Resistance to UV-Irradiation of DNA Synthesis in Fibroblast Cell Lines Derived from LEC Strain Rats

Kazuhisa UEHARA, Soichiro ARAI, Toyo OKUI[1)], and Masanobu HAYASHI*

Department of Veterinary Radiology, Faculty of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069, and [1)]Hokkaido Institute of Public Health, Sapporo 060, Japan

(Received 25 June 1996/Accepted 2 September 1996)

ABSTRACT. After UV-irradiation, no difference in the survival curves was observed among cell lines derived from LEC strain (LEC) rats and WKAH strain (WKAH) rats. The dose-response curves for inhibition of DNA synthesis in WKAH-derived cells showed a sharp decline at lower doses and a mild decline at higher doses of UV-rays. In contrast, the dose-response curves in LEC-derived cell lines had no sharp component, and were almost identical to the mild component of the curves in WKAH-derived cells. These results show that DNA synthesis in the cell lines of LEC rats was more resistant to UV-irradiation than that of WKAH rats. — KEY WORDS: LEC strain rat, resistant DNA synthesis, UV-irradiation.

J. Vet. Med. Sci. 59(1): 67–69, 1997

In humans, there are several syndromes such as ataxia telangiectasia (AT) with enhanced sensitivity to some chemical and physical mutagens and carcinogens, including ionizing radiation [16]. These disorders are frequently associated with increased spontaneous and induced chromosome aberrations [2, 8], and a genetic predisposition to cancer [18, 24]. The cells from AT patients are sensitive to X-irradiation, but not UV-irradiation [11, 16]. It is well known that double-strand breaks of DNA produced by X-irradiation [17] and pyrimidine dimers induced in DNA by UV-irradiation [21] cause major lethal damage to cells unless repaired. The AT cells are also characterized by the resistance of DNA synthesis to X- and UV-irradiation [13–15], although different types of DNA damages are produced by X- and UV-irradiation. Thus, the cellular sensitivity is segregated from the resistant phenotype of DNA synthesis to DNA-damaging agents [25]. The resistance of DNA synthesis in AT cells is primarily due to the abnormality of many or all of the transient cell cycle arrests which occur in normal cells following the treatment with DNA-damaging agents [9, 19]. Therefore, the study of the response of cellular sensitivity and DNA synthesis to such agents holds the key to revealing some of the biological processes involved in DNA metabolism, such as repair, replication and cell cycle regulation.

LEC strain rats have been established at the Center for Experimental Plants and Animals, Hokkaido University [22]. This strain suffers from spontaneous fulminant hepatitis associated with severe jaundice at about 4 months of age. Other characteristics of LEC rats are a high incidence of spontaneous liver cancer in long-surviving individuals [26] and an increased sensitivity to whole-body X-irradiation [4, 5]. We have reported that the hypersensitivity of LEC rats to whole-body X-irradiation is controlled by a single autosomal recessive gene, xhs [7], and the frequencies of all types of chromosome aberrations induced by X-irradiation in the bone marrow cells of LEC rats are approximately 2- to 3-fold higher than those of WKAH rats [12]. Recently, we reported the resistance of DNA synthesis to X-irradiation in LEC rat cells [6], however, it remains unknown yet whether or not DNA synthesis in LEC cells is resistant to other types of DNA-damaging agents such as UV-rays, and whether or not two phenotypes to DNA-damaging agents, enhanced sensitivities of cell killing and resistance of DNA synthesis, are segregated in LEC rat cells. Therefore, we examined the effects of UV-irradiation on the cellular sensitivity and DNA synthesis in cell lines derived from the lung fibroblasts of LEC and WKAH rats.

The rat fibroblasts were initiated from lungs of LEC and WKAH rats at 2 weeks of age as described previously [7]. Cells were grown in a monolayer culture in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS). Cell cultures were kept at 37°C in an atmosphere containing 5% CO_2 with ambient humidity. Cells were co-transfected with pEF-T that expresses simian virus 40 (SV40) large T-antigen under the control of human elongation factor 1a promoter [10] and pSV2-neo using the standard calcium phosphate precipitation procedure [3]. Briefly, 5×10^5 cells in 6-cm dishes were treated with 10 μg of pEF-T and pSV2-neo DNA, and were replated on the following day at a density of 1×10^5 cells per dish in a selection medium containing 1 mg G418/m*l*. G418-resistant clones were established 2 months later.

Cell survival was determined using the conventional colony-forming assay. Expanded cells were collected by trypsinization and $2–50 \times 10^2$ cells were plated into 6-cm dishes. After adhesion, the medium was removed from the plates, and the cells were washed with phosphate-buffered saline, pH 7.2 (PBS) and then irradiated by far-UV-rays (254 nm) from a Toshiba Germicidal Mercury vapor lamp at fluence rate of 1.0 J/m^2/sec. Immediately after irradiation, a fresh medium was added and the plates were incubated again. After 2 weeks of incubation, the colonies were fixed with methanol, stained with Giemsa and then counted. A colony containing more than 50 cells was counted as a

* CORRESPONDENCE TO: HAYASHI, M., Department of Veterinary Radiology, Faculty of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069, Japan.

survivor under a dissecting microscope.

Logarithmically growing cells (5×10^4) were incubated at 37°C for 24 hr in MEM containing [^{14}C]thymidine (3.7 kBq/m*l*, ICN Radiochemical Inc.). After removal of the medium, the cells were washed twice with PBS. After the cells were exposed to 0–30 J/m^2 of UV-rays, the cells were pulse-labeled with [^{3}H]thymidine (74 kBq/m*l*, ICN Radiochemical Inc.) at 37°C for 30 min. After the incubation, [^{3}H]thymidine was removed and the cells were washed twice with PBS. After the addition of MEM without [^{3}H]thymidine, the cells were incubated at 37°C for 2 hr. The cells were subsequently harvested, lysed with 2% SDS and then precipitated with 5% TCA. Relative incorporation of [^{3}H]thymidine was determined as the ratio of ^{3}H disintegration per minute (dpm) to ^{14}C dpm.

Sixteen cell lines were each established from lung fibroblasts of LEC and WKAH rats. We show here that there was no difference in the survival curves among the cell lines from LEC and WKAH rats after UV-irradiation. The typical survival curves of the cell lines are shown in Fig. 1. It is well known that pyrimidine dimers induced in DNA by UV-irradiation cause lethal damage to cells unless repaired, and that most of the pyrimidine dimers are repaired by a nucleotide excision repair system in normal cells [20, 21]. Since no difference in the survival curves was observed among LEC and WKAH rat cell lines, it is suggested that there is no deficiency in the nucleotide excision repair system of LEC rat cells.

The dose-response curves for inhibition of DNA synthesis in WKAH-derived cell lines, W2 and W16, showed a sharp decline at low doses and a mild decline at high doses of UV-rays (Fig. 2). In contrast, the dose-response curves in LEC-derived cell lines, L4 and L9, showed no sharp component, but were almost identical to the mild component of the curves in W2 and W16 cells (Fig. 2). The resistance of DNA synthesis to UV-irradiation shown in L4 and L9 cells was also observed in other LEC cell lines (data not shown). These results indicate that DNA synthesis in the cell lines derived from LEC rats was more resistant to UV-irradiation than that of WKAH rats.

Recently, we have shown that cell lines from LEC rats are more sensitive in cell killing and more resistant in DNA synthesis than WKAH rat cells to X-irradiation [6]. In addition, the present study shows that DNA synthesis in LEC rat cells is more resistant to UV-irradiation than that in WKAH rat cells. Therefore, DNA synthesis in LEC cells shows resistance to different types of DNA-damaging agents, X- and UV-rays, and the response of DNA synthesis in LEC cells shows a resemblance to that in AT cells to DNA-damaging agents [13–15].

AT cells represent resistance of DNA synthesis to X- and UV-irradiation, although AT cells are susceptible to X-irradiation, but not UV-irradiation [13–15]. Thus, the enhanced cellular sensitivity is segregated from the resistant phenotype of DNA synthesis to DNA-damaging agents [25]. In the present report, LEC rat cells showed normal cellular

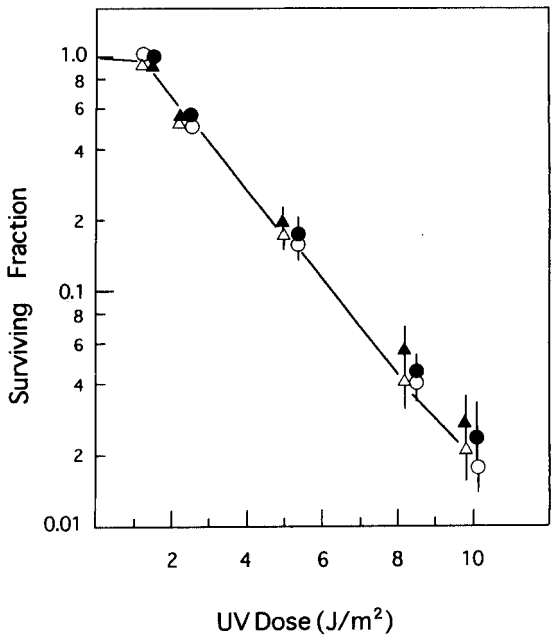

Fig. 1. Survival curves of cell lines from LEC rats, L4 (●) and L9 (○), and WKAH rats, W2 (▲) and W16 (△), after exposure to UV-rays. Points represent the average from four separate experiments. Error bars represent the standard deviation of the mean values.

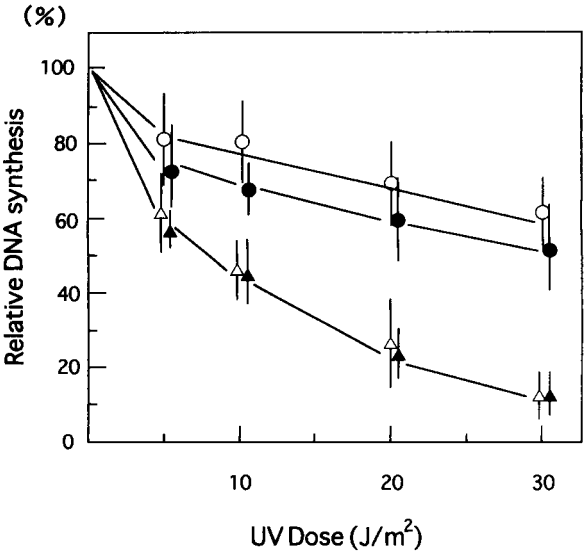

Fig. 2. Inhibition of DNA synthesis in cell lines from LEC and WKAH rats by UV-irradiation. ^{14}C-labeled L4 (●), L9 (○), W2 (▲) and W16 (△) cells were UV-irradiated, and the cells were labeled with [^{3}H]thymidine for 30 min. The ratio of ^{3}H to ^{14}C dpm represents the rate of DNA synthesis per cell, which is plotted as a percentage of this ratio in unirradiated cells. Points represent the average of four separate experiments. Error bars represent the standard deviation of the mean values.

susceptibility and resistance of DNA synthesis to UV-irradiation. These results suggest that two phenotypes, cellular sensitivity and resistance of DNA synthesis to DNA-damaging agents in LEC rat cells might be regulated by different genes. Since the resistance of DNA synthesis to DNA-damaging agents in AT cells seems to be primarily due to the abnormality of many or all of the transient cell cycle arrests which occur in normal cells following the treatment with DNA-damaging agents [9, 14, 19], the resistance of DNA synthesis to UV- and X-rays might be affected by the same mutant gene. It has recently been reported that a mutant gene in AT is a human phosphatidyl inositol-3'-kinase (PI-3'K) homologue [23]. A study concerning the PI-3'K homologue gene of LEC rats is now in progress.

LEC rats are characterized by radiosensitivity to whole-body X-irradiation similar to the ataxia telangiectasia mutation in humans [16] and scid mutation in mice [1]. LEC rat cells also exhibit resistance of DNA synthesis to different types of DNA-damaging agents such as X- and UV-rays. Thus, the LEC rat could provide a useful animal model for understanding the mechanism of the repair process of DNA damage and the regulation of cell cycle.

ACKNOWLEDGEMENT. This work was supported in part by Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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