Ten Years of Clinical and Laboratory Work on Xeroderma Pigmentosum

Shinichi MORIWAKI

Department of Dermatology, Division of Medicine for Function and Morphology of sensory Organs, Osaka Medical College, Takatsuki-city, Osaka 569-8686, Japan

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ABSTRACT

Xeroderma pigmentosum (XP) is a rare autosomal recessive photosensitive disorder with extremely high frequency of skin cancers in sun-exposed areas associated with reduced DNA repair ability of UV induced DNA damage. There are genetically different seven nucleotide excision repair (NER) complementation groups (A through G) and a NER proficient form (XP variant). More than 50% of Japanese XP patients (mostly XPA) may also have XP • related neurological abnormalities caused by unknown pathogenesis. The laboratory diagnosis of XP can be performed by DNA repair tests such as autoradiography, cell survival study and molecular biological analyses including polymerase chain reaction (PCR), plasmid host cell reactivation assay and DNA sequence. DNA damage by UV is repaired by NER system; mainly two DNA repair pathways, (1) transcription-coupled repair and (2) global genome repair. More than 20 factors involved in these pathways are related to the pathogenesis of XP and other photosensitive diseases, Cockayne syndrome (CS) and trichothiodysmophy (TTD). In XP (especially XPA), genotype-phenotype relation has been observed. Clinical management consists of early diagnosis followed by sun protection. XP patient/family support groups have been established in Japan that provides information and resources of UV protection for patients, their families and teachers having XP children.

INTRODUCTION

Ultraviolet (UV) light existed in sunlight is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than x-rays, in the range of 100 to 400 nm. As an ionizing radiation it can cause chemical reactions to DNA or cell membrane. Most people are aware of the effects of UV through the experience of sunburn, but the UV has many other effects on human health., both beneficial such as Vitamin D synthesis and vicious such as systemic/local immunosuppression, acute inflammation, tanning, cutaneous aging and skin carcinogenesis. UV light can directly cause DNA damage;
mainly cyclobutane pyrimidine dimer (CPD) and (6-4) pyrimidine-pyrimidone photoproducts (6-4PP), especially between adjacent pyrimidine bases [1]. Recent studies suggested that such direct damage induced by UVC/UVB or indirect damage caused by UVB/ UVA through the generation of free radicals and reactive oxygen species (ROS) in DNA contributed to aging and carcinogenesis of the skin if these damages are not repaired correctly by DNA repair system [2].

XERODERMA PIGMENTOSUM

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease characterized by severe photosensitivity, abnormal pigmentation and a more than 1000 fold increase in frequency of skin cancers in areas exposed to sunlight compared to normal population [3]. The estimated frequency of the disorder is 1/one million and 1/40~100 thousands newborns in Western countries and Japan, respectively. As patients with XP are very sensitive to ultraviolet (UV), not visible light, the anterior portion of the eye, lips and the tip of the tongue are also affected in some cases. The posterior portion of the eye receives only visible light since UV is blocked by the cornea and lens and usually intact in XP patients. About 55% of Japanese patients (20% in Western cases) with XP have XP neurological disease such as mental retardation, sensorineural deafness, abnormal motor function in association with a primary progressive neuronal degeneration [4,5].

Cultured cells derived from XP patients showed hypersensitivity to killing by UV irradiation, reduced DNA repair capacity and high frequency of UV-induced mutations. By cell fusion technique there are genetically different seven complementation groups, A through G, of impaired excision repair type and one excision repair proficient form (XP variant) with deficient post-replication DNA repair by impaired capacity of translesion DNA synthesis. Among these groups, the clinical findings such as the frequency of skin cancer and the severity of the neurological abnormalities are different.

Table 1 shows the characteristics of each complementation group of XP summarized by Dec. 2008. The total number of registered patients with XP in Japan is 351, especially dominated by XP group A, the severest form of XP in Japan. XP group F and XP variant are also more common in Japan than other countries.

Complementation groups assignments were determined for these Japanese XP patients by the genetic complementation test or molecular biological methods. Among them 188 cases (55%) belong to XP group A and about 25% are XP variants. The early diagnosis of XP group A is very important in dermatology and pediatrics, permitting care of progressive cutaneous and neurological symptoms in XP patients. Among the 8 types of XP, patients with groups A, B, D and G of XP may have progressive neurodegenerative symptoms. The pathogenesis of the neurological abnormalities in XP has not yet been proven irrespective of the energetic XP research with the fact that no abnormalities of central and peripheral nervous systems have been found in XP mice. There are evidences that some XP patients have impaired ability of repair of DNA damages induced by ROS (e.g. thymine glycols, 8-oxoguanine) [6,7]. Various kinds of ROS are generated through the metabolic and respiratory pathway. These observations may imply some of the neurological symptoms appeared in XP. In Western countries most of the XP patients belong to XPC. This group often has severe cutaneous lesions without any neurological findings if they do not protect their skin from the sun. The distribution of XP genotypes

Table 1 Characteristics of xeroderma pigmentosum complementation group

<table>
<thead>
<tr>
<th>Complementation Group</th>
<th>Unscheduled DNA Synthesis (% of Normal)</th>
<th>Skin Cancer</th>
<th>Neurological Abnormalities*</th>
<th>Number of Registered Cases in Japan</th>
<th>Responsible Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;10%</td>
<td>+</td>
<td>+</td>
<td>188</td>
<td>XPA</td>
</tr>
<tr>
<td>B</td>
<td>3~7%</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>XPF/ERCC3</td>
</tr>
<tr>
<td>C</td>
<td>10~20%</td>
<td>+</td>
<td>-</td>
<td>12</td>
<td>XPC</td>
</tr>
<tr>
<td>D</td>
<td>25~50%</td>
<td>+</td>
<td>+</td>
<td>28</td>
<td>XPD/ERCC2</td>
</tr>
<tr>
<td>E</td>
<td>40~60%</td>
<td>+</td>
<td>-</td>
<td>10</td>
<td>DDB2/XPF/p48</td>
</tr>
<tr>
<td>F</td>
<td>10~20%</td>
<td>+</td>
<td>+</td>
<td>24</td>
<td>XPF/ERCC4</td>
</tr>
<tr>
<td>G</td>
<td>&lt;5%, 35%</td>
<td>+</td>
<td>+</td>
<td>3</td>
<td>XPG/ERCC5</td>
</tr>
<tr>
<td>Variant</td>
<td>75~100%</td>
<td>+</td>
<td>-</td>
<td>86</td>
<td>XPV/hrAD30</td>
</tr>
</tbody>
</table>

Total - 351

* + present in most patients in the complementation group; - absent in most patients
is much different between Japanese and Caucasians.

DNA REPAIR OF UV-INDUCED DNA DAMAGE

CPD and 6-4PP can influence cellular death, aging, mutagenesis and carcinogenesis when they are not functionally corrected by the DNA repair machinery of the cells. The nucleotide excision repair (NER) system (Fig. 1) is mainly responsible for correcting UV-induced damage in DNA [8]. More than 20 factors are involved in this system, several of which are also related to the transcription. DNA damage of actively transcribed areas is repaired more rapidly and more completely than that in those entire genome or in the non-transcribed strand of active genes. The former is called transcription-coupled repair (or TCR) and the latter, global genome repair (or GGR). At the first recognition of DNA damage, CSA and CSB protein are important in TCR and XPC-hHR23B is a specific factor in GGR. XPA, TFIIH, replication protein A (RPA) and damaged DNA binding protein (DDB) are related both in TCR and GGR. DDB is a heteroduplex protein and one part of it (DDB2) is defective in XPE patients.

The GGR pathway begin with the first recognition of DNA damage by XPC-hHR23 complex, followed by the complete recognition by XPA-RPA complex. This complex can combine with TFIIH, a transcription factor containing two kinds of DNA helicase ac-

![Diagram of DNA repair system](image)

Fig. 1 Nucleotide excision repair system [8]
tivity; XPB and XPD with opposite direction (3’→5’, 5’→3’, respectively). Those play a role for DNA unwinding of the double strand DNA.

After the recognition of the DNA damage, two endonucleases XPF-ERCC1 and XPG, make incisions 5’- and 3’ to the lesion releasing a 25~27 nucleotide DNA fragment containing the photoproduct. The resulting gap can be filled by DNA polymerases, ε/δ, proliferating cell nuclear antigen (PCNA), and replication factor C (RFC), followed by sealing with DNA ligase I.

LABORATORY DIAGNOSIS OF XP BY DNA REPAIR TESTS

There is marked clinical heterogeneity among groups of XP (Table 1). Possible XP patients should be diagnosed precisely as early as possible for the quality of life of patients and their families.

The diagnosis of XP has been made by DNA repair tests such as the measurement of post-UV unscheduled DNA synthesis (UDS) by autoradiography, UV survival by colony formation and the analysis of the recovery of post-UV DNA/RNA synthesis (Table 2). Classically, complementation groups have been assigned by somatic cell fusion using polyethylene glycol (PEG). Cells in different complementation groups correct the reduced post-UV unscheduled DNA synthesis (UDS) in heterokaryons formed by fusing skin fibroblasts from two different patients [9]. Although this assay is reliable, it requires complicated techniques and usually takes several months to determine the complementation group of XP, meaning that it is difficult to handle many samples in the laboratory at once.

Recently, host cell reactivation (HCR), an easier, more rapid and more sensitive laboratory assay for the diagnosis of XP than the cell fusion assay has been developed [5] (Fig. 2). HCR utilizes an ultraviolet (UV)-treated plasmid expressing a reporter gene such as luciferase and has been performed in our laboratory. The plasmid, pLuc, is treated with UV and transfected into XP cell lines from patients with cloned XP genes (XPA·XPG, control plasmid). After repair and expression of pLuc, luciferase activity was measured and DNA repair capacity (DRC) was calculated as percentage of the residual pLuc gene expression after repair of damaged DNA compared to undamaged DNA which is considered as 100%. XP complementation is assigned by determining which XP vector resulted in an enhanced post-UV pLuc expression. This assay is sensitive, easy and reproducible and each laboratory doing XP research in each country performs this assay for the diagnosis of XP.

Table 2 DNA repair tests for the diagnosis of XP

<table>
<thead>
<tr>
<th>Methods</th>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Post UV unscheduled DNA synthesis (UDS)</td>
<td>Skin</td>
</tr>
<tr>
<td>2. UV survival by colony formation</td>
<td>Skin</td>
</tr>
<tr>
<td>3. Post UV DNA/RNA synthesis</td>
<td>Skin</td>
</tr>
<tr>
<td>4. Complementation test</td>
<td>Skin</td>
</tr>
<tr>
<td>Cell fusion</td>
<td></td>
</tr>
<tr>
<td>Host cell reactivation of the plasmid</td>
<td></td>
</tr>
<tr>
<td>5. Gene analysis</td>
<td>Skin, blood</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td></td>
</tr>
<tr>
<td>DNA sequence</td>
<td></td>
</tr>
</tbody>
</table>
A human gene responsible for XP group A, isolated in Japan, is 25kb long and contains 6 exons [10]. The protein has zinc finger domains, which serve to bind DNA [11]. First, twenty one Japanese XPA cell lines were analyzed but only 3 sites of mutations in XPA gene were recognized [12]. Now there are at least 6 types of XPA genotypes in Japan (Table 3). Surprisingly, 85% of the Japanese XPA patients are homozygous or heterozygous for a G>C transversion at the 3' splice acceptor site in intron 3, which causes abnormal splicing [5, 13, and 14]. Conveniently, this mutation creates a new cleavage site for a restriction enzyme, AbluNI. This founder mutation can be easily and rapidly detected by restriction fragment length polymorphism (or RFLP) of PCR amplified DNA fragment of XPA gene after the digestion by AbluNI by use of patients' blood cells or skin specimens. We can thus confirm the diagnosis of XPA without the above-mentioned complementation test. Of 117 Japanese XPA cases that we could examine in the literature or in our laboratory, 91 (78%) patients were homozygous and 19 (16%) patients were heterozygous for this mutation (Table 3).

The second most common mutation found in Japanese XPA patients is a nonsense mutation of exon 6, which alters the 228th Arg codon to a stop codon. This mutation also creates a new restriction site for HphI and can be detected by PCR-RFLP. We have found two homozygotes and 10 heterozygotes for the mutation of the HphI site in exon 6 (Table 3). Japanese patients with this mutation have less severe clinical disease than patients with the more common XPA mutation of AbluNI site.

In Japan we can diagnose most Japanese XPA patients easily, rapidly and precisely by PCR-RFLP using several restriction enzymes. PCR-RFLP is very useful not only for diagnosis of patients but also to apply early prenatal diagnosis using amniotic fluid or chorionic villi as a source from a fetus and the detection of XPA carriers.

Table 4 summarizes all of the cases who might be XP or should be differentiated from XP, analyzes in our laboratory since Oct. 1998 for 10 years. We confirmed newly 106 XP patients as well as 14 CS cases, a first Japanese XPD/CS complex and a first Japanese TTD (TTD-A) patient.

**CARRIERS OF XP**

The frequency of XP and XPA heterozygotes in Japan (XPA and XP carriers) is estimated by Hiraia et al to be 1 in 100 and 1 in 34, respectively [15].

Clinically parents of XP patients (the obligate heterozygotes) do not have any cutaneous or neurological symptoms or hypersensitivity to sunlight. Cells from these XPA carriers have no DNA repair abnormality after UV irradiation, measured by colony formation or UDS [16]. There are several observations of an increased frequency of chromosomal abnormalities after UV irradiation and high frequency of spontaneous chromosome aberrations in XP carriers. Most clinical and laboratory investigations based on their DNA repair abnormality have failed to show the clinical and biological differences between XP carriers and normal subjects. Based on the research of transgenic mice, heterozygous for XPA and heterozygous for XPC, a higher frequency of tumors of the skin and internal organs, respectively, was reported [17, 18, and 19]. However, there is no direct evidence that human carriers of XP genes have a higher risk of skin cancers or cancer of internal organs than normal subjects.
CARE AND TREATMENT OF XP PATIENTS

Extensive protection from the sunlight and intensive care by various kinds of experts in various fields (dermatology, pediatrics, ophthalmology, orthopedics, dentistry, as well as school teachers) is often necessary. Clinical management consists of early diagnosis followed by a rigorous program of sun protection including avoidance of unnecessary UV exposure, wearing UV blocking clothing, and use of sunblocks on the skin. As there is no cure for the genetic disorder like XP, the main policy of the treatment involves the prompt and complete removal of skin cancers by skin surgeons. XP patients often have problems with their eyes such as conjunctivitis, corneal clouding and neoplasms at a much younger age than in the general population. To avoid these ocular symptoms patients should use sunglasses that block UV light.

In patients with XPA, B, D or G, progressive neurological symptoms may be difficult to manage. Hearing aids may be helpful. Patients should be evaluated for the rehabilitation of muscles of the whole body periodically by orthopedics.

Patient support groups have been established in several countries including Japan that provide information and resources for patients and their families and have practiced summer camps with a UV protected environment. The XP Family Support Groups are located in Kobe, Osaka and Tokyo. Recently all three groups consolidated, which has a common website: http://xp-japan.net/. The booklet "Understanding Xeroderma Pigmentosum" by National Institutes of Health in USA was translated to Japanese, which provides essential information about XP to patients, their families, doctors and educators.

REFERENCES


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