DNA Repair Pathways in Mammalian Cells Analyzed by Isolation of ACNU-Sensitive Chinese Hamster Ovary Cells

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NUMATA, M., HATA, H., TOHDA, H., YASUI, A and OIKAWA, A. *DNA Repair Pathways in Mammalian Cells Analyzed by Isolation of ACNU-Sensitive Chinese Hamster Ovary Cells.* Tohoku J. Exp. Med., 1992, 168 (2), 123-128 — 1-[4-amino-2-methyl-5-pyrimidinyl)methyl]-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU) causes chloroethylation of DNA strand followed by cross linking through an ethylene bridge. We recently isolated two ACNU sensitive mutants from mutagenized Chinese hamster ovary cells, and found them to be new drug sensitive recessive mutants (Hata et al. 1991). The *O*⁶-methyl guanine DNA methyl transferase (MT) activities of these cells were undetectable as the parental cell line, indicating that the sensitivity of the mutant cell lines to ACNU was not due to the decreased cellular level of this enzyme. By complementation analysis with the 7 established UV-sensitive CHO cell lines, one of the mutants, UVS1, turned out to complement their UV-sensitivity and, therefore, build a new complementation group among all the CHO cell lines ever reported. The other mutant, CNU1 showed hypersensitivity only to chloroethylating agents (ACNU, CCNU) and exhibited a slightly reduced unscheduled DNA synthesis (UDS) induced by UV. It is, therefore, suggestive that this mutant is defective in a specific step of DNA repair systems, which is important for the processing of DNA damages produced by ACNU. Only cell lines from the complementation group 1 and 4 out of 7 established complementation groups of UV-sensitive CHO mutants were more sensitive to ACNU than UVS1 and CNU1, indicating some steps of excision repair pathways as well as specific repair system play important roles in repairing ACNU-induced DNA damages. ——— chloroethylating agent; ACNU; cross linking; DNA repair; CHO mutant

ACNU, a hydrophilic chloroethylnitrosourea, is clinically useful against a variety of malignant diseases, particularly brain tumors and Hodgkin's disease. Its anti-tumor activity appears to be a result of chloroethylation at *O*⁶-position of guanine residues, followed by interstrand crosslinks formation (Kohn 1977; Erickson et al. 1980; Tong et al. 1982). In mammalian cells *O*⁶-methyl guanine DNA methyl transferase (MT) removes chloroethyl moieties by transferring to its own cysteine residues. Recently it was reported there was a good correlation between MT activity and sensitivity to ACNU in cultured cell lines from various

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animal species (Ikenaga et al. 1987).

We were interested in the repair mechanism of ACNU-induced DNA damages in mammalian cells and isolated two novel ACNU-sensitive CHO cell lines (Hata et al. 1991). We characterized further these mutant cell lines by comparing their sensitivity with those of other UV-sensitive CHO cell lines and analyzed DNA repair pathways in CHO cells.

MATERIALS AND METHODS

Cells and culture conditions. The UV-sensitive CHO cell lines 43-3B, UV5, 27-1, UV47, UV135, UV61, V-B11 represent the complementation groups 1 to 7. Complementation groups 2 to 6 were kindly provided by Dr. L. Thompson. The V-B11 cells were provided by Dr. Zdzenicka. All cells were routinely maintained as monolayer cultures at 37°C in DMEM/F12 medium (Sigma, St. Luis, MO, USA) supplemented with 10% fetal bovine serum.

Chemicals and irradiation. ACNU was a generous gift from Sankyo Co., Tokyo. Irradiation of UV light of 254 nm was performed using Hitachi germicidal lamp GL10 at a fluence rate of 0.44 J/m² per sec.

Survival assays. All survival experiments were carried out as previously described (Hata et al. 1991). For measuring colony forming ability, an appropriate number of cells in plastic dishes were incubated at high humidity under 5% CO₂ in air. After about 8 hr incubation at 37°C, the cells were treated for 1 hr at 37°C with ACNU or irradiated with UV. ACNU was dissolved in Hanks' balanced salt solution (Nissui, Tokyo) immediately before use.

Assay of MT activity. The MT activity was measured as previously described with some modification (Karran et al. 1979; Morohoshi and Munakata 1987). The reaction mixture containing substrate DNA pretreated with ³H-MNU (1.0 Ci/mmol; New England Nuclear Corp., Boston, MA, USA) and extracts were incubated for 1 hr at 37°C. After precipitation with 0.8 M trichloroacetic acid, the precipitate was collected and treated with proteinase K (Boehringer Mannheim). The supernatant was collected and the radioactivity was counted by liquid scintillation counter (Beckman, Fullerton, CA, USA).

Measurement of UV-induced UDS. UDS was measured as previously described (Hata et al. 1991). In short, the UV-irradiated cells were incubated in the common medium for various periods, followed by incubation in the medium containing 10 µCi/ml ³H-thymidine for 1 hr. After the conventional treatment of cells for UDS, grains were counted and the mean number and the standard error were calculated.

RESULTS AND DISCUSSION

Fig. 1a and 1b show the sensitivity of UVS1 and CNU1 mutant cells against ACNU and UV, respectively. CNU1 was especially sensitive to ACNU and slightly sensitive to UV, whereas UVS1 is cross-sensitive to UV. Since chloroethylated guanines, primary DNA adducts produced by ACNU on DNA, are efficiently repaired by O⁶-alkylguanine alkyltransferase (Ikenaga et al. 1987), we determined the activity of this enzyme in the cell extracts of UVS1 and CNU1 and compared them with those of CHO9, the parental cell line. The enzymatic activity was not detectable in all the cell extracts. This result coincided well with the earlier results that these cell lines were equally sensitive to other alkylating agents, methylnitrosourea, ethylnitrosourea and ethyl methanesul-

Fig. 1. Dose effect of ACNU (A) and UV (B) on colonyforming ability of the isolated UVS1 (triangles) and CNU1 (squares) cell lines and the parental cell line CHO9 (circles).

fonate (Hata et al. 1991). The sensitivities of both mutants to ACNU, therefore, were not due to decreased cellular levels of the alkyltransferase.

In order to identify the deficient function in both mutants, we determined UV-induced unscheduled DNA synthesis (UDS) of UVS1, CNU1, 43-3B and CHO9 cells (Fig. 2). 43-3B was used as a negative control of UDS, because it was shown to be defective in an incision step of the excision repair process resulting in no detectable UDS after UV-irradiation. UDS was measured for one hour after various period of post-UV incubation. Both UVS1 and 43-3B showed a basal level of UDS. CNU1, on the other hand, exhibited a reduced level of UDS as compared with that of CHO9, especially immediately after UV-irradiation and after 6 hr. Since fused cells between both mutants were resistant against ACNU, the above results suggest that both mutants are defective in two different steps of

Fig. 2. Unscheduled DNA synthesis (UDS) after UV irradiation (10 J/m^2) of UVS1 (triangles) and CNU1 (squares). UVS1 shows low level of UDS as 43-3B (diamonds) which is assigned as the first complementation group of excision repair deficient CHO mutant. UDS of CNU1 appears to be slightly low comparing wild type CHO9 (circles).

excision repair. We analyzed, whether the UV-sensitivity of UVS1 can be complemented by fusion with the established UV-sensitive CHO cell lines of complementation groups 1 through 7 and two CHO cell lines recently isolated (Stefanini et al. 1991), and found that the mutation in UVS1 can be complemented by all of these CHO cell lines, indicating UVS1 builds a new complementation group among UV-sensitive CHO cell lines. By transferring human DNA into UVS1 cells, we are currently trying to identify the gene responsible for the sensitivity.

Fig. 3a depicts the sensitivity of the established UV-sensitive cell lines, complementation groups 1 to 7 and UVS1 against ACNU. The sensitivity of these cell lines to ACNU are quite different from those to UV (Fig. 3b). Since it is reasonable to assume that all cell lines possess no alkyltransferase activity against alkylated DNA produced by ACNU (Fritz et al. 1991), the difference of sensitivity among these cells is probably due to different excision repair capacity of the cells to ACNU-induced DNA damages. 43-3B and UV47 are known to be very sensitive to mitomycin C, a cross-linking agent. It may be, therefore, reasonable to assume that the sensitivity of these cell lines to ACNU is due to the defect in repairing cross-links produced by ACNU. UVS1 is not so sensitive to ACNU as these cells, but it is much more sensitive than UV5 or UV135, which are hypersensitive to UV. Since UVS1 is also sensitive to other cross-linking agents like mitomycin C and *cis*-platinum, this cell line is supposed to be defective in a step of excision repair, which plays an especially important role in the repair of cross-links. CNU1, however, was as resistant as the parental cell line CHO9 to

Fig. 3. Dose effect of ACNU (A) and UV (B) on colony forming ability of UV-sensitive CHO mutants.

various cross-linking agents (Hata et al. 1991). Therefore, we suppose that CNU1 is defective in special step of ACNU-induced DNA damages. Since it was shown that chloroethylated DNA built cross-links 3 to 4 hr after monoadducts formation, CNU1 may be defective to remove this monoadduct by an excision pathway. Further analysis is necessary to identify the defects of the isolated CHO cell lines.

DNA repair activity against DNA cross-links provides cells with resistance to anticancer drugs like ACNU or *cis*-platinum. Understanding of DNA repair mechanism of DNA cross-links may lead to the finding of new category of cancer chemotherapy.

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