Diagnosis of Xeroderma Pigmentosum by Measuring Unscheduled DNA Synthesis

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The unscheduled DNA synthesis assay has become the most important laboratory method for confirmative diagnosis of xeroderma pigmentosum (XP). Cases of XP diagnosed on clinical grounds alone have been reported in Korea.

We experienced a boy who showed typical features of XP clinically and tried to confirm the diagnosis of XP experimentally.

Fibroblasts were cultured from the patient and from a normal healthy volunteer. Unscheduled DNA synthesis (UDS) after UVB irradiation to the fibroblasts was measured autoradiographically. In addition, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with AlwNI in exon 4 of XP group A complementing (XPAC) gene was done after an extraction of genomic DNA from the patient and his parents.

The level of UDS in our patient was 63% of that of the normal control. No cleavage site for the AlwNI was found in the PCR samples.

The diagnosis was confirmed in vitro by measuring UDS autoradiographically. Our patient was believed to belong to the variant group based on clinical findings and on our experimental results. (Ann Dermatol 8:(2)135~139, 1996).

Key Words : Xeroderma pigmentosum, Unscheduld DNA synthesis assay, PCR-RFLP with AlwNl

Xeroderma pigmentosum (XP) is a rare genetic disease with clinical and cellular hypersensitivity to ultraviolet (UV) radiation and defective DNA repair. Clinically patients with XP show typical pigmentary changes on the exposed skin in the early stage of XP but soon experience UV-induced cutaneous and ocular abnormalities, including neoplasia[1-3].

Cleaver[4] found that XP cells in culture showed markedly diminished rates of unscheduled DNA synthesis (UDS), which represents excision repair, following ultraviolet exposure. Epstein et al[5] demonstrated reduced UDS in vivo in patients with XP. Ever since the UDS assay has become the most important laboratory method for confirming a diagnosis of XP.

De Weerd-Kastelein et al[6] reported genetic heterogeneity of XP demonstrated by cell fusion technique and seven genetic complementation groups, A through G, of excision repair-deficient types and one excision repair-proficient type, variant group, have been identified[3]. Recently, a human gene, designated as XP group A complementing (XPAC) gene was cloned, three types of mutations in the XPAC gene were elucidated, and a rapid diagnostic method for group A using

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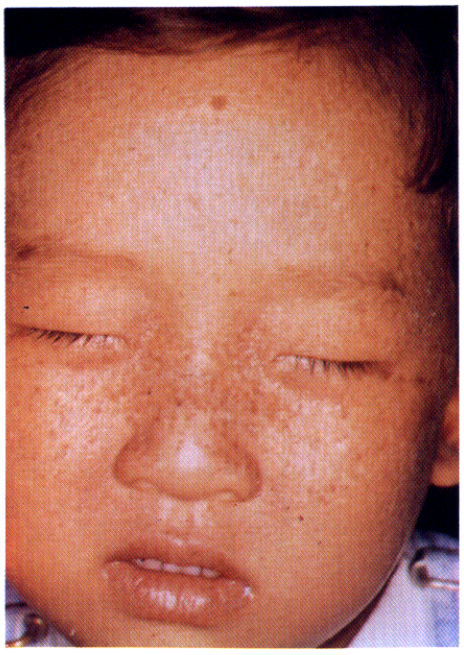

Fig. 1. Multiple freckles on the face.

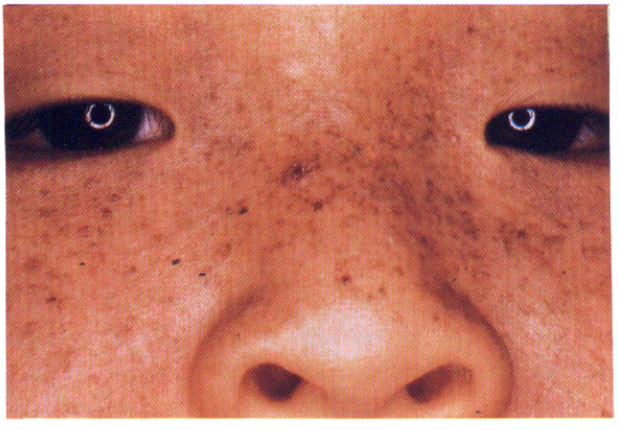

Fig. 2. Slightly scaly papule on the nasal bridge.

polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was reported in Japan[7-9]. However, in Korea only cases of XP diagnosed on clinical grounds alone have been reported[10-20].

We experienced a boy who showed quite typical features of XP clinically and tried to confirm the diagnosis of XP experimentally by measuring the rate of excision repair after UV irradiation, i.e. UDS. We also applied the PCR-RFLP technique which proven useful for the rapid diagnosis of XP group A in our patient.

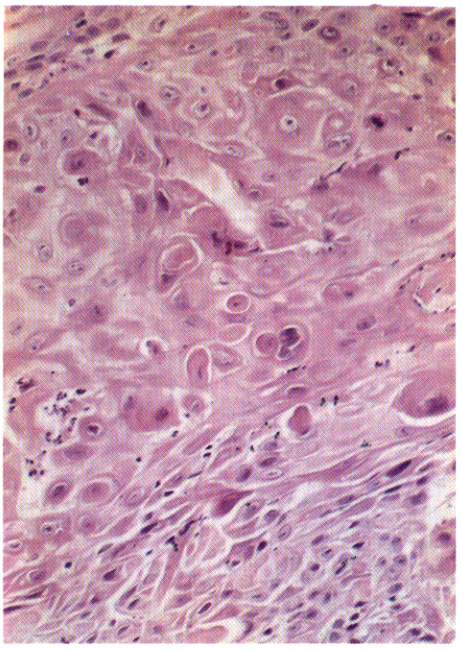

Fig. 3. Histopathologic examination of the papule on the nasal bridge showed squamous cell carcinoma (H & E ×400).

REPORT OF A CASE

boy at the age of 3 years and 10 months e to our hospital complaining of multiple ıented macules on his face which had ap-ed since 7-8 months after birth. He had a ıry of severe sunburning and photophobia. umor was found at his initial visit and his th was normal. His parents were not consan-eous and looked clinically normal. His elder sister looked completely normal without any freckles on her face. However, his father had a few adult relatives with similar pigmented skin lesions.

Physical examination showed multiple freckles on his face(Figure. 1) and a mild conjunctival injection. One month after his initial visit two slightly scaly papules developed on the nasal bridge and the left cheek(Figure. 2), which were proved to be squamous cell carcinomas by histo pathologic examination(Figure. 3). Neither ophthalmologic examination nor neurologic examination showed specific abnormalities except for the conjunctival injection.

Fibroblasts were cultured from the patient and from a normal healthy volunteer. Unscheduled

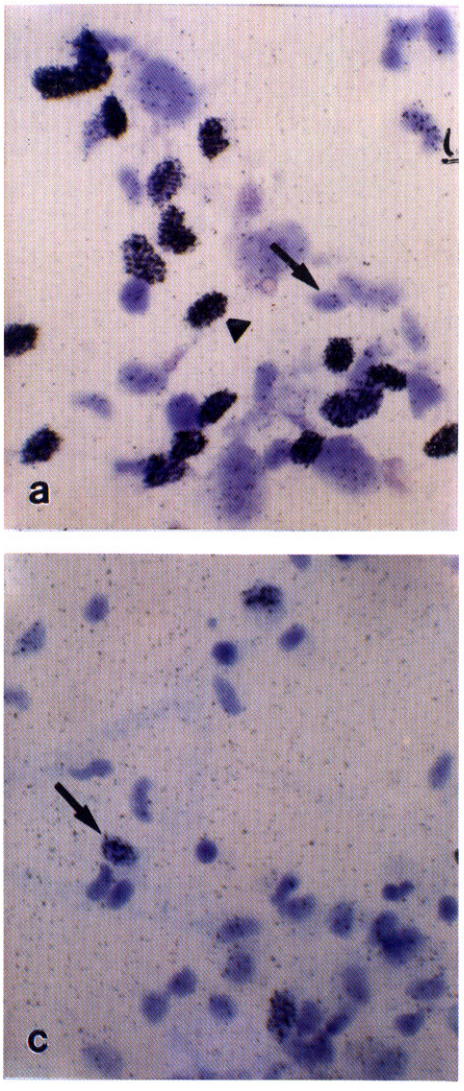

Fig. 4. Autoradiogram of normal and XP fibroblasts. "Heavily-labeled" cells (arrowhead) in scheduled, S-phase DNA synthesis and "lightly-labeled" cells (arrow) in non-S-phase, unscheduled DNA synthesis in normal nonirradiated cells (Figure 4a.). Figure 4b. showed normal cells after UVB irradiation; all the cells were "lightly-labeled" and labeling in the nucleus increased. However, in the cells of XP patient after UVB irradiation the amount of labeling was less than that of normal cells(Figure 4c.).

DNA synthesis (UDS) after UVB irradiation to the fibroblasts was measured autoradiographically with a slight modification as described by Cleaver. The average number of grains per nucleus was determined by counting 50 nuclei in each specimen. Figure 4 showed an autoradiogram of normal and XP fibroblasts. Normal nonirradiated cells after a 3-hour incubation with tritiated thymidine (^{3}HTdR) showed "heavily-labeled" cells (arrowhead) in scheduled, S-phase DNA synthesis and "lightly-labeled" cells (arrow) in non-S-phase, unscheduled DNA synthesis representing excision repair synthesis(Figure 4a). Figure 4b showed normal cells after a 3-hour postirradiation incubation with ^{3}HTdR; all the cells were "lightly-labeled" and labeling in the nucleus increased owing to the UV-induced incorpora-

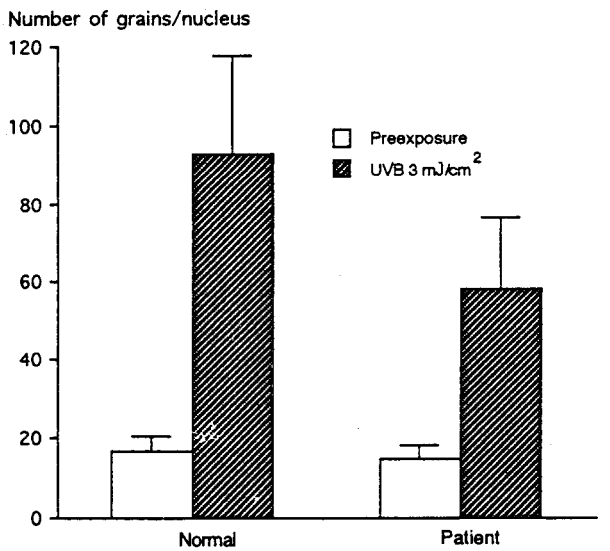

Fig. 5. Unscheduled DNA synthesis (UDS) after 3 mJ/cm^2 of UVB irradiation to the cultured fibroblasts was measured by counting graunules in 50 nuclei in each specimen. The level of UDS in our patient was 63% of normal control.

tion of ^{3}HTdR during DNA repair synthesis. However, in the cells of the XP patient after a 3-hour postirradiation incubation with ^{3}HTdR the amount of labeling was less than that of the normal cells(Figure 4c) of the control.

The number of grains of the nonirradiated normal fibroblasts were 16.52 ± 4.26 and those of the nonirradiated fibroblasts of patient were 14.80 ± 3.34. However, following UVB irradiation the number of grains of normal fibroblasts increased to 92.72 ± 24.94 compared to 58.50 ± 18.11 of XP fibroblasts. The level of UDS in our patient was 63% of that of the normal control(Fig. 5).

Also polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with AlwNI in exon 4 of XP group A complementing (XPAC) gene was done after an extraction of genomic DNA from the patient and his parents. PCR products of the DNAs from the normal subject, the patient, and his parents gave uniform 328-base pair bands(data not shown).

A point mutation at the splicing junction of intron 3 and exon 4 that was found to be the most predominant mutation in Japanese patients with XP group A (XPA) creates a new cleavage site for the AlwNI by transversion (G to C) at the 3' splice acceptor site of intron 3 of XPAC gene, and thus 2 bands of 244 and 84 base pairs would appear in the mutated homozygote state with AlwNI. However, in our normal subject, the patient and his parents no PCR samples were digested with AlwNI indicating that our XP patient is less likely to belong to the XPA.

DISCUSSION

Our patient showed the typical clinical features of XP and the diagnosis was confirmed in vitro by measuring UDS autoradiographically.

To know a patient belongs to a certain complementation group depends on complementation group analysis. Testing standard XP cell lines against sample cells by measuring UDS autoradiographically after cell fusion allows groups of patients whose cells fail to complement each other in culture to be grouped within a single complementation group[1]. We could not perform complementation group analysis and classify our patient exactly.

Among 8 subtypes (A through G, and variant) groups A, C, D, and variant comprised 90% of reported XP patients. Group A, the most common type in Japan, is characteristically accompanied by an early onset of severe neurologic abnormality and group D shows a clinical diversity ranging from patients with no neurologic symptoms to patients with a late onset of neurologic symptoms. Group C, E, F, and variant show no neurologic abnormality[1-3].

Each complementation group has a characteristic range of UDS rates and variant group shows proficient UDS[3]. Group C is not associated with neurologic symptoms but shows a very low rate of UDS of 10-20%, and group D also shows a low rate of UDS (25-50% of normal control)[3]. Our patient had skin cancer but no neurologic symptoms at least until now and showed the level of UDS to be 63% of that of the normal control.

A rapid diagnostic method for group A using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) reported in Japan[8,9] was also applied to our case but no cleavage site for the AlwNI by transversion (G to C) at the 3' splice acceptor site of intron 3 of XPAC gene was found. This was another indirect evidence that our XP patient is less likely to belong to the XPA. We concluded that our patient belonged to the variant group based on clinical findings and our experimental results.

From now on the diagnosis of XP in Korea must be made by typical clinical features and confirmed by laboratory methods in vitro. Laboratory confirmation especially in cases with ambiguous clinical features will make the early diagnosis of XP and early genetic counseling possible. In the future we will be able to subgroup Korean patients with XP according to their genetic heterogeneity by doing complementation group analysis.

REFERENCES

1. Lambert WC : Genetic diseases associated with DNA and chromosomal instability. In Alper JC : Dermatologic Clinics : Genodermatoses. vol 5, no 1. WB Saunders Co., Philadelphia, 1987, pp. 85-108.
2. Kraemer KH, Lee MM, Scotto J : Xeroderma pigmentosum. Arch Dermatol 123:241-250, 1987.

3. Kraemer KH, Levy DD, Parris CN et al : Xeroderma pigmentosum and related disorders: examining the linkage between defective DNA repair and cancer. J Invest Dermatol 103:96-101, 1994.
4. Cleaver JE : Defective repair replication of DNA in xeroderma pigmentosum. Nature 218:652-656, 1968.
5. Epstein JH, Fukuyama K, Reed WB et al : Defect in DNA synthesis in skin of patient with xeroderma pigmentosum demonstrated in vivo. Science 168:1477-1478, 1970.
6. De Weerd-Kastelein EA, Keijer W, Bootsma D : Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. Nature 238:80-83, 1972.
7. Satokata I, Tanaka K, Miura N et al : Three nonsense mutations responsible for group A xeroderma pigmentosum. Mutation Res 273:193-202, 19 92.
8. Kore-eda S, Tanaka T, Moriwaki S et al : A case of xeroderma pigmentosum group A diagnosed with a polymerase chain reaction (PCR) technique. Arch Dermatol 128:971-974, 1992.
9. Nishigori C, Moriwaki S, Takabe H et al : Gene alterations and clinical characteristics of xeroderma pigmentosum group A patients in Japan. Arch Dermatol 130:191-197, 1994.
10. Kim PM, Hong JI, Lim SD : Xeroderma pigmentosum complicated by squamous cell epithelioma and its treatment with bleomycin. Kor J Dermatol 9:35-40, 1971.
11. Rim SJ, Park JS, Kim YK : Two cases of xeroderma pigmentosum in brothers. Kor J Dermatol 9:81-84, 1971.
12. Lee JB, Woo TH : Xeroderma pigmentosum: the treatment of associated skin cancer. Kor J Dermatol 10:99-105, 1972.
13. Kim MS, Kim WS : Xeroderma pigmentosum: report of a case. Kor J Dermatol 12:187-190, 1974.
14. Kim WJ, Song JN, Kim JY et al : A case of xeroderma pigmentosum. Kor J Dermatol 12:191-193, 1974.
15. Hong NS, Lim TP : Xeroderma pigmentosum: report of a case. Kor J Dermatol 15:447-451, 1977.
16. Suh MS, Haam JH, Kook HI : Two case of xeroderma pigmentosum. Kor J Dermatol 16:261-267, 1978.
17. Hwang SW, Yoo YE, Kim YP : The genetic and clinical studies of xeroderma pigmentosum. Kor J Dermatol 20:879-884, 1982.
18. Lim SC, Oh S, Kim NI et al : A case of xeroderma pigmentosum. Kor J Dermatol 23:59-62, 1985.
19. Lim GS, Hong CK, Ro BI et al : A patient with xeroderma pigmentosum developing malignant melanoma and basal cell epithelioma. Kor J Dermatol 24:826-831, 1986.
20. Lee SB, Lee GB, Cho BK et al : A case of xeroderma pigmentosum with various skin tumors. Kor J Dermatol 26:206-211, 1988.