



DNA repair diseases: What do they tell us about cancer and aging?

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Abstract

The discovery of DNA repair defects in human syndromes, initially in xeroderma pigmentosum (XP) but later in many others, led to striking observations on the association of molecular defects and patients' clinical phenotypes. For example, patients with syndromes resulting from defective nucleotide excision repair (NER) or translesion synthesis (TLS) present high levels of skin cancer in areas exposed to sunlight. However, some defects in NER also lead to more severe symptoms, such as developmental and neurological impairment and signs of premature aging. Skin cancer in XP patients is clearly associated with increased mutagenesis and genomic instability, reflecting the defective repair of DNA lesions. By analogy, more severe symptoms observed in NER-defective patients have also been associated with defective repair, likely involving cell death after transcription blockage of damaged templates. Endogenously induced DNA lesions, particularly through oxidative stress, have been identified as responsible for these severe pathologies. However, this association is not that clear and alternative explanations have been proposed. Despite high levels of exposure to intense sunlight, patients from tropical countries receive little attention or care, which likely also reflects the lack of understanding of how DNA damage causes cancer and premature aging.

Keywords: xeroderma pigmentosum, ultraviolet, DNA damage, oxidative stress, DNA repair.

Introduction

During evolution, the stability of genetic material plays a fundamental role in the maintenance of life. The equilibrium between the benefits of changes in the blueprint of life, which facilitates diversity, and the perpetuation of genetic information is fundamental for evolution. In multicellular organisms, this equilibrium faces other problems, such as the transmission of genetic instability to new generations of cells (gametic cells) and components of the organism structure (somatic cells). For example, in humans, genetic instability in somatic cells has been directly associated with the formation of cancer, and cells may die to avoid these deleterious effects. However, cell death may trigger processes associated with organism aging.

The long DNA molecules, that compose genetic material, are not stable. Exogenous factors (such as ultraviolet, UV, from daylight), chemical products (such as smoke, pollution, and natural and artificial drugs), endogenous agents, and normal byproducts of cell metabolism, including radical oxygen species (ROS) generated during respiration, constantly threaten DNA stability (Andressoo and Hoeijmakers, 2005; Iyama and Wilson 3rd, 2013). These

agents damage the DNA molecule, and cells must attend to these DNA lesions to properly survive and evolve. To maintain the equilibrium necessary for survival and diversity, evolution has equipped all living beings with the tools to either remove or tolerate DNA damage. In general, these tools, called DNA repair mechanisms or pathways, take care of the DNA molecule, avoiding the deleterious consequences of the different types of DNA lesions. DNA repair pathways are highly conserved in nature, indicating these mechanisms are as old as life on Earth.

The importance of DNA repair mechanisms is dramatically demonstrated in certain human syndromes that are characterized as being defective in DNA repair processes, exacerbating the clinical effects of the unrepaired DNA damage, such as xeroderma pigmentosum (XP). Most XP patients are defective in nucleotide excision repair (NER), which removes DNA lesions induced by the UV component of sunlight, although some XP patients have also shown defects in the replication of UV-damaged DNA. The main clinical symptoms of XP patients include the high frequency of tumors in the exposed areas of skin, indicating a clear causative relationship between unrepaired DNA lesions, genetic instability (XP cells are highly mutable) and cancer. Some XP patients, however, present more severe clinical phenotypes, including developmental problems, neurodegeneration and premature aging (Lehmann, 2003; Lehmann *et al.*, 2011; DiGiovanna

and Kraemer, 2012). These severe symptoms have been associated with other syndromes, such as Cockayne syndrome (CS), that have also been shown to be defective in NER (see below) (Cleaver *et al.*, 2013). The reasons for these more severe clinical aspects are still not clearly understood, although, in general, these symptoms implicate unrepaired DNA damage in the process of neurological degeneration and aging. In this work, we discuss the historical data associated with the lack of DNA repair and its deleterious consequences on the human organism, focusing on the scientific contribution of the findings in XP and NER-related human diseases associated with cancer and aging. Other human syndromes, such as ataxia telangiectasia, Fanconi Anemia, Werner syndrome, among others, associated with defects in other DNA repair pathways or DNA damage responses, also show symptoms associated with cancer, aging and developmental and neurological problems. These syndromes also support the relevance of DNA damage in these processes and also indicate that different DNA repair pathways correspond to networks that protect the genome. However, these syndromes will not be reviewed here, and the reader is invited to search for more information in other recent reviews (Kim and D'Andrea, 2012; Knoch *et al.*, 2012; Marinoglou, 2012; Moraes *et al.*, 2012; Shiloh and Ziv, 2013). Moreover, mouse models with defective DNA repair mimic some human clinical phenotypes, including many aging symptoms. Indeed, animal models have provided several clues and numerous data to support this debate, but this information will only be commented in this work when they are applied directly to some aspects of DNA repair. For more information concerning animal models, the reader is referred to recent reviews (Diderich *et al.*, 2011; Goss *et al.*, 2011; Niedernhofer *et al.*, 2011; Gredilla *et al.*, 2012; Jaarsma *et al.*, 2013).

Most studies concerning XP patients have been performed in developed countries (North America and Europe) at high latitudes, with the exception of African-born patients studied in Europe. The doses of UV in the sunshine at high latitudes are low compared with tropical countries, such as Brazil and other Latin American countries. Thus, although exposed to a more aggressive environment, the patients in these countries have primarily remained unattended, except for a few recent studies. More than just identifying gene mutations that affect XP patients in tropical countries, these studies have contributed to the understanding of how DNA repair defects correlate with genotype-phenotype associations. A prospective approach using next-generation sequencing to diagnose the mutations of XP and NER-related patients may help these individuals and their families to address the disease and may eventually contribute with new data for understanding how DNA lesions lead to cancer, neurodegeneration and/or aging.

XP and the Discovery of Molecular Defects in DNA Repair

XP is a rare autosomal recessive disease; most patients exhibit high sensitivity to sunlight. The patients present strong skin freckle-like pigmentation and lesions in areas exposed to sunlight. These skin-aging symptoms may also appear as precancerous skin lesions, such as actinic keratosis, and most XP patients, when unprotected, develop skin cancer (non melanoma and melanoma) before the age of 10. Compared with the entire population, XP patients have a 10,000-fold increase in the incidence of non-melanoma skin tumors and a 2,000-fold increase in the incidence of melanoma before the age of 20 (Kraemer *et al.*, 1994). Certain exposed areas of the face, such as the eyes and mouth, may also suffer from the sensitivity to sunlight and develop lesions, including tumors. Many XP patients present photophobia. The frequency of internal tumors also shows a 10-fold increase in XP patients. Interestingly, 20 to 30% of XP patients develop progressive neurological problems (DiGiovanna and Kraemer, 2012; Sepe *et al.*, 2013). The estimated incidence of XP in the population varies: 1 per 250,000 in USA (Robbins *et al.*, 1974) and 1 per 450,000 in Western Europe (Kleijer *et al.*, 2008). In some places, such as Japan and North Africa, the incidence of XP may be higher (1 per 20,000), likely reflecting the high prevalence of certain mutations due to founder effects (Hirai *et al.*, 2006; Soufir *et al.*, 2010).

XP was the first syndrome associated with a defect in the DNA processing pathway. The dermatologists Moritz Kaposi (Hungarian) and Ferdinand Ritter von Hebra (Austrian) provided the first clinical description of XP patients (Hebra and Kaposi, 1874). By the nineteenth century, the syndrome was correctly considered as an inherited disease and was associated with sunlight exposure. This condition was referred to as xeroderma pigmentosum (for dry and pigmented skin, from the Greek words *xero* and *derma*; and Latin word pigmentosum). Subsequently, DeSanctis and Cacchione (1932) described a more severe form of XP, DeSanctis-Cacchione syndrome (DSC), which was associated with neurological degeneration.

In 1968, James Cleaver identified the molecular defects associated with XP, showing that XP cells were hypersensitive to UV light and unable to perform unscheduled DNA synthesis (UDS) after UV irradiation (Cleaver, 1968). As described below, UDS is part of the NER pathway after the removal of DNA damage. This outstanding discovery provided the first evidence that a cell defect in DNA metabolism is associated with a clinical phenotype, explaining, in part, the high photosensitivity of XP skin.

Soon, clever experiments, fusing cells from different patients, facilitated the identification of complementation groups (De Weerd-Kastelein *et al.*, 1972). Specifically, if the fusion of two different cells resulted in the restoration of UDS, then the cells were deficient in different comple-

mentation groups or genes. If the fusion did not restore UDS, the cells were deficient in the same gene and belonged to the same complementation group. These types of experiments led to the classification of XP in seven different complementation groups (XP-A to XP-G), corresponding to seven genes with mutations that might result in XP. Years later, the cells of some XP patients, however, were demonstrated as NER proficient and capable of UDS. The inability of these cells to replicate DNA after UV irradiation (daughter-strand repair) indicated other problems in terms of dealing with DNA lesions, although these cells maintained the ability to remove these lesions (Lehmann *et al.*, 1975). These cells were referred to as XP variants (XP-V). The subsequent identification of DNA polymerase activity associated with the XPV protein (DNA polymerase ϵ) confirmed that XP-V patients are deficient in translesion synthesis (TLS) (Kannouche and Strydom, 2003; Masutani *et al.*, 2008).

The cells from all XP patients show an increased frequency of mutagenesis after exposure to UV light that is correlated with molecular defects in DNA damage processing, leading to mutagenic consequences in the cells and increased tumorigenesis in patients. Thus, these correlations were the first clear demonstration that DNA damage and mutations cause cancer (van Steeg and Kraemer, 1999; Cleaver, 2000).

Other NER-Defective Genetic Disorders

As described above, approximately 20 to 30% of XP patients also develop progressive neuronal impairment and premature aging. The symptoms include hearing loss, swallowing difficulties and mental retardation. Neuronal degeneration has been identified as the primary cause for these clinical features; however, in some cases, severe developmental problems, such as growth retardation and microcephaly, have been observed. These more severe symptoms are associated with the DSC as well as XP in combination with Cockayne syndrome (CS), and this combined disorder is referred to as XP/CS. Indeed, CS is a rare autosomal recessive disorder, first described in 1936 (Cockayne, 1936). CS patients suffer from sun sensitivity and exhibit many premature aging features, including cachectic dwarfism, kyphosis, arrested sexual development, thin hair, subcutaneous fat loss, osteoporosis and a characteristic “bird-like” face (deep sunken eyes and prominent ears). The neurological symptoms include microcephaly, progressive hearing and visual loss, progressive ataxia, delayed psychomotor development, and mental retardation. These features reflect dysmyelination and neurodegeneration, and the brain shows prominent calcification (Iyama and Wilson 3rd, 2013; Jaarsma *et al.*, 2013). Similar to XP cells, initial studies with CS cells revealed hypersensitivity to UV irradiation, although normal levels of UDS and daughter strand repair were observed (Schmickel *et al.*, 1977; Andrews *et al.*, 1978a). A few years later, the nature of the molecular repair

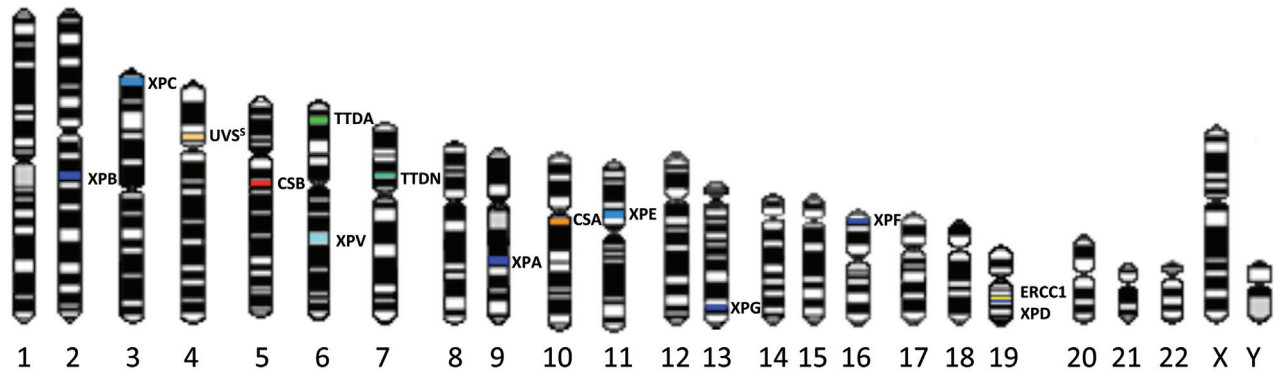
defect in CS cells was identified as a failure to recover RNA synthesis after DNA damage induced through UV-irradiation (Mayne and Lehmann, 1982), suggesting a problem with the transcription of a damaged template. Importantly, this RNA synthesis impairment was used to identify two different complementation groups for CS cells (CS-A and CS-B) and an independent complementation group in XP/CS cells (associated with the XP-B group) with similar molecular defects (Lehmann, 1982). This important discovery revealed two NER sub-pathways associated with global genome repair (GGR) and RNA transcription (transcription coupled repair-TCR).

Subsequently, other genetic disorders were described as the direct results of NER defects. In some cases, the patients present neuronal and/or developmental problems, such as trichothiodystrophy (TTD), where the patients show features similar to CS (short stature, skeletal abnormalities, impaired intelligence, decreased fertility, and photosensitivity) and fragile and brittle hair due to the sulfur-deficiency and reduced levels of cysteine-rich matrix proteins (Stefanini *et al.*, 2010). Cerebro-oculo-facio-skeletal syndrome (COFS) is considered to be a severe form of CS, with symptoms appearing at birth (Suzumura and Arisaka, 2010), and XPF-ERCC1 progeroid (XFE) syndrome is associated with renal and liver abnormalities (Niederhoffer *et al.*, 2006). However, UV-sensitive syndrome (UVSS) has been described as a mild genetic disorder leading to skin photosensitivity (Spivak, 2005; Oh and Spivak, 2010). Curiously, all these syndromes show skin photosensitivity and defects impairing NER, but among their clinical features, there has been no report of increased cancer frequency.

With the advent of DNA recombinant techniques in the 1980s, many research groups have attempted to clone the mutated genes in these NER-related diseases, although this proved to be a difficult task because DNA transfection in human cells was not trivial. In the 1990s, however, the use of mutated rodent cells deficient for different DNA repair pathways facilitated the cloning of XP genes, contributing to the understanding of the mechanisms of NER and TLS, described in more detail below. As human genes showed cross complementation with rodent cells, these genes have been, in many cases, referred to as ERCC (for excision cross-complementing) genes (Boulikas, 1996). The human genes identified in NER-related diseases and TLS, with their chromosomal locations and summarized functions, are shown in Figure 1.

The XP Molecular Defects: Removing DNA Damage via NER or Replicating Damaged DNA through TLS

Classical XP (XP-A to XP-G) cells are defective in the removal of DNA damage through NER, and XP-V cells tolerate the lesions by replicating damaged templates



Gene	Disease	Chromosomal location	Protein size (aa)	No. of exons	Protein function	Defective pathway	Clinical features
XPA	XP, DSC	9q22.33	273	6	Position of the DNA repair machinery	NER	Exaggerated sunburn; Skin Cancer; Mild to severe neurological abnormalities
XPB/ERCC3	XP, XP/CS	2q14.3	782	15	Helicase 3'-5', TFIIH subunit	NER	Exaggerated sunburn; Skin Cancer; Mild neurological abnormalities
XPC	XP	3p25.1	940	16	DNA damage recognition	NER (GGR)	No abnormal sunburn reaction; Skin Cancer; No neurological abnormalities
XPB/ERCC2	XP, XP/CS, TTD	19q13.3	760	23	Helicase 5'-3', TFIIH subunit	NER	Exaggerated sunburn; Skin Cancer, No neurological abnormalities
XPE/DDB2	XP	11p11.2	427	10	DNA damage recognition	NER (GGR)	No abnormal sunburn reaction; Skin Cancer; No neurological abnormalities
XPF/ERCC4	XP, CS, COFS, XFE	16p13.12	916	11	Endonuclease 5'-incision	NER	Exaggerated sunburn; Skin Cancer; No neurological abnormalities to severely affected patients
XPG/ERCC5	XP, XP/CS	13q33.1	1186	15	Endonuclease 3-incision	NER	Exaggerated sunburn; Skin Cancer; may present developmental abnormalities
XPV/POLH	XP	6p21.1	713	11	Bypass polymerase (Pol η)	TLS	No abnormal sunburn reaction; Skin Cancer; No neurological abnormalities
CSA/ERCC8	CS, UVSS	5q12.1	396	12	RNA Pol II cofactor	NER (TCR)	Mental retardation, microcephaly, and growth failure
CSB/ERCC6	CS, COFS, UVSS, DSC	10q11.21	1493	21	RNA Pol II cofactor	NER (TCR)	Mild sun sensitivity; No neurological abnormalities
UVSSA(KIAA1530)	UVSS	4p16.6	709	7	RNA Pol II cofactor	NER	
TTD-A/GTF2H5	TTD	6p25.3	71	3	TFIIH subunit	NER	Brittle hair, delayed development, significant Intellectual disability, and recurrent infections.
TTDN1/PLK1	TTD not UVB sensitive	7p14	179	2	Maintenance of cell cycle integrity	???	
ERCC1	COFS, XFE	19q13.32	297	3	Endonuclease 5'-incision (XPF partner)	NER	Mental retardation; growth failure

Figure 1 - Human chromosomal location and general description of the genes encoding proteins involved in nucleotide excision repair (NER) and translesion synthesis (TLS). Mutations in these genes cause human syndromes, listed in the table.

through TLS. These mechanisms are schematically represented in Figures 2 and 3.

Although NER was first described for the removal of UV-induced lesions (such as cyclobutane pyrimidine dimers, CPDs, and 6-4 pyrimidine-pyrimidone, 6-4PPs), this pathway is recognized as a versatile and flexible mechanism. Specifically, NER removes structurally unrelated bulky lesions, which promote DNA distortions in the double helix. These lesions include DNA adducts or intra-strand crosslinks induced by chemical agents (such as benzo(a)pyrene, a product of tobacco smoke, and cisplatin, an anticancer chemotherapeutic drug) that react with DNA or injuries induced by endogenous agents, such as lesions generated by oxidation through ROS produced in the cells as a potential byproduct of respiration. There are more than 30 proteins that act in a sequential and concerted manner to remove DNA damage (Costa *et al.*, 2003). Notably, defective activity in seven of these proteins (from XPA to XPG) may result in a clinical XP phenotype. Moreover, the defective activity of CSA and CSB may result in severe CS symptoms (Jaarsma *et al.*, 2013). Other NER proteins have

also been recently described, where mutations cause NER syndromes: TTD/p8 (part of TFIIH complex, Giglia-Mari *et al.*, 2004), causing TTD; ERCC1 (partner of XPF endonuclease), causing XFE, CS and COFS (Suzumura and Arisaka, 2010, Kashiyama *et al.*, 2013); UVSSA causing UVSS (Schwertman *et al.*, 2012; Zhang *et al.*, 2012).

NER can be summarized as having four sequential steps, including i) lesion recognition, ii) unwinding the damaged double helix, iii) cleavage and excision of the damaged strand and iv) filling the gapped molecule, followed by DNA synthesis and final ligation (Figure 2). TLS through XPV also facilitates tolerance of UV-induced photoproducts, such as CPDs, and other types of DNA damage induced through other DNA damaging agents (Figure 3).

Two different sub-pathways have been implicated in the recognition step of NER, depending on the location of the lesion. The global genome repair (GGR) sub-pathway involves the recognition of DNA lesions throughout the genome, and it depends on the XPC/HR23B protein complex as the primary DNA damage detector. For some types of le-

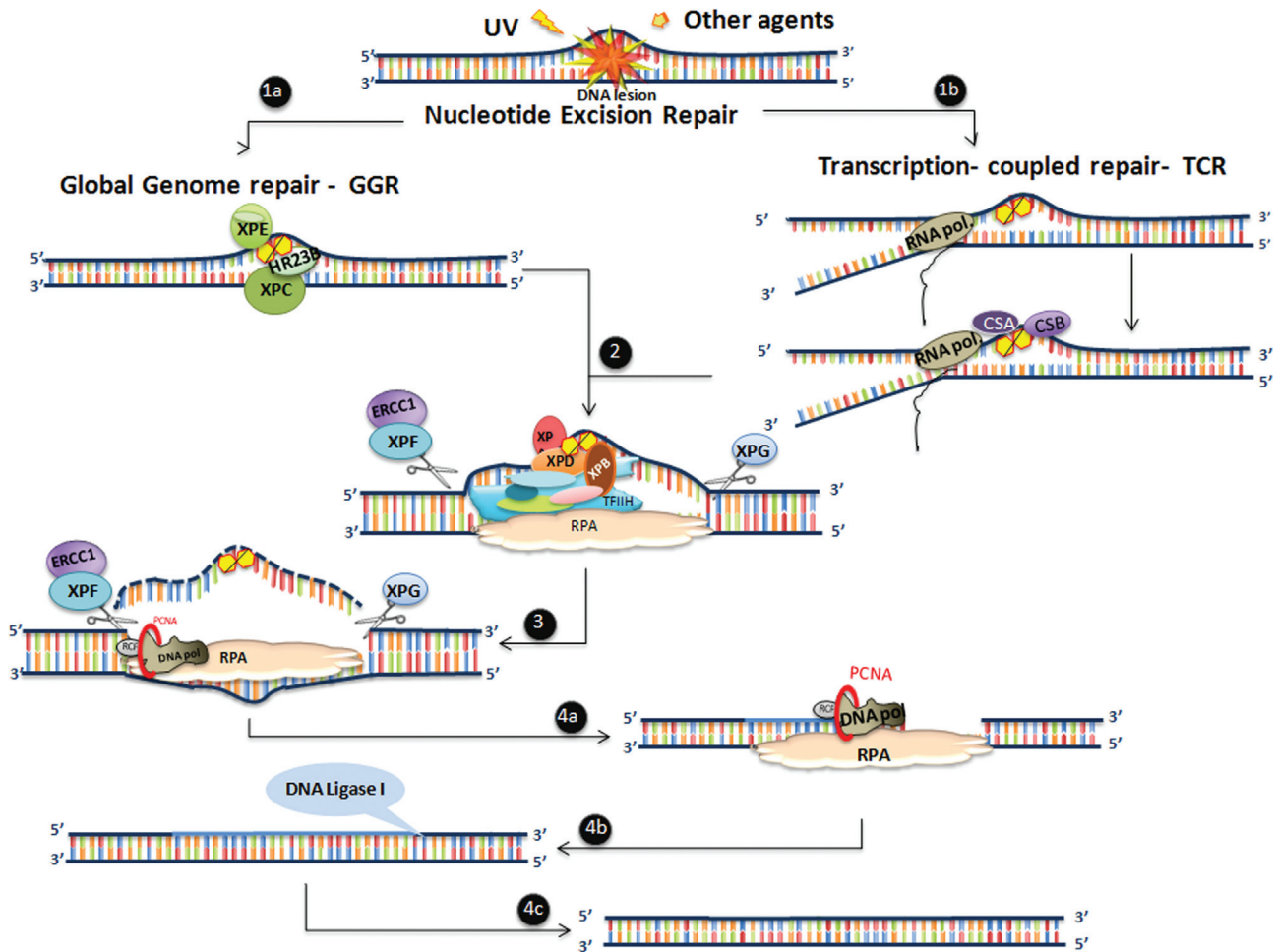


Figure 2 - Schematic representation of nucleotide excision repair (NER): the proteins associated with human diseases are highlighted. The downstream NER pathway and each of the sub-pathways (GGR and TCR) for lesion recognition are indicated, showing the different steps for DNA lesion removal in an error-free manner.

sions, such as CPDs, the damaged DNA binding (DDB) complex (composed of two proteins, including XPE) is required for improving the efficiency of damage recognition and removal. The transcription-coupled repair (TCR) sub-pathway acts on the transcribed strand of actively expressed genes. The existence of this sub-pathway was first observed when DNA damage was not homogeneously removed from the genome of mammalian cells, and the preferential repair of CPD lesions was detected in active genes (Bohr *et al.*, 1985). Subsequently, this preferential repair system was demonstrated as being selective for the transcribed strand, implicating the transcription machinery in the recognition of DNA damage (Mellon *et al.*, 1987). Subsequent studies with CS cells demonstrated that these cells were deficient in the preferential repair of active genes (Mullenders *et al.*, 1988; Venema *et al.*, 1990). Indeed, the CSA and CSB proteins are important players in the recognition of DNA lesions in the TCR pathway, triggered by the blockage of RNA polymerase II during transcription.

The next step, common to both GGR and TCR sub-pathways, is initiated through the recruitment of the tran-

scription factor TFIIH, a multiprotein complex, which includes XPB and XPD proteins, 3'-5' and 5'-3', respectively, ATP-dependent DNA helicases. This complex unwinds ~30 bp at the damaged DNA site, which is covered by a single RPA (from Replication Protein A) complex (a single-stranded DNA binding complex, composed of three sub-unities). RPA and XPA proteins are likely involved in the correct three-dimensional assembly of the NER machinery prior to the endonucleolytic cleavage step. XPG proteins and the XPF/ERCC1 complex are the main endonucleases that nick DNA at both sides, some nucleotides away from the damage. Although XPG is required for XPF/ERCC1 activity, this heterodimer makes the initial 5'-incision, followed by the XPG incision at the 3' side of the lesion (Fagbemi *et al.*, 2011). The final step of NER is the gap filling through repair synthesis. Replicative DNA polymerases (Pol δ and Pol ϵ) use the 3'-hydroxyl extremity, generated by the XPF/ERCC1 incision, as a primer to synthesize DNA at the 30-nucleotide-gap. The resulting nick at the 5' end of the gap is sealed through DNA ligase I

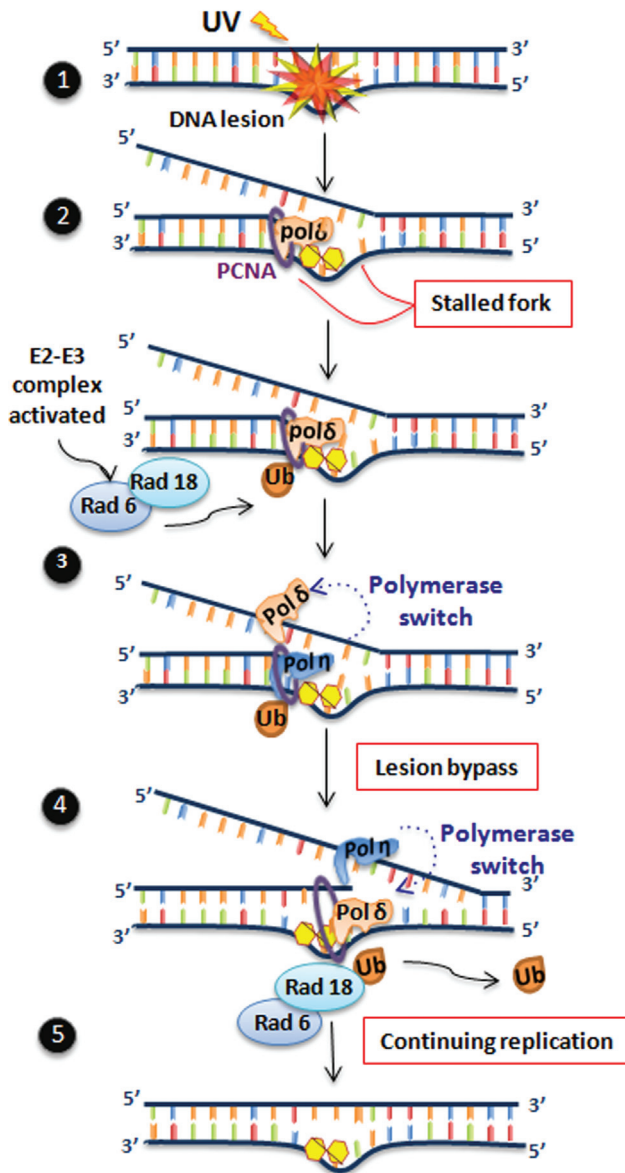


Figure 3 - Schematic representation of translesion synthesis (TLS) by Pol η . The replication fork is blocked by DNA damage, and after a series of events (including the monoubiquitination of PCNA), a polymerase switch provides DNA polymerase eta (XP-V) with an opportunity to bypass the lesion. In this figure, only one strand (leading strand) is represented. At the end of the process the lesion remains in the DNA, but the replication of this molecule can proceed.

or III (Moser *et al.*, 2007). Thus, normal NER restores the original DNA molecule in an error-free manner.

The replication machinery may encounter unrepaired DNA lesions, potentially resulting in a blockage in DNA synthesis, as replicative DNA polymerases do not have the capacity to use damaged templates. This important blockage may lead to the collapse of the replication fork, production of double-strand breaks, and, ultimately, cell death (Batista *et al.*, 2009). To tolerate DNA damage in replicating templates, nature has evolved several alternative DNA polymerases that replace the replicating polymerases (also

termed *polymerase switching*) and replicate the lesions through TLS (Figure 3). Among the many alternative DNA polymerases described during the last two decades (Sale *et al.*, 2012; Sale, 2013), XPV protein, or DNA polymerase eta (Pol η), deserves special attention. Mutations in this DNA polymerase have been implicated in the human syndrome XP. The defective activity of this protein in XP-V cells results in sensitive to UV-light or cisplatin treatment, particularly when the cells are transformed with SV40 or HPV, inhibiting the activity of p53 protein. These cells are also highly mutable after UV-light, which explains the high incidence of skin tumors in XP-V patients. Pol η bypasses certain types of lesions, such as the CPDs induced through UV-light, although the activity of this polymerase through 6-4PPs is controversial. The lack of Pol η leads to a strong blockage of the replication forks in XP-V cells, but replication can still recover through the action of other alternative polymerases (Kannouche and Stry, 2003; Quinet *et al.*, 2014). Interestingly, Pol η is an error prone DNA polymerase, showing reduced replication fidelity. However, this polymerase seems to be capable of bypassing thymidine CPDs correctly through the insertion of adenosines in the daughter strand. Thus, the increased mutagenesis observed in UV-irradiated XP-V cells may reflect the activities of other polymerases that replicate this lesion in an error prone manner (Kannouche and Stry, 2003; Masutani *et al.*, 2008).

DNA Damage and Repair vs. Cancer

Skin photosensitivity is a common clinical feature of all patients showing defective NER responses. The increased sensitivity to UV-light is also observed in cells from XP, CS, TTD, or UVSS patients. Thus, the defective removal of UV photoproducts likely results in this photosensitivity (Lehmann, 2003; Oh and Spivak, 2010). However, although these syndromes reflect defects on the same DNA processing pathway, apart from photosensitivity, they are clinically highly heterogeneous, including increased carcinogenesis, and more severe clinical features, such as abnormal development, neurodegeneration and premature aging. This clinical heterogeneity raises several unanswered questions concerning how unrepaired DNA damage leads to a plethora of different symptoms.

The highly increased mutagenesis induced by UV irradiation in XP cells is well correlated with the high levels of skin cancer observed in XP patients for all XP complementation groups, including NER-defective, and TLS-defective (Figure 4). Indeed, UV-induced signature mutations were observed in specific tumor suppressor genes (such as p53 and patched) in skin tumors from XP patients, demonstrating the causal role of unrepaired lesions and mutations in the formation of tumors in these patients (Dumaz *et al.*, 1993; Bodak *et al.*, 1999; D'Errico *et al.*, 2000; Giglia-Mari and Sarasin, 2003). Even internal tumors observed in XP patients carry mutations provoked by unre-

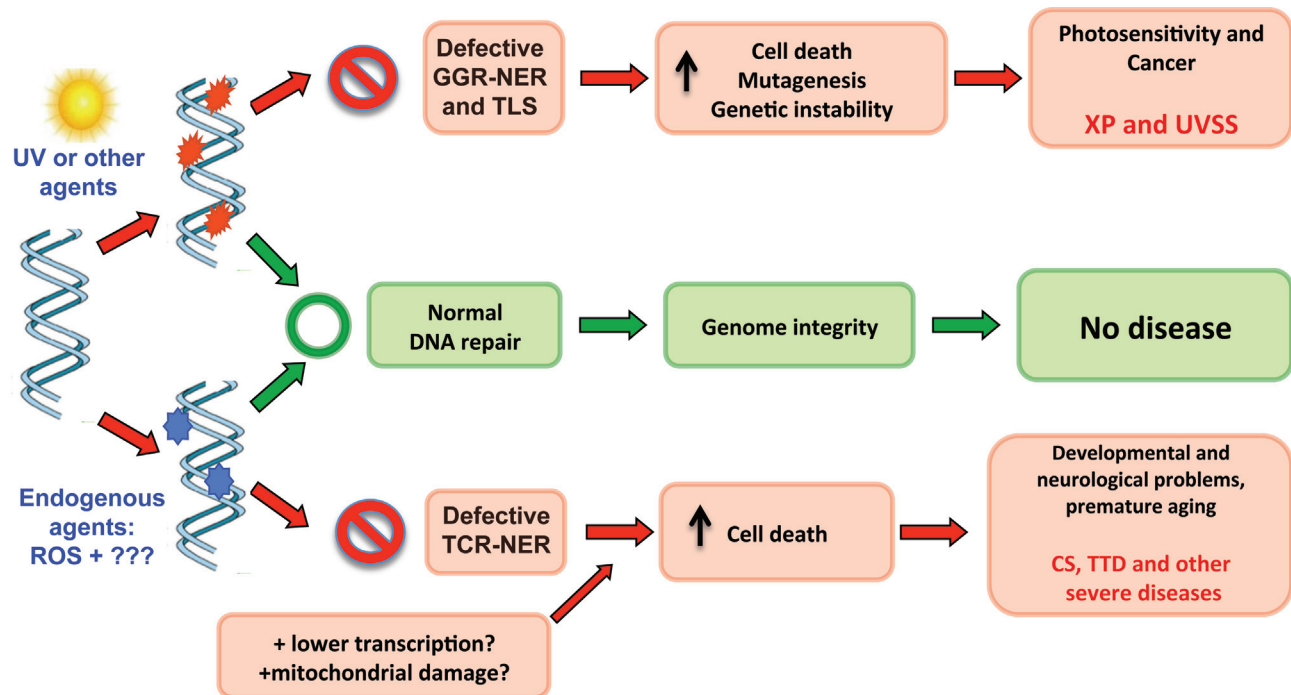


Figure 4 - The consequences of DNA damage and how defective NER and TLS may explain certain clinical phenotypes. Different types of DNA damage can be induced by a variety of endogenous and exogenous agents. NER removes several of these lesions and TLS helps to replicate them, aiming maintenance of the genome stability, and guaranteeing normal cell proliferation, and normal human life (represented in green). When these mechanisms fail, errors in DNA replication increase mutagenesis and genetic instability, resulting in higher photosensitivity (observed in UVSS and XP patients) and risk of cancer development (observed in XP patients). Defective GGR-NER and TLS better explain these phenotypes (in red, superior part of the figure). Alternatively, endogenous DNA damage (including those induced by ROS) may disturb the transcription and/or cause replication blockage, leading to cell death, thus defective TCR-NER better explain the more severe phenotypes (including premature aging) of XP/CS, CS and other syndromes. Other effects, such as impaired transcription or damaged mitochondria may also be involved in these phenotypes (in red lower part of the figure).

paired lesions, likely originating from oxidative stress (Giglia *et al.*, 1998).

Thus, there are clear indications that unrepaired DNA damage induces mutations, and these mutations cause cancer. Nevertheless, the lack of tumors in CS and TTD patients is puzzling. Although the cells from CS patients are defective in a sub-pathway of NER (TCR), the cells from these patients (Parris and Kraemer, 1993) and rodent cells with mutations in the CSA homolog (Vreeswijk *et al.*, 1998) show increased mutations after UV irradiation. Moreover, mouse models defective for CSA and CSB show skin cancer predisposition after UVB irradiation (van der Horst *et al.*, 1997, 2002), unlike their human counterparts. As GGR is more efficient in human cells compared with rodent cells (Hanawalt, 2001), this sub-pathway could protect CS patients from developing skin tumors. Alternatively, it has been proposed that CS cells have a strong signal for cell death after DNA damage, likely reflecting higher levels of transcription blockage (McKay *et al.*, 2001), which may have a reverse effect on cancer development, as dead cells cannot induce cancer. However, XP-A cells are also highly sensitive to DNA lesions, but XP-A patients always develop skin tumors. Notably, the lifetime of severely affected CS, TTD, COFS and XFE patients is typically short and may require living indoors (due to severe clinical fea-

tures), reducing their exposure to sunlight and the probability of developing tumors.

Until recently, most mutation studies have investigated base substitution and small point mutations, deletions and insertions, although certainly larger changes in the DNA sequence, including gross rearrangements, copy number variations and chromosomal anomalies and other types of genomic instabilities, are affected by unrepaired DNA damage, representing known alterations in human cancer (Amiel *et al.*, 2004). Moreover, even with clear evidence of sunlight being responsible for skin cancer, many questions remain concerning what is the part of the spectrum acting in tumorigenesis, either in XP patients or the normal population. Although the results strongly suggest a role for CPDs, the main lesion induced by UVB (280-320 nm), in skin carcinogenesis, this type of lesion is also generated after UVA (320-400 nm) irradiation, and other lesions induced by this spectrum of sunlight should also be considered (Douki *et al.*, 2003; Cadet *et al.*, 2012; Schuch *et al.*, 2013). Indeed, a recent study implicated an important role for both CPDs and 6-4PPs in the biological consequences of UVA light in XP cells (Cortat *et al.*, 2013). Skin tumors are the most frequent types of cancer in human populations, particularly in countries near the tropics; thus, a detailed understanding of how these cancers are induced

through exposure to sunlight is highly relevant to the general public health. Further investigations with XP patients and XP cells can provide important contribution to this field.

Unrepaired DNA Damage and the Aging Process

Concerning other symptoms, such as developmental problems, neurological abnormalities and premature aging, observed in more severely affected XP patients and other NER-related syndromes (such as CS, COFS, TTD and XFE), the causal effects remain far from being understood. This puzzling question was first raised in the 1970s, when the UV-survival of XP cells from several complementation groups were compared to understand the neurological abnormalities (Andrews *et al.*, 1976, 1978b; Kraemer *et al.*, 1976). In general, the cells from patients with more severe symptoms were more UV sensitive than those with no neurological abnormalities. Similar results were soon described for CS-derived cells, indicating the sensitivity of these cells to UV damage (Andrews *et al.*, 1978a). Ultimately, it was concluded that extremely inefficient DNA repair could not protect these neurons (or supporting glial cells) from DNA damage in some of the patients, and the neurons would die prematurely.

Interestingly, in the first reports, XP-C patients were already noticed as less affected by the severe symptoms. Indeed, XP patients mutated in the XPC, XPE (both participate in the initial steps of GGR pathway of NER, see Figure 2) and XPV (TLS, Figure 3) genes present symptoms associated with skin lesions, but not affecting the nervous system. This observation indicates that defects in the GGR sub-pathway of NER and TLS through Pol η could explain the increased tumorigenesis observed in the skin of these patients, which prevents mutagenesis, as described above. Thus, patients with mutations in these genes and other genes (XPA, XPB, XPD, XPF and XPG) that participate downstream in the GGR sub-pathway may present only XP phenotypes associated with skin lesions, which may be avoided by preventing sun exposure.

On the other hand, TCR impairment is clearly associated with more severe symptoms in NER-related syndromes (Figure 4). The high levels of cell death of TCR impaired cells in response to DNA damage, likely triggered by a blockage in RNA transcription, have been associated with the severe symptoms in CS patients (McKay *et al.*, 2001). A similar explanation may also be associated with XP/CS, COFS, TTD, and XFE syndromes. However, the nature of the DNA damage resulting in this increased cell death remains unknown. Endogenously induced lesions generated by free radicals, including ROS, are the strongest candidates for these effects. Indeed, CS-B cells have been demonstrated as deficient in the repair of DNA damage generated through oxidative stress under many circum-

stances. Initially, CSB was shown to be important for the removal of 8-oxoguanine (8-oxoG), a known lesion produced by oxidative stress, in human cells irradiated with ionizing radiation (Tuo *et al.*, 2001, 2003). Subsequently, this effect was also described for CS-A human primary fibroblasts (D'Errico *et al.*, 2007). Mouse models defective in CSB protein were shown to accumulate oxidized purine modifications (8-oxoG and (5'S)-8,5'-cyclo 2'-deoxyadenosine) in certain tissues with age (Osterod *et al.*, 2002; Kirkali *et al.*, 2009). More recently, CS-A and CS-B primary human cells and CS-B reprogrammed iPS cells were shown to have altered metabolic redox balance, with increased levels of intracellular ROS and basal (and induced) levels of DNA damage (Andrade *et al.*, 2012; Pascucci *et al.*, 2012).

Consistent with this model, CS-A and CS-B cells are more sensitive to hydrogen peroxide treatment compared with cells derived from UVSS patients, who present only photosensitivity symptoms. Host cell reactivation after the transfection of damaged plasmids in human cells yields similar results. This approach measures the expression of a reporter gene, and this expression is impaired after DNA damage, which is recovered through DNA repair in the transfected cells. Although both CS-B and UVSS cells are defective in the recovery of UV-irradiated plasmids, only CS-B cells recover gene expression through plasmids carrying oxidized base damage (Spivak and Hanawalt, 2006). Moreover, a UVSS patient was demonstrated to have a specific mutation in the CS-A gene, and cells derived from this patient were highly sensitive to UV irradiation; however, in contrast to CS-A cells from CS patients, these UVSS cells were not sensitive to potassium bromate or other agents that generate oxidative stress (Nardo *et al.*, 2009). Similar results were obtained in XP patients carrying missense mutations in the XPG gene. Specifically, the cells from mildly affected patients (clinically showing only photosensitivity) were sensitive to UV-light but not to the treatment of photoactivated methylene blue (which generates singlet oxygen), in contrast to XP-G/CS cells that were sensitive to this oxidative stress. Host cell reactivation also confirmed that the XPG alleles containing missense mutations fully reactivated the expression of reporter genes from plasmids damaged with photoactivated methylene blue in XP-G/CS deficient cells, while only partial reactivation was obtained in UV-irradiated plasmids (Soltys *et al.*, 2013).

Thus, defective transcriptional repair of DNA damage generated through oxidative stress is associated with more severe clinical features, such as developmental and neuronal abnormalities and premature aging, while the defective repair of UV induced DNA damage is associated with photosensitivity phenotypes (Andressoo and Hoeijmakers, 2005). However, other published data challenge this model: XPC protein has also been implicated in the repair of DNA damage induced through oxidative stress, although XP-C patients present only clinical features associated with

skin photosensitivity, with no symptoms involving neurological or developmental problems. Human XP-C primary keratinocytes and fibroblasts are hypersensitive to treatment with oxidizing agents and accumulate oxidized DNA damage after oxidant exposure (D'Errico *et al.*, 2006). A similar defect was shown in the host cell reactivation of photoactivated methylene blue damaged adenovirus vectors (Kassam and Rainbow, 2007). The participation of both XPA and XPC proteins in the repair of photoactivated methylene blue-induced DNA damage was also investigated in cells treated with this agent or in the host cell reactivation of damaged plasmids. The results suggest that the NER pathway directly participates in the repair of DNA lesions generated through oxidative stress (Berra *et al.*, 2013). However, live imaging data indicates that proteins that initiate NER (XPC and CSB) are rapidly recruited to sites of the cell nuclei containing oxidized base damage, with the absence of the detectable recruitment of other downstream NER factors. These results indicate that the binding of XPC and CSB to the sites of DNA damage is independent on a further NER reaction (Menoni *et al.*, 2012).

Therefore, as the participation of XPC protein in cell protection from oxidative stress is confirmed, the lack of oxidative stress-mediated DNA damage repair may be necessary but not sufficient to explain the severe pathology of neurological and developmental symptoms and the accelerated aging observed in NER-related syndromes. Further defects are needed to construct these severe symptoms, and many possibilities are being investigated. Thus, two potential hypotheses emerge that may help to understand and eventually answer this question: mitochondrial DNA damage and general deficiency in transcription.

Indeed, the participation of NER factors in mitochondria is gaining support. CS-B protein was shown to stimulate the repair of 8-oxoG in the mitochondrial DNA (Stevnsner *et al.*, 2002, Aamann *et al.*, 2010). Moreover, CS-B cells are deficient in autophagy, leading to increased oxidative stress and the accumulation of damaged mitochondria. Interestingly, these effects could be reversed through the addition of autophagy stimulators, such as lithium chloride and rapamycin, to the cell culture medium (Scheibye-Knudsen *et al.*, 2012). Moreover, the participation of many of the proteins in TCR in the global transcription machinery may also be important. A general reduction in transcription through RNA polymerase II has been observed in CSB cells (Balajee *et al.*, 1997). The participation of CSB in transcription elongation and TFIIH as an initiation transcription factor should be considered for these "transcription syndromes" (Compe and Egly, 2012; Velez-Cruz and Egly, 2013). The need for the repair of DNA damage to achieve the effects observed in these pathologies has also been questioned, as the defects on transcription through RNA polymerases I and II may solely explain the severe symptoms of CS patients (Brooks, 2013).

Some patient cases still add more confusion to the genetic basis of carcinogenesis and neurological abnormalities resulting from NER defects. Although CS patients do not present skin tumors, at least one case of siblings showing a severe XP phenotype (diagnosed as the DeSanctis-Cacchione variant of XP) were identified with a missense mutation in the CSB gene (Colella *et al.*, 2000). In contrast, a UVSS patient has been demonstrated as completely null for this same CSB gene (Horibata *et al.*, 2004). The understanding of the molecular basis underlying these clinical phenotypes may help to understand the intricate role of DNA damage and repair in the human pathology of cancer and aging.

XP Under the Tropical Sun

Although sunlight is much more intense at low latitudes, with higher intensity of UVB light and more severe DNA lesions (Schuch *et al.*, 2012), most studies with XP patients has been performed in Europe, USA and Japan, under high latitudes. In tropical and poorer countries, these patients are typically, and unfortunately, unattended. Difficulties in diagnosis, including the lack of information from local medical dermatologists and complications in obtaining appropriate rigorous photoprotection, are just some of the problems faced by these patients and families.

Most of the reports of gene identification and attention to XP families in these countries depend on studies performed in USA or Europe. These studies showed interesting data of genetic clusters, such as the one identified in Guatemala for an isolated community, where 12 children carried a new mutation in the XPC gene, likely reflecting consanguineous marriage (Cleaver *et al.*, 2007). Another genetic cluster was also recently described in patients from a French Comorian Island near the Indian Ocean in West Africa. Among the black Mayotte population, more than 32 XP patients carried a mutation in the XPC gene (Cartault *et al.*, 2011), likely resulting from a founder effect from the African continent. Although black skin protects these patients, these individuals still suffer from the heavy sunlight intensity, with the development of skin tumors and early ocular injuries. Another important finding was the prevalence of a specific mutation (c.1643_1644delTG, p.Val548AlafsX25) in the XPC gene in North Africa. This mutation was observed in 87% of the XP-C patients in this location, primarily in unrelated families, reflecting a founder mutation aging 50 generations or 1250 years (Soufir *et al.*, 2010). It is curious how this deleterious mutation can still be observed, despite a strong negative selection, especially in these sunny environments. Certainly, the mutated allele is kept in heterozygote individuals, as they present normal repair phenotype.

Recently, evidence has been obtained from the direct assistance of patients in the tropical countries, showing sporadic cases (Halpern *et al.*, 2008) and more complete studies in the genetic identification of mutations (mostly

novel) in XPC (Leite *et al.*, 2009), XPV (Ortega-Recalde *et al.*, 2013) and XPG (Soltys *et al.*, 2013) genes. One of the mutations described for the XPC gene in Brazil (Leite *et al.*, 2009) is the same as the highly prevalent mutation reported in North Africa (Soufir *et al.*, 2010), indicating the potential migration from Africa to Europe and subsequently to Brazil (likely from Portugal). The two novel XPG missense mutations observed in the two siblings in Brazil were shown to produce proteins with low repair of UV-photoproducts, but normal repair of lesions generated through oxidative stress, likely explaining the mild phenotype of these patients (Soltys *et al.*, 2013). Moreover, a pioneering effort for the systematic identification of mutations that affect XPC and XPA genes has recently been underway in Brazilian patients, and these results will contribute to both the assistance to these patients and the scientific knowledge on the distribution of these pathological mutations (K. Santiago and MI Achatz, unpublished data). Interestingly, among those XP patients some have a Native American Indian ori-

gin, as the XP community described in Guatemala (Cleaver *et al.*, 2007) and the XP severely affected children belonging to the Navajo Reservation in Southwest of USA (<http://globalgenes.org/xeroderma-pigmentosum-suddenly-arises-on-navajo-reservation/>). Most likely, these will not be the only cases for Native Americans, suggesting mutated alleles of XP genes, causing the disease, have possibly existed before the human migration to the Americas, from Asia.

Moreover, another genetic cluster has been reported in the Brazilian state of Goias (a sunny central area of this country), with more than 20 affected XP patients diagnosed in an isolated community (Chaibub, 2011). Genetic identification of the mutation is underway, and preliminary data indicate that the XPV gene is affected in this population (unpublished results).

Conclusions and Perspectives

The wide spectrum of symptoms and diseases caused by NER deficiency reflects the relevance of this repair

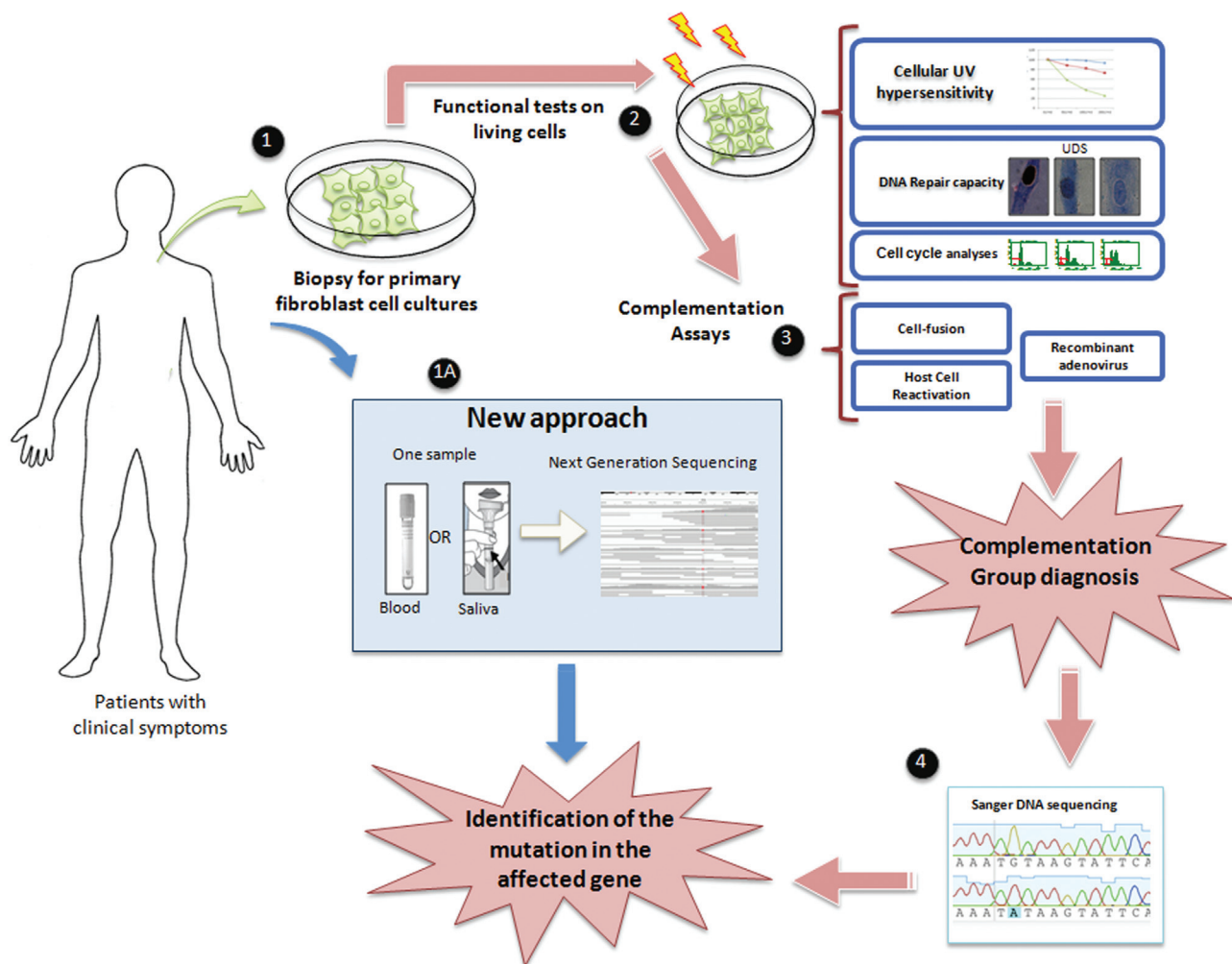


Figure 5 - Strategies for molecular diagnosis of XP patients. Identification of XP gene mutation based on a series of biological and biochemical assays using primary cells obtained from patient skin biopsies. Although the information from these cells has always been rich, the direct sequencing of DNA repair genes using next generation sequencing techniques may facilitate the molecular diagnosis using blood or saliva samples.

pathway in the maintenance of genome stability in humans. Since 1968, the molecular defects affecting XP patients and other syndromes have been described. Studies with cells from these patients have contributed to our current understanding of the causes of cancer and the development of tools to fight tumors (Morales *et al.*, 2012). We have also learned a great deal concerning aging, which might improve the health and quality of life of the elderly. Thus, the human society is indebted to NER-defective patients, although little can really be done to help them. Gene therapy for these patients is at its infancy (Magnaldo and Sarasin, 2004; Menck *et al.*, 2007), and not even the simple identification of the genetic mutation is provided in many countries worldwide, particularly in developing countries. This identification could, at least, provide some prognosis for the disease and support for genetic counseling for the affected families. Until recently, there was a strong need for biological essays for the identification of the genetic complementation group in XP patients, requiring skin biopsies, which are painful for the patient, and cell culture, which is time consuming and requires specific skills. The direct sequencing of exomes (Ortega-Recalde *et al.*, 2013) or specific genes using next generation sequencing techniques of DNA isolated directly from saliva samples provides an excellent opportunity for the rapid diagnosis of these mutations (Figure 5). Thus, new horizons may bring hope for the XP patients and their families.

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