sunlight cannot prevent the development of neurological disorders. In tropical countries with bright weather and frequent sunny days, skin problems become of greater concern. Unfortunately, these countries are often poor and medical care is often deficient, with both the lack of information and late diagnosis (if any) often leading to dramatic consequences.

The need for alternative treatments has led to vigorous basic research in the field, thereby giving rise to a certain hope for XP patients. Easy access plus a large surface make the skin an attractive target for developing new systems for delivering proteins, or even genes themselves, capable of correcting the specific gene defect.

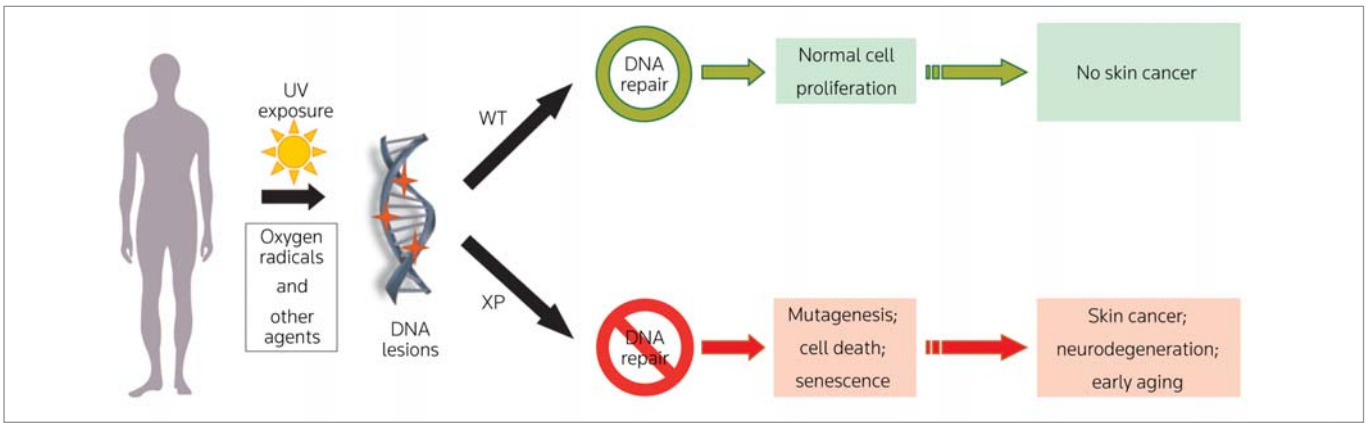

Figure 1. Xeroderma pigmentosum patients have defective DNA repair processes that render them sensitive to sunlight, with several skin manifestations, including skin cancer. Some of the patients also present other clinical features, such as early aging and neurodegeneration. The latter symptoms are thought to be caused by other DNA-damaging agents normally present throughout life, such as oxygen radicals.

Herein, we will initially focus on the main biochemical and cellular disturbances leading to clinical symptoms in XP patients, and then describe strategies that may lead to a definitive cure, or at least improve quality of life.

THE DANGER HIDDEN IN THE SUN

The human genome is constantly being exposed to a number of damaging agents from endogenous or exogenous sources, capable of causing lesions in DNA. These lesions can lead to cell death and senescence, which ultimately contribute to cancer and aging processes in multicellular organisms. Sunlight is one such genotoxic agent, since it is also composed of ultraviolet (UV) light, a well-known DNA-damaging agent. UV light is normally classified according to wavelength in UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm). The stratospheric ozone layer entirely blocks the UVC component of sunlight, but part of the UVB and most of the UVA reach the Earth's surface, and thus can cause damage to living organisms. UVB produces mainly two types of photoproducts, cyclobutane pyrimidine dimer (CPD) and pyrimidine-pyrimidone(6-4) (6-4PP). In the case of UVA, oxidative DNA damage is also produced, in addition to less frequent CPD and 6-4PP (1).

To deal with these and other genotoxic forms of assault, cells have evolved mechanisms for removing lesions, known as DNA repair. In humans, UV-induced photoproducts are mainly repaired by nucleotide excision repair (NER), a flexible mechanism that eliminates a broad spectrum of lesions and includes the direct and/or indirect participation of more than 30 proteins. Briefly, processing DNA lesions by NER (Fig. 2) involves five steps: 1) recognition of the lesion; 2) opening of the DNA helix; 3) incision of the damaged DNA strand followed by displacement of the lesion-containing oligonucleotide; 4) DNA resynthesis; and 5) ligation (reviewed in [2] and [3]). Although we will mainly discuss strategies for the potential therapy of the XP syndrome, deficiencies in NER can also lead to other inherited developmental genetic diseases, such as trichothiodystrophy (TTD), the Cockayne syndrome (CS), the cerebro–oculo–facial–skeletal syndrome (COFS) and the UV-sensitive syndrome ($UV^{S}S$), all of which have photosensitivity as a common feature.

THE CHILDREN OF THE MOON

XP was first described in 1874, although its molecular basis was only recognized almost a century later, with the demonstration of reduced DNA repair replication in cells from XP patients (4). In fact, XP patients are NER-deficient and can be assigned to eight complementation groups: XPA through G (classical XP) and a variant form, XPV. As XPA to XPG proteins participate in the NER cascade, classical XP cannot properly perform this repair pathway. On the other hand, XPV patients exhibit normal NER activity, their phenotype being related to defective translesion DNA polymerase eta (5), an important protein in cell tolerance to damaged DNA.

This syndrome is rare and hereditary, and is transmitted as an autosomal recessive trait. Its frequency varies worldwide and is estimated to be 1 per million in the U.S. and Europe (6), although it can be higher in other countries such as Japan, with 1 per 100,000 (7). The clinical hallmarks used for diagnosis of the XP syndrome include severe photosensitivity, poikiloderma, dryness (xerosis), premature skin aging and malignant tumors (squamous cell cancers, basal cell cancers and melanoma), in addition to various neurological and ophthalmological symptoms (8). Apart from photosensitivity, patients also show impaired immune responses. The incidence of skin tumors is almost 1,000 times higher than in the general population, with a 30-year reduction in life expectancy (9). Intriguingly, the range of clinical manifestations is highly heterogeneous, varying from mildly to severely affected, and includes impaired neurological capabilities. The latter are observed in XPA patients and in individuals where the phenotype of XP is combined with CS (XP/CS), and with defective *XPB*, *XPD* or *XPG* genes. Therefore, the identification of the complementation group in a patient (which corresponds to the gene affected) may be helpful for the prognosis of the disease itself and, for those defective in *XPC*, *XPE* and *XPV* genes, treatment of the skin manifestations may alleviate the main problems.

THERAPEUTIC STRATEGIES FOR XP PATIENTS

As stated above, the best way to prevent skin problems for XP patients is to avoid exposure to sunlight. Eventually, if protection is insufficient, actinic keratoses and cutaneous malignancies may still

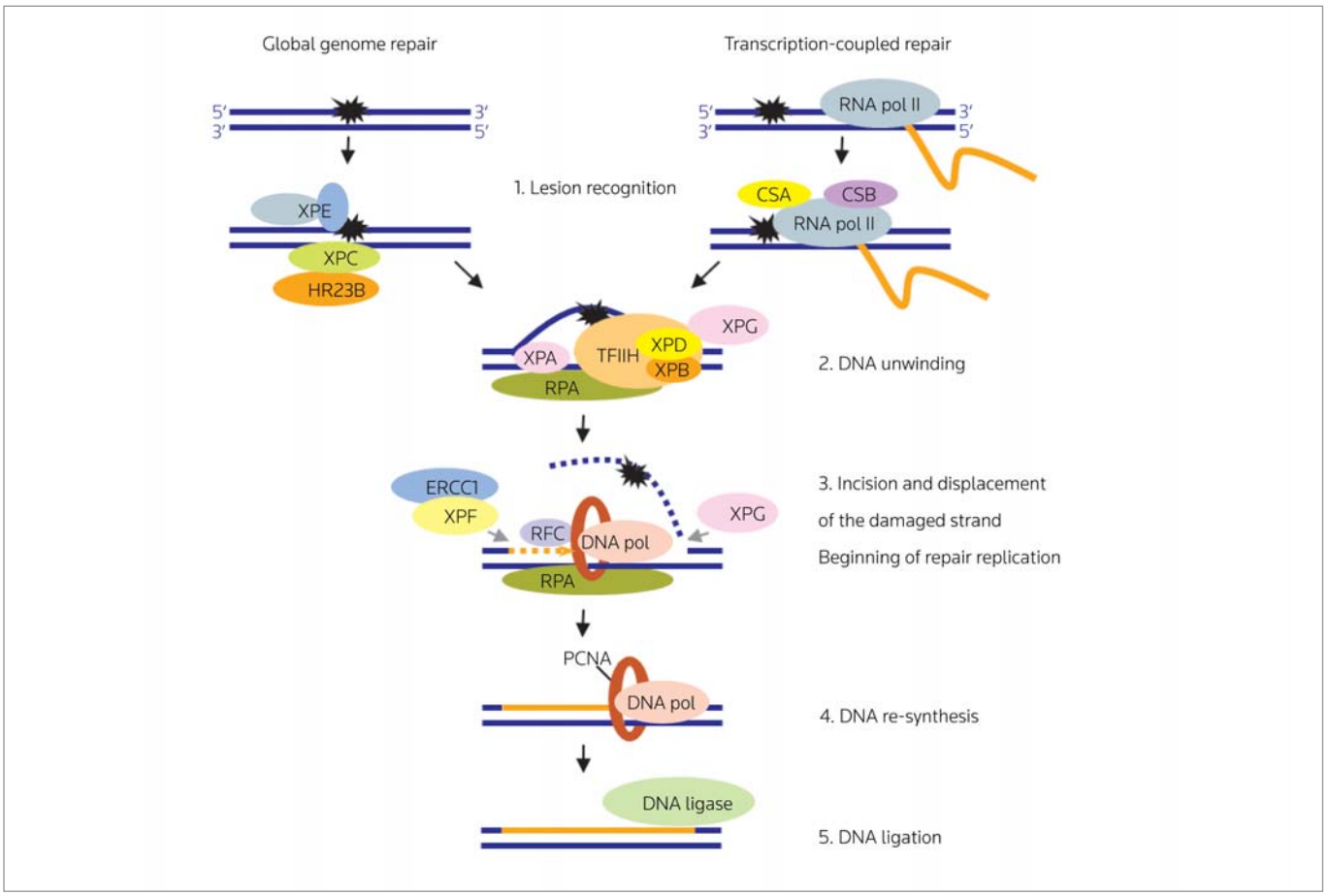

Figure 2. Representation of repair of DNA lesions by nucleotide excision repair (NER). This repair mechanism is comprised of two subpathways —global genome repair (GGR) and transcription-coupled repair (TCR)— that basically differ in the lesion recognition step. For GGR, this function is mainly performed by the heterodimer XPC-HR23B, with the participation of XPE for certain lesions. On the other hand, lesion recognition by TCR is linked to transcriptional arrest due to the blockade of RNA polymerase II by the distorting lesion. Subsequent steps are common to both subpathways.

appear. Chemotherapy and ionizing radiation should be avoided or very carefully planned, with minimal doses, as these patients are more susceptible to many forms of classical treatment. Thus, tumors should preferentially be removed surgically, followed by resurfacing with autologous skin grafts. However, as multiple lesions may often appear on the face, the risk of scarring is high.

Chemoprevention has been tried with limited success. Retinoids are often used for improving aged skin and thus have been proposed for XP patients. Oral isotretinoin is effective for reducing the development of skin cancer in some patients (10), although it is also associated with strong systemic side effects, and treatment interruption results in the rapid appearance of many new lesions. Thus, dermatologists have almost completely abandoned the use of retinoids in XP therapy.

More recently, the topical use of imiquimod cream has yielded promising results, with healing of tumor lesions and improvement in skin pigmentation and texture, with very few side effects (11, 12). The efficacy of imiquimod is probably due to immunomodulating effects and restoration of immunological memory, which possibly results in a prolonged therapeutic effect, which continues even after interruption of therapy.

The topical application of enzymes capable of correcting the defect in most XP patients was proposed as an alternative therapy. Small DNA repair enzymes (CPD photolyase and T4-endonuclease from bacteriophages) were encapsulated into liposome nanoparticles, which provide efficient cutaneous delivery. CPD photolyase simply reverses CPDs back to monomers by using visible light as the energy source. In the case of T4-endonuclease, it nicks the DNA at the site of the lesion, which is then removed by other DNA repair pathways that are not defective in XP cells. Clinical trials in XP patients were carried out, where both enzymes were shown to prevent UV light-induced immunosuppression. After daily application of a T4-endonuclease liposome-encapsulated lotion in XP patients, a decrease in the formation of new actinic keratoses and carcinoma lesions was reported, without adverse effects (13). Continuous lifelong treatment was recommended in this preventive strategy. However, it should be noted that none of these alternatives provided direct correction of genetic defects in XP patients. Approaches for gene transfer and correction in skin cells of XP patients are being tested in preclinical experiments and are described below.

GENE THERAPY FOR XP PATIENTS

Although many different approaches to gene transfer have been developed, viral vectors are still the most efficient available gene transduction system, mainly because they have evolved to enter cells by receptor-mediated endocytosis and escape from endosomes, delivering their genome into nuclei in the process. We will therefore focus on these vectors and describe their potential, limitations and attempts to develop XP gene therapy protocols.

Most efforts have targeted the skin, due to easy access, and also with a mind to preventing many of the problems caused by sunlight (14). Basically, correction can be undertaken using at least three different methods, independent of the gene delivery system (Fig. 3): 1) the gene may be transiently transduced directly into the cells (and in vivo into the skin), but, as it does not integrate in the genome, application must be repeated periodically; 2) the gene may be delivered to skin cells ex vivo, whereupon it integrates randomly in the genome, so that corrected cells can be transferred to reconstruct the patient's skin; and 3) XP cell genes may be directly corrected by targeting homologous recombination.

Adenovirus- and adeno-associated virus-mediated transient gene transduction to the skin

Gene transfer vectors based on adenoviruses have been successfully used over the past years, as they are efficient in transducing genes to many cell types. Furthermore, after entering the cells, adenovirus genomes remain extrachromosomally in the nuclei, thereby avoiding the genotoxicity usually related to insertional mutagenesis (15).

Experiments using recombinant adenovirus have been successful in the complementation of XP-A, XP-C, XP-D and XP-V cells (16). Indeed, the expression of the respective heterologous functional proteins in transduced cell populations was high, thereby resulting in recovery of DNA repair ability and increased resistance to UV radiation (17-19). Furthermore, by employing similar complementation assays, these same recombinant adenoviruses have also been used for determining the XP complementation group of cells obtained from skin biopsies of three Brazilian XP patients (20). Recently, these exciting results obtained in vitro could be reproduced in vivo by using XPA knockout mice, animal models that mimic the human XP phenotype (21, 22). The subcutaneous injection of recombinant adenovirus into the mouse dorsal region led to an extremely efficient expression of the human XPA protein in basal keratinocytes in the epidermis, dermal fibroblasts and hair follicle cells, where skin stem cells are normally encountered. Consequently, the repair ability of these cells in the transduced mice was recovered, thus preventing UVB-induced deleterious skin effects, such as persistent scars and skin hyperkeratosis. More importantly, adenovirus transduction directly into the skin prevented the formation of squamous cell carcinomas, as observed in most of the control knockout mice (23).

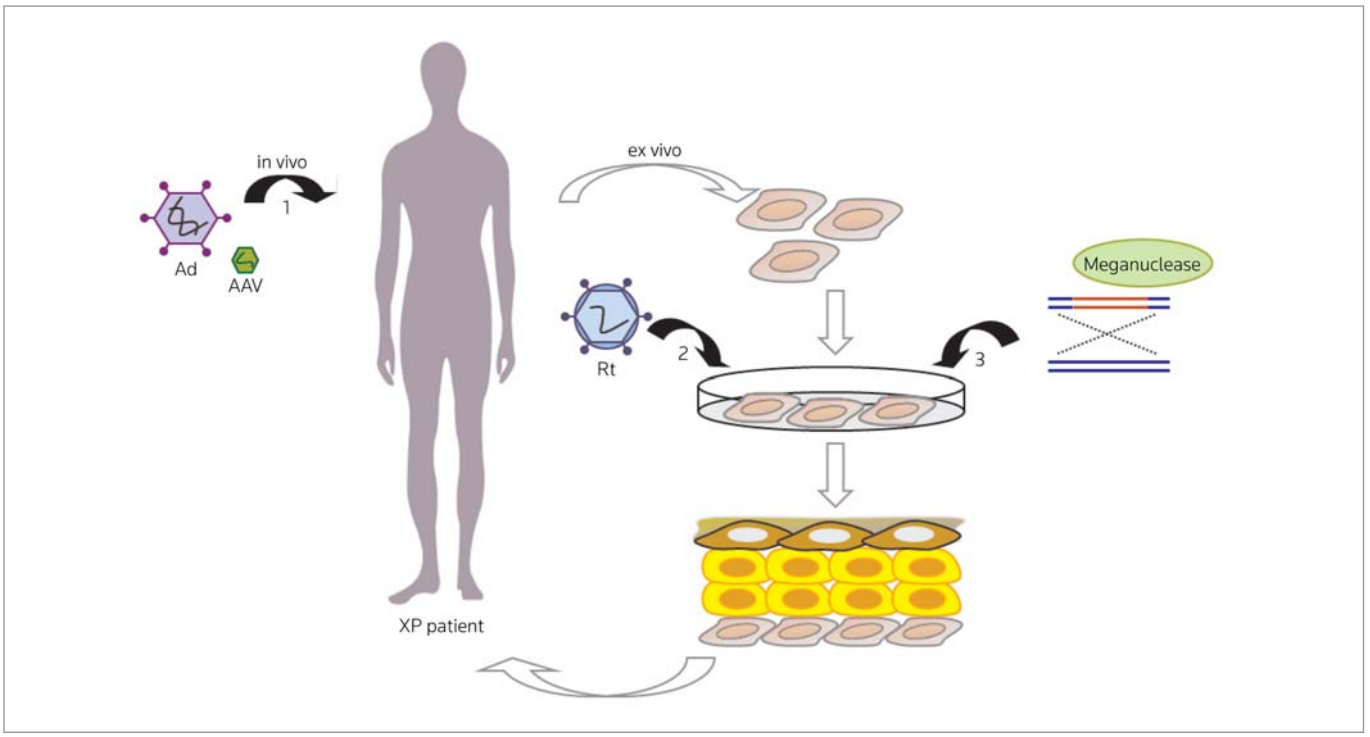

Figure 3. Gene therapy strategies with potential for the treatment of xeroderma pigmentosum (XP) syndrome. Gene therapy protocols aimed at treating XP patients may be performed either in vivo or ex vivo. In the first case, recombinant adenoviral (Ad) or adeno-associated viral (AAV) vectors carrying the wild-type gene would be injected directly into the skin (1), promoting an efficient but transient correction requiring periodic readministration. Ex vivo protocols are based on the construction of complemented equivalent XP skin from autologous skin stem cells, which would be grafted back onto the patient. Complementation of patient's cells could either be performed by employing recombinant retroviral (Rt) vectors (2) or changing the defective gene sequence for the respective functional gene by using enzymes known as meganucleases, which improve homologous recombination (3).

Although this particular study demonstrated the potential use of adenoviral vectors as an efficient means for introducing gene delivery vehicles into skin cells, their use in humans still raises discouraging issues, especially those related to innate and pre-existing immunity, which may limit the effectiveness of subsequent reapplication. As a result, much effort is under way to develop innovative strategies aimed at circumventing adenovirus antigenicity, for example, the use of vectors based on alternative adenovirus serotypes instead of Ad5 (24), chemical modification of viral capsid proteins (25) or modulation of host immune responses using immunosuppressants (26).

On the other hand, the use of adeno-associated viral (AAV) vectors has emerged as a plausible alternative to adenovirus-derived systems. AAV is a nonpathogenic, nonenveloped parvovirus bearing a single-stranded DNA (ssDNA) molecule as the genome, which requires a helper virus, such as an adenovirus or a herpes simplex virus, for productive infection of dividing and nondividing cells (27). Due to their broad cell tropism, AAVs are capable of infecting a vast number of tissues and organs, including nerve cells and the skin. In fact, bioengineered human skin stably expressing green fluorescent protein (GFP) mediated by an AAV-derived vector has already been generated (28). Furthermore, detectable amounts of laminin 5 in basement membranes of newborn mouse skin affected by Herlitz junctional epidermolysis bullosa, a lethal inherited genodermatosis, were obtained following AAV-mediated prenatal gene therapy (29). Another interesting study reported the efficient AAV-mediated transduction of β-galactosidase into porcine skin, reaching not only dividing and postmitotic keratinocytes, but also various skin appendages (30). These findings represent a hopeful picture for employing AAV-derived vectors as an alternative to highly immunogenic adenoviral vectors.

Retrovirus vectors for the permanent correction of XP cells

Retroviruses are enveloped viruses with an ssRNA genome. These RNA molecules are replicated by reverse transcription to a linear double-stranded DNA (dsDNA), which randomly integrates into the host chromosome. Thus, in contrast to adenoviruses, vectors based on retroviruses potentially permit permanent expression of the therapeutic gene, since they are transmitted through generations of cells (31). Retroviral vectors represent an important hallmark in studies aimed at a cure for XP patients. Using such viruses, for the first time, a French research group was able to fully complement XP-A, XP-B and XP-C cells, thereby restoring defective DNA repair activity and UV resistance (32). Moreover, the equivalent to ex vivo XP skin, emulating the normal form and stably expressing the human functional XPC protein, has been obtained by employing retroviral transduced XP-C keratinocytes as founder cells (33). This approach opens up promising possibilities for grafting permanently complemented bioengineered skin back onto XP patients from autologous cells.

In recent years, the use of lentiviruses, a subclass of retroviruses, as a gene delivery system has been motivated as much by their ability to infect quiescent cells as their high transduction efficiency. This feature may be especially interesting for XP patients who suffer neurodegenerative disorders, as well as skin tumors. Encouraging results have shown long-term expression of a variety of transgenes following lentiviral vector-mediated transduction into several different tissues, including neuronal tissues and the skin (34). Indeed, recombinant lentiviruses were capable of fully correcting the XP phenotype in both immortalized and primary cells derived from XP-A, XP-C and XP-D patients, thereby recovering DNA repair ability and increasing cell survival after UV irradiation (35). Furthermore, an alternative XP gene therapy protocol based on lentiviral-mediated catalase overexpression resulted in a marked reduction of sunburn cell formation, caspase-3 activation and p53 accumulation in UV-irradiated XP skin models (36).

Engineering the genome with meganucleases

Gene targeting induced by homologous recombination (HR) is an alternative for correcting cells from XP patients, with the added advantage of truly rectifying the mutated gene locus. In addition to restoring NER capacity, this approach facilitates placing the exogenous sequence into the same genomic context as the endogenous gene, by being submitted to the same regulation as under natural conditions and avoiding insertional mutagenesis and transgene expression silencing.

The strategy is based on the introduction into the cell of an exogenous DNA fragment that shares homology with the target gene and carries the correct sequence to replace the mutation present in the endogenous locus by employing the HR machinery. However, the frequency of HR events in human cells is very low, a crucial matter when dealing with application in gene therapy. The use of meganucleases appeared as an attractive tool for improving HR frequency at least 1,000-fold and with specificity (37), since these enzymes break DNA at specific loci in the cells (38). Several of these nucleases have been identified, but the probability of finding a target site in a chosen gene is very low. Many laboratories have been working on increasing the repertoire of recognition sites by meganucleases through protein engineering (39-43), and custom-designed meganucleases that recognize sequences inside the *XPC* locus gene have been developed (44). Researchers have demonstrated that these recombinant enzymes are specific and can induce high levels of gene correction in mammalian cells. The molecular basis for recognition of the *XPC*-targeted sequence by these engineered meganucleases has been ascertained from further investigation (45). The potential of this methodology to correct cells from XP patients through bioengineered autologous skin therapy opens up new avenues in the search for gene therapy directed to individuals suffering from this syndrome.

STEM CELLS AND CELLULAR THERAPY

Classically, stem cells are defined as having both the potential to self-renew and the ability to give rise to additional differentiated populations (46, 47). Human embryonic stem cells (hESCs) and the recently described induced pluripotent stem (iPS) cells have the intrinsic ability to differentiate in vitro and in vivo into tissues derived from all three embryonic layers (48). More specifically, hESCs have been reported to give rise to skin progenitor cells, such as keratinocyte precursors (49), and to multiply those cell lineages needed for the production of stratified epithelial tissues (50). Human keratinocyte progenitor cell and epidermal progenitor cell grafting have been shown to improve healing in chronically injured patients, and thus could be promising alternatives to the use of cadaver skin allografts (51-55).

Recently, iPS cells were successfully generated from adult human dermal fibroblasts by viral transduction of reprogramming factors that initially included the oncogene c-Myc (48). Further research showed that a process referred to as "footprint-free" reprogramming could also be achieved without c-Myc or viral vector random genome integration (48, 56, 57). These cells could be potentially useful in the reconstruction of autologous tissue-engineered skin in XP therapy. Specifically, fibroblast cultures could be developed from punch biopsies performed on non-UV-exposed areas from XP patients, whereupon the defect could then be corrected by the addition of the missing functional DNA repair gene. Recovery of the DNA repair ability of XP-deficient fibroblasts by adding the missing gene, and without causing toxicity to the cell itself, is well documented in the literature (17, 18, 20, 35). Subsequently, footprint-free and genetically corrected pluripotent stem cells could be derived and further differentiated into keratinocyte precursors that would be used in skin replacement/grafting. These now corrected skin precursor cells would be autologous to the patients, and in their pluripotent undifferentiated form represent an unlimited source of material. Moreover and ideally, the cells could also be cryogenically preserved and expanded whenever needed.

Stable and safe corrective gene transfer is essential to ensuring proper grafting in cutaneous gene therapy. Recently, skin cells derived from a patient with junctional epidermolysis bullosa were expanded and corrected ex vivo. The corrected cells were then successfully employed for preparing epidermal grafts, which were then used in transplanting the patient's own skin (58). The work opened up possibilities for engineered autologous tissue skin therapy in diseases where a mutation is still present in the grafted tissue. Much effort is currently under way regarding the design of compatible viral vectors (i.e., those lacking immunoreactive selection markers, such as neomycin) and the selection of the population of interest (i.e., that incorporating keratinocyte-specific markers when sorting for skin transplantation) with the perspective of autologous grafting onto XP volunteers (59).

CONCLUSIONS AND PERSPECTIVES

Investigations on the cells and clinical history of XP patients have substantially aided in the understanding of the mechanisms underlying the way cells deal with DNA lesions in the genome, thereby leading to knowledge on the intricate network of those DNA repair processes that continuously protect organisms. More importantly, they were crucial for determining the main causes of carcinogenesis, and more recently, for furnishing clues on the aging process. Unfortunately, little success has been achieved in effectively improving the quality of life of these patients and their families. Nevertheless, recent efforts show that stimulation of the immune system may alleviate their skin problems. Moreover, different approaches in gene and cell therapy are under preclinical investigation, and protocols are being proposed to begin clinical trials. Hopefully, these new approaches will allow these patients to lead a more normal life.

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DISCLOSURE

The authors state no conflicts of interest.

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