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REVIEW

Hereditary Disorders with Defective Repair of UV-Induced DNA Damage

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Abstract: Nucleotide excision repair (NER) is an essential system for correcting ultraviolet (UV)—induced DNA damage. Lesions remaining in DNA due to reduced capacity of NER may result in cellular death, premature aging, mutagenesis and carcinogenesis of the skin. So, NER is an important protection against these changes. There are three representative genodermatoses resulting from genetic defects in NER: xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD). In Japan, CS is similarly rare but XP is more common and TTD is less common compared to Western countries.
In 1998, we established the system for the diagnosis of these disorders and we have been performing DNA repair and genetic analysis for more than 400 samples since then. At present, there is no cure for any human genetic disorder. Early diagnosis and symptomatic treatment of neurological, ocular and dermatological abnormalities should contribute to prolonging life and elevating QOL in patients.

Keywords: UV-induced DNA damage, nucleotide excision repair, genodermatosis, DNA repair, skin cancer

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Introduction

Deoxyribonucleic acid (DNA), the basic substance of life, changes over the long term in the process of evolution, but strict homeostasis of DNA is important over the short term for the "maintenance of individual organisms."

DNA damage is thought to occur at the rate of tens of thousands events daily in each cell (endogenous DNA damage) while it is carrying out basic activities (normal metabolic processes) due to replication errors and oxidative damage. Exogenous factors (ultraviolet [UV] light, ionizing radiation, and environmental mutagens created by humans [tobacco smoke, exhaust fumes, etc.]) also cause DNA damage, so it is constantly occurring within living organisms. If the damage affects an important part of the genome, mutation, replication arrest, or inhibition of transcription may occur, leading to impairment of cellular function, cell death, aging, carcinogenesis, or even death of the organism. However, organisms are not defenseless against DNA damage, because various "DNA repair systems" have been developed in the course of evolution to efficiently repair "harmful" DNA damage via very precise mechanisms that involve many proteins functioning in an integrated fashion.

Xeroderma pigmentosum (XP) is a "human" mutation that causes hypersensitivity to UV radiation, resulting in inherited severe photosensitivity, which was initially described by the Austrian dermatologist Kaposi at the end of the 19th century.[1,2] The first breakthrough in the study of XP, however, was only achieved when the radiation biologist Cleaver found that it was caused by abnormal removal/repair of UV-induced DNA damage.[3] Like XP, Cockayne syndrome (CS) and trichothiodystrophy (TTD) are also diseases caused by a human mutation leading to defective DNA repair.

UV-Induced DNA Damage

Human DNA consists of 3 billion base pairs. It is constantly exposed to exogenous factors that cause damage (UV light, ionizing radiation, environmental mutagens, drugs, etc.), coupled with endogenous factors such as metabolites, reactive oxygen species, and replication errors, resulting in new DNA damage at every moment. DNA damage can be classified into the following 8 types based on the structural changes that occur (Fig. 1). Among these, type 1 is caused by solar

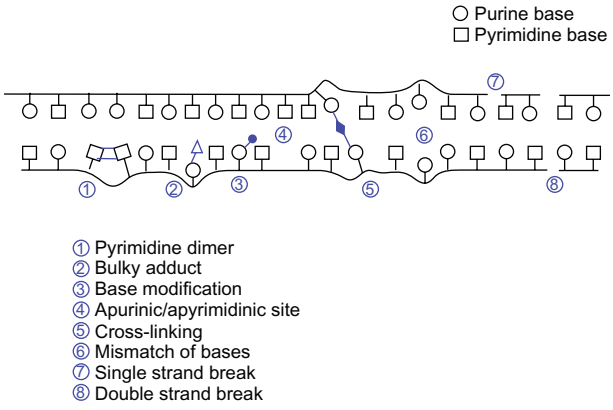

Figure 1. Spontaneous and environmental damage to DNA.

UV radiation (UVC, UVB and UVA2) (not visible light) that leads to dimerization of two adjacent pyrimidine bases. There are two known types of pyrimidine bases (Fig. 2). Successive pyrimidine bases can be activated by UV radiation, resulting in dimerization via a covalent bond between the C5 and C6 positions to create a cyclobutane pyrimidine dimer (CPD). Alternatively, a bond between the C6 position on the 5′ side and the C4 position on the 3′ side causes distortion, generating a (6-4) pyrimidine pyrimidone dimer photoproduct (6-4PP). Of all DNA damage caused by UV irradiation, the former type accounts for 75% and the latter for 25%. Repair of CPD is a relatively slow process, and damage still persists at 24 hours after UV irradiation. On the other hand, repair of 6-4PP is rapid and the damage is almost completely eliminated after 3 hours. Furthermore, lethality is frequent with the former type of mutation, whereas the latter is associated with a high rate of mutagenicity. In Figure 1, ③ is called base modification, and this change does

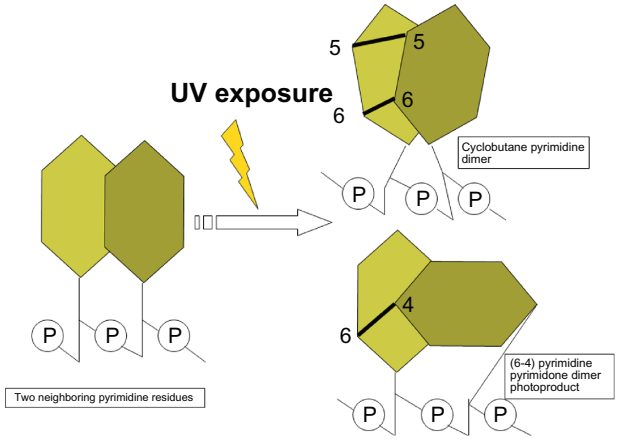

Figure 2. Two major alterations in DNA induced by UV.

not cause distortion of DNA. Oxidation of a guanine base to 8-oxo-guanine is a typical example.

DNA Repair of UV-Induced DNA Damage

Living organisms have DNA repair systems in order to maintain the integrity of DNA that carries the genetic code for life. These repair systems can cope with various types of DNA damage and are divided into 6 categories, including photoreactivation, dealkylation of alkylated bases, direct repair of damage (eg, repair of single-strand or double-strand breaks), excision repair, recombination repair, or post-replication repair (translesion DNA synthesis).

Photoreactivation utilizes long-wavelength UV light and visible light to repair pyrimidine dimers produced by exposure to UV radiation. Depending on the type of DNA damage, a covalent bond of a dimer is cut by electron transfer through the activity of two enzymes (CPD photolyase and 6-4 photolyase). However, this system does not exist in placental mammals such as humans.

The excision repair system includes a base excision repair (BER) mechanism and a nucleotide excision repair (NER) mechanism. Most oxidative DNA damage is repaired by the former mechanism. Recently, an association between the onset of neurological symptoms of XP and abnormalities of this repair mechanism has been pointed out.[4] The latter is the most important DNA repair mechanism (Fig. 3), and it plays a role in the removal of CPDs and 6-4PPs. This repair mechanism can remove relatively large DNA regions encompassing dozens of bases as a complete unit, and is the mechanism most frequently involved in the pathogenesis of XP, CS and TTD. In NER, there are two main pathways, global genome repair (GGR) and transcription-coupled repair (TCR) and each pathway includes 4 steps; these are damage recognition, DNA unwinding, incision/DNA excision and de novo synthesis.[5] Post-replication repair (ie, translesion DNA synthesis) is a back-up repair system for the NER mechanism that acts slowly and attempts to bypass residual CPD sites. In XP variant (XPV), the NER mechanism functions properly, but there are defects of the post-replication repair system (Fig. 3).[6]

Xeroderma Pigmentosum (XP)

Xeroderma pigmentosum is a rare photosensitive dermatosis with autosomal recessive inheritance that is caused by abnormalities of the repair mechanisms for UV-induced DNA damage and is associated with a high frequency of skin cancer.[7,8] The frequency of XP in Japan is 1 person in tens of thousands, but this

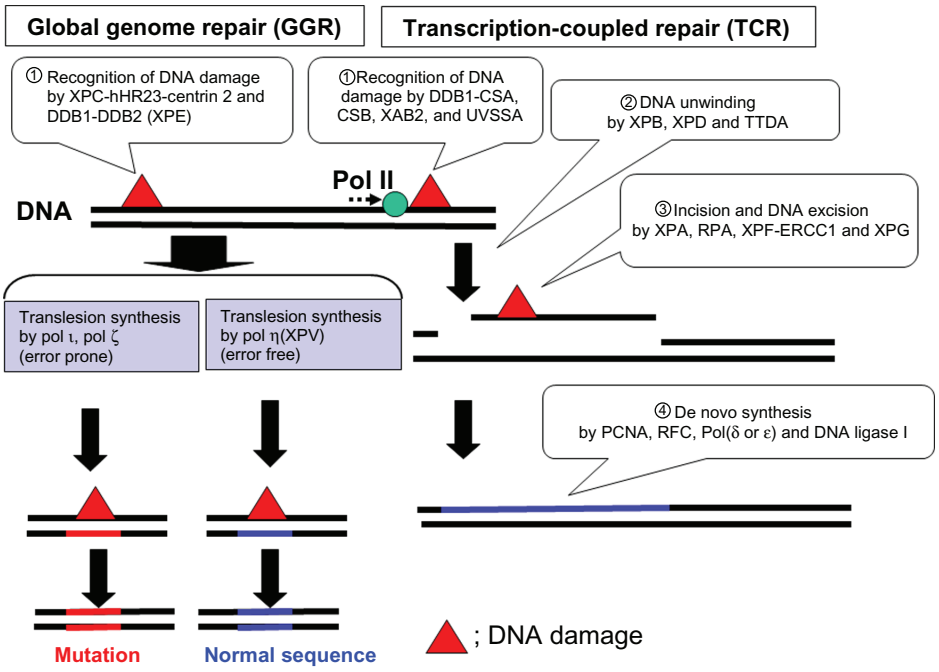

Figure 3. The pathway of nucleotide excision repair and translesion synthesis.

prevalence is more than 10 times higher compared with that in Europe and the United States.[9]

In a typical case, the skin of the face and other sun-exposed areas is affected by repeated severe sunburn from early childhood (Fig. 4A), resulting in abnormal freckle-like pigmentation (Fig. 4B). A phototest will reveal a marked decrease of the minimal erythema dose and a severe delayed erythema reaction. Should patients fail to carry out strict sun protection, skin tumors such as basal cell carcinoma, solar keratosis, squamous cell carcinoma and melanoma will occur frequently from an early age, with the risk being more than 1,000 times higher than in healthy individuals. Progressive central and peripheral neurological degeneration are observed in 30% of all XP patients (60% of Japanese patients), but the underlying mechanisms remain unknown. There are several genetically distinct types of XP that are categorized into a total of eight groups. These include seven groups (A to G) with NER abnormalities and one variant that has normal NER function but defective post-replication repair. The progression of symptoms, severity, and prognosis are different for each group.[9] The groups that present with characteristic neurological symptoms of XP are XPA, XPD, and XPG. In XPB, all patients have CS (see below), whereas some XPD patients have both CS and TTD (see below). Some XPG patients also have CS. In Japan, XPA patients with severe dermatological and neurological symptoms accounts for 54%, followed by the XP variant with only dermatological symptoms, accounting for 25%. On the other hand, XPC patients without neurological symptoms are common (40%) in Europe and the United States. Almost all XPA patients develop central or peripheral neurological complications, including impaired psychomotor development, and the severity of their neurological defects influences the prognosis. Based on the clinical diversity of XP, it can be classified as "the cutaneous type" with skin symptoms only, "the neurological type" with XP neurological symptoms, "the CS type" with symptoms of CS, and "the TTD type" with symptoms of TTD.[10] However, the association between clinical features/biological characteristics of XP (decrease of UDS and marked decrease of cell viability after UV irradiation) (phenotype) and the type or location of the *XP* gene mutation (genotype) has not been clarified.

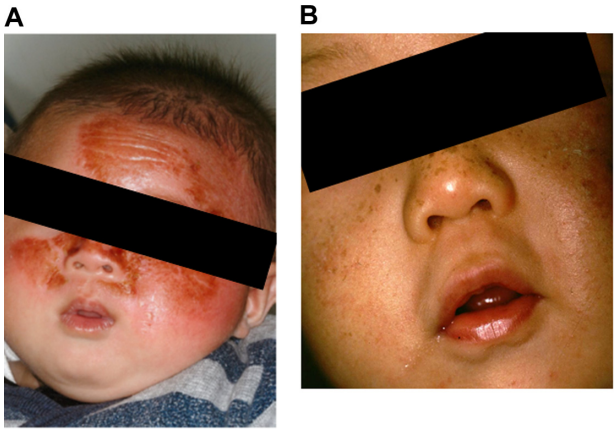

Figure 4. Clinical features of patients with xeroderma pigmentosum group A (XPA). (**A**) Severe sunburn (a newborn baby). (**B**) Abnormal pigmentation appears after repeated episodes of sunburn (a 3-month-old boy).

Cockayne Syndrome (CS)

CS was first reported in 1946 by the British pediatrician Cockayne as "a case with a marked decrease in growth accompanied by atrophy of the optic nerve and hearing loss".[11] Similar to XP, it is an extremely rare autosomal recessive disorder (1 in 0.5–1 million) that occurs due to failure of the NER mechanism, a major repair system for UV-induced DNA damage, especially for damage at sites of transcription. Over 200 cases have been reported in Europe and the United States, whereas there have been about 70 cases reported in Japan.

Symptoms of photosensitivity start to occur around six months after birth. Diverse features can be noted, such as microcephaly, a distinct facies (an aged look, sunken eyes, beak-like nose, big ears, protruding upper jaw), short stature, malnutrition, poor growth, pigmentary retinal degeneration, hearing loss, and mental retardation. These findings are not at all apparent immediately after birth, but start to appear around the age of 2 years and progress with aging. Freckle-like pigmentation of sun-exposed areas, as seen in patients with XP, and skin cancer are absent in patients with CS, except for those with XP/CS. Calcification of the brain is observed on head computed tomography (CT) and this finding is of high diagnostic value. Impaired liver and kidney function and diabetes mellitus occur as complications, and 80% of patients die before the age of 20 years due to infections such as pneumonia.[12] There are also rare mild cases where the onset is delayed.[12] Clinically, CS is classified into 3 types: a classic

type (type 1) in which patients survive until around the time of puberty, a severe type (type 2) in which patients die in infancy, and a delayed or adult-onset mild type (type 3). Types 2 and 3 are very rare.

2 genetically different types exist (groups A and B), with 25% being CSA and 75% being CSB. The defective proteins CSA (also called ERCC8; the gene responsible is at chromosome 10q11.23) and CSB (ERCC6; the gene responsible is at chromosome 5q12.1) are essential for the NER mechanism and both act in the early phase of TCR.

Diseases that exhibit the CS phenotype can be classified into 5 types according to the genes responsible, which are (1) CSA, (2) CSB, (3) CS/XPB, (4) CS/XPD, and (5) CS/XPG. Among these, types 3, 4, and 5 are referred to as "XP/CS complex", with each type being attributed to mutation of the *XPB* gene, *XPD* gene, and *XPG* gene, respectively. In types 1 and 2, patient cells maintain normal GGR, but TCR defects lead to impaired cell viability and a markedly decreased ability to synthesize ribonucleic acid (RNA) after UV irradiation, despite normal unscheduled DNA synthesis (UDS), which is an indicator of GGR. Types 3, 4, and 5 are complicated by XP, and occur due to mutations of the *XPB*, *XPD*, and *XPG* genes, respectively, with the clinical picture sometimes including abnormal facial pigmentation and malignant skin tumors in addition to features of CS. Because many of the factors associated with the NER system involved in the pathogenesis of CS also affect transcription, these patients can have various symptoms in addition to symptoms related to premature aging.

Cerebro-oculo-facio-skeletal (COFS) syndrome is a disorder with the main characteristics of congenital microcephaly, congenital cataract, microphthalmia, progressive arthrogryposis, and severe growth failure. Recently, a genetic mutation of *CSB* has been found, that is considered to represent a subtype of CS, and its relation to the *XPD* or *ERCC*1 genes has been suggested in some reports.[13–15]

Trichothiodystrophy (TTD)

TTD is known as sulfur-deficient brittle hair syndrome, since the main symptoms of this extremely rare autosomal recessive congenital disease include hair abnormalities due to a decreased sulfur content, accompanied by various other symptoms such as short stature, ichthyosis, mental retardation, abnormal nail plates, abnormal teeth, and infertility. Photosensitivity is also observed in 40% of these patients. The characteristic of this disease is short and brittle hair (trichorrhexis nodosa or trichoschisis) due to a low content of cysteine, one of the sulfur-containing amino acids. Observation under a polarizing microscope reveals a yellow and black striped pattern known as tiger tail banding. According to statistics from the United States, TTD patients also have mental retardation (86%), short stature (73%), and ichthyosis (65%).[16,17] Genetically, there are three types of the photosensitive form of TTD: (1) the TTDA type with no functional GGR or TCR, high sensitivity to UV radiation, and low UDS; (2) a type with *XPG* gene mutation; and (3) a type with *XPD* gene mutation. Of these, the third type is the most common (85%), while the first and second types are very rare. The protein responsible for TTDA is a component of TFIIH, which has recently been revealed to be TFB5 (GTF2H5) involved in both transcription and NER.[18] Because TTDA, XPB, and XPD are all components of TFIIH, it is speculated that symptoms of TTD other than hypersensitivity to sunlight may be due to abnormalities of transcription. On the other hand, in the non-photosensitive form of TTD, the responsible gene is TTDN1 (C7orf11), which is only involved in transcription and is not a component of TFIIH. The frequency has been reported to be 1 in 1 million persons for Europe and the United States, while only 2 cases have been reported in Japan (unconfirmed group and TTD-A in 1 case each).

Diagnosis of XP, CS, and TTD

Definitive diagnosis of the above-mentioned diseases is mainly achieved by using cultured fibroblasts from the patient's skin to perform the following tests: (1) measurement of UDS after UV irradiation, (2) assessment of UV light sensitivity (with or without caffeine), (3) assessment of the level of DNA repair and a complementation test, and (4) genetic or protein analysis. In patients with XP (excluding XPV) and TTD, cells are hypersensitive to killing by UV, and UDS is reduced to less than 50% of that in normal cells. Cells from CSA and CSB patients are highly sensitive to UV radiation and show normal levels of UDS/impaired synthesis of RNA after UV irradiation. The possibility of XPV becomes higher if caffeine increases UV sensitivity. XPA accounts for the

majority of XP in Japan, and a homozygous mutation (G to C) at the 3′ splice acceptor site of intron 3 of the *XPA* gene is detected in 79% of patients, while a heterozygous mutation is detected in 16%. In addition, there is a homozygous mutation involving exon 6 (R228X) in 2% and a heterozygous mutation in 9%. These abnormalities (IVS3-1G > C, R228X) represent *XPA* gene mutation hot spots for Japanese patients (the former is the major hot spot and the latter is the minor hot spot), and both mutations can be easily identified by PCR-restriction fragment length polymorphism analysis (*AlwN I, Hph I*).[9,19] Due to the strong founder effect, accurate genetic testing can be performed rapidly in most Japanese patients with XPA, and this is also utilized in genetic services such as carrier detection[20] and prenatal diagnosis. Definitive diagnosis of other XP groups, CS and TTD can be achieved by a genetic complementation test that assesses the ability of patient cells to reactivate a reporter gene (eg, a luciferase expression vector) after UV damage. It is difficult, however, to obtain a definitive diagnosis of XPE and XPV with this complementation test, so protein and genetic analyses are required.[22]

Figure 5. Sun protective hood used in a case of XPA.

Patient Management (eg, Protection Against Ultraviolet Radiation)

Since XP, CS, and TTD are all genetic disorders, a cure cannot be expected. Therefore, strict and complete lifetime protection from UV radiation for prevention of complications is the basic policy for patients with these diseases.

As measures for protection against sunlight, patients are instructed to use a topical sunscreen with a high SPF value and high PA grade, and are told to wear tops with long sleeves, long pants, a hat, UV protective clothing, and UV protective glasses when they go out. Achieving complete UV protection stops the progression of freckle-like pigmentation and suppresses the development of malignant skin tumors (Fig. 5).

As for the neurologic defects associated with XP and CS, there are no effective evidence-based treatment measures because the pathogenesis is still unclear. Intake of a diet rich in vitamin C, vitamin E, and catechin (which have an antioxidant effect) stimulation of the brain, and encouragement of movement from early childhood may prevent neurological symptoms from advancing. Older children should regularly attend a rehabilitation service for the purpose of delaying movement disorder and preventing contractures.

Due to the need for "lifelong protection from sunlight," patients with inherited photosensitivity diseases have an impaired quality of life (QOL). Because patients with XP, CS, and TTD also have various specific complications, QOL is further decreased for these patients and their families. In other words, patients and families suffer from severe physical, mental, and economic stress due to the heavy burdens of "strict lifetime UV protection," "complications, children with disabilities," "incurable disease," and "genetic problems". Adequate care for patients with such diseases and support for their families cannot be provided by physicians alone. Under such circumstances, there is an important role for patient and family advocacy groups, which are founded with the objectives of sharing knowledge about diseases and ideas or information for daily living, sharing enjoyment, and making

society aware of these rare diseases in order to improve the healthcare environment. The activities of such patient and family advocacy groups are naturally patient/family-driven, but physicians and researchers involved with these diseases also offer positive support through provision of information and other assistance.

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