Jpn. J. Genet. (1983) **58**, *pp.* 393–403

Radiation sensitivity and chromosomal changes in human leukemic T-cell lines[1)]

By Takahiro TAGUCHI and Yukimasa SHIRAISHI

Laboratory of Human Cytogenetics, Department of Anatomy, Kochi Medical School, Nankoku City, Kochi 781-51

(*Received March* 24, 1983)

ABSTRACT

Radiosensitive and resistant cell lines were studied with regard to radiation survival and chromosomal changes. A radioresistant cell clone (CCRF-HSB2-M) was spontaneously separated from a radiosensitive human T-cell line (CCRF-HSB2) which was originated from the peripheral blood of the patient with an acute lymphoblastic leukemia (ALL). Cell growth and dose-survival curves after X-irradiation indicated that HSB2-M cells were as resistant as normal cells, though the frequency of chromosomal aberrations in irradiated HSB2-M cells was slightly higher than that in irradiated normal cells. Higher frequencies of chromatid and chromosome deletions (breaks) were observed in irradiated HSB2 cells, whereas those of deletions in HSB2-M and normal cells showed a relatively low frequency and the chromosomal aberrations in these two cell lines decreased with time. The karyotype of radiosensitive HSB2 cells was XY, 46, t(1;7)(p32;q32). The radioresistant mutant clone (HSB2-M) gained some other changes in addition to the original translocation: 47, XY, t(1;7)(p32;q32), +t(6;6)(p11;q13), t(12;16)(q24;q11), −16p, +22. The acquirement of radioresistant character in HSB2-M cells could be closely related to the additional chromosomal changes (+iso6q and +22). The emergence of a radioresistant clone from a radiosensitive cell line is discussed in relation to human leukemia.

1. INTRODUCTION

The exact mechanism determining radiosensitivity of normal and neoplastic cells remains unknown, though a great deal of studies have been aimed at a better understanding of the mechanism underlying cellular radiosensitivity (Bromer *et al.* 1982; Chen *et al.* 1978; Cleaver *et al.* 1982; Knox *et al.* 1981; Kohn *et al.* 1982; Painter and Young 1980; Rahmsdorf *et al.* 1981; Richard *et al.* 1982). In previous studies (Shiraishi *et al.* 1976, 1978), we have shown that a human T-cell line (CCRF-HSB2), which was originated from the peripheral blood of a patient with acute lymphoblastic leukemia (ALL), was extremely radiosensitive with regard to cell survival and chromosomal aberrations, whereas a normal B-cell line and normal T-lymphocytes were relatively

1) This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan, and from the Nissan Science Foundation.

radioresistant. An increased sensitivity of the T-cell line to X-ray irradiation, consisting of remarkable chromosomal aberrations and a high percentage of nonviable cells, suggested that the T-cell line (HSB2) was defective in its DNA repair mechanism necessary to overcome the lesions produced by X-ray irradiation. In the course of the study of the radiosensitivity of the T-cell line, a radioresistant cell clone (CCRF-HSB2-M) was spontaneously separated from the original cell line (HSB2). Even though the original HSB2 cells grew forming cell aggregates during the course of *in vitro* subcultures, the cell population with negative cell aggregates survived and proliferated as a suspension of free cells without forming cell aggregates. Interestingly, cell clone (HSB2-M) with negative aggregations was radioresistant and gained some additional chromosomal changes in Nos. 6, 12, 16 and 22, in addition to the original balanced translocation between chromosomes Nos. 1 and 7. It is of special interest to clarify the mechanism underlying in radiation sensitivity by using these two T-cell lines (HSB2 and HSB2-M), derived from the same patient. In the present study, we examined the differences in karyotype, cell killing rate and chromosomal aberrations following X-irradiation in these cell lines. The radiation sensitivity, changes in karyotype and gene dosage effect are discussed.

2. MATERIALS AND METHODS

Two human T-cell lines, radiosensitive (CCRF-HSB2) and radioresistant (CCRF-HSB2-M) cell line and a normal B-cell line (KS 64), which was derived from a normal healthy volunteer, were used in the present study. The radiosensitive HSB2 cell line (Shiraishi *et al.* 1976) was originated from the peripheral blood of an acute lymphoblastic leukemia (ALL) patient and the HSB2-M cell line was radioresistant mutant clone which was separated from the HSB2 cell line in *in vitro* culture. The radioresistant cell clone (HSB2-M) proliferated as a suspension of free cells *in vitro* (Fig. 1a), though radiosensitive cells (HSB2) grew forming cell aggregates (Fig. 1b). Cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) in a CO_2 (5%) incubator at 37°C. After two days of subculture, these cells (3×10^5 cells/ml) were irradiated with 100, 200, 300, 400 and 500 rads of X-ray in a Falcon plastic flask (25 cm^2), using a 250 KV X-ray source (Shin-ai 250-2M: RX-250) with a 1 mm aluminum filter, at room temperature. Irradiated and non-irradiated cells were studied cytogenetically and with respect to cell survival. The cells were counted and fixed at 0, 12, 24, 48, 72, 96 and 120 hrs after irradiation. Chromosome preparations were made as follows, colcemid was added for the final 2 hr of culture and cultures were harvested by a 10 min treatment with warm (37°C) hypotonic (0.075M) KCl followed by two changes of fixative (3:1=methanol: acetic acid) and slides

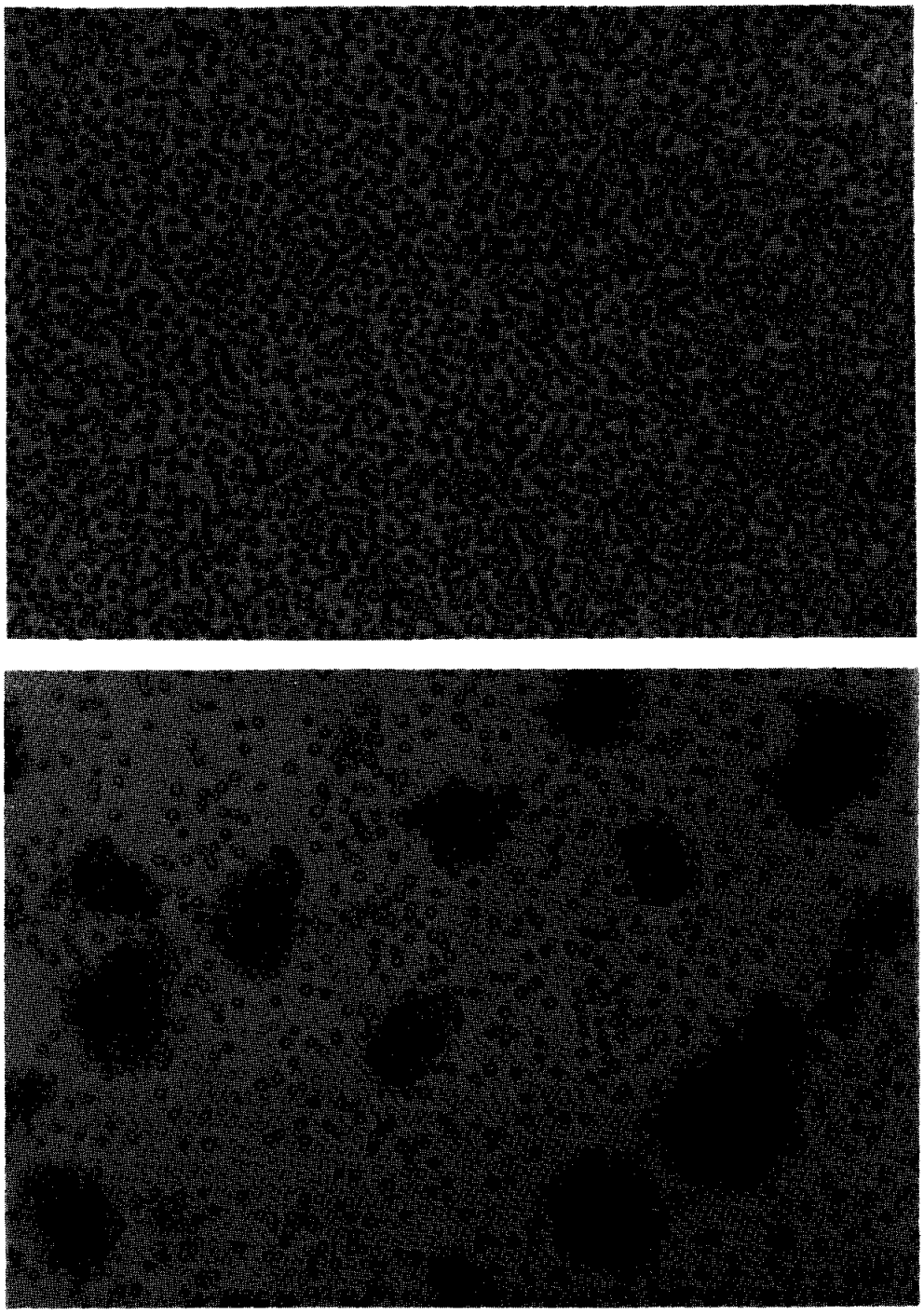

Fig. 1. The different appearance of CCRF-HSB2-M cells as a suspension of free cells (a) and CCRF-HSB2 cells growing with forming cell aggregates (b).

were prepared by the air drying method. Chromosome banding studies were carried out according to G-, Q- and C-bandings (Shiraishi and Yoshida 1973; Caspersson *et al.* 1972; Sumner 1972). Chromosomal aberrations were counted in 100–200 metaphases of the irradiated cells. Chromosome analysis was only performed in cell cultures irradiated with 100 rad of X-ray, since the dose of 100 rad was suited for the analysis of chromosomal aberrations and chromosome analysis was not possible over 100 rad of X-ray because of rare mitoses in the HSB2 cells. Experiments were repeated three or more times, with qualitatively similar results. The evaluation of cell growth and cell killing after irradiation was performed using the trypan blue dye exclusion test.

3. RESULTS

Karyotype analysis

The karyotype of the radiosensitive HSB2 cells had a male karyotype with a typical Y-chromosome, 46, XY, t(1;7)(p32;q32). Karyotype analysis of the mutant clone (HSB2-M) was performed by conventional staining, G-, Q- and C-bandings of air dryed preparations. On the basis of the banding patterns, we have analysed and identified a karyotype of 47, XY, t(1;7)(p32;q32), +t(6;6)(p11;q13), t(12;16)(q24;q11), −16p, +22 (Figs. 2 and 3). By compar-

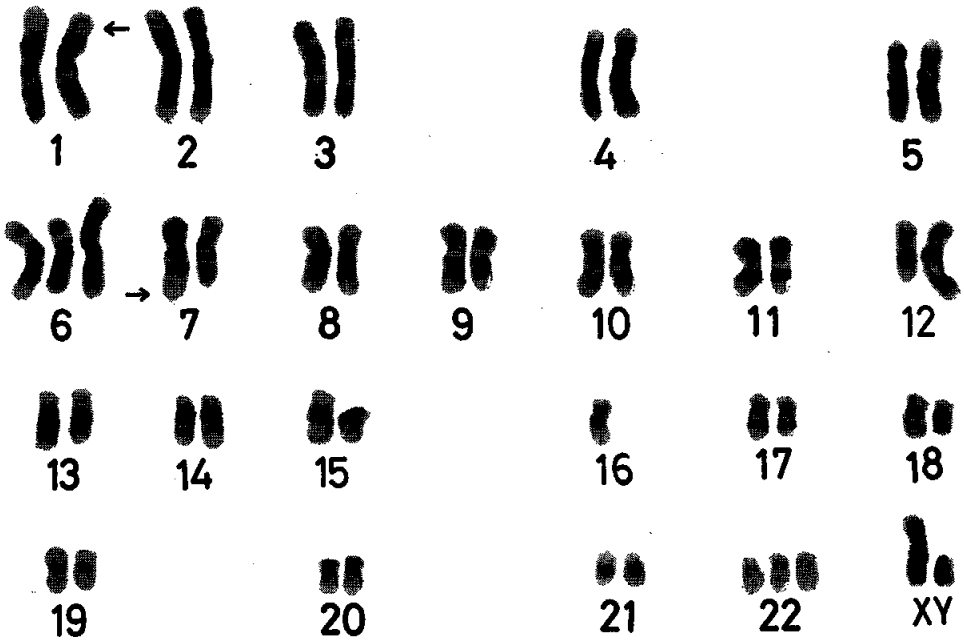

Fig. 2. G-banding karyotype of the radioresistant clone (CCRF-HSB2-M) showing, 47, XY, t(1;7)(p32;q32), +t(6;6)(p11;q13), t(12;16)(q24;q11), −16p, +22. The arrows indicate the balanced translocation between No. 1 and No. 7.

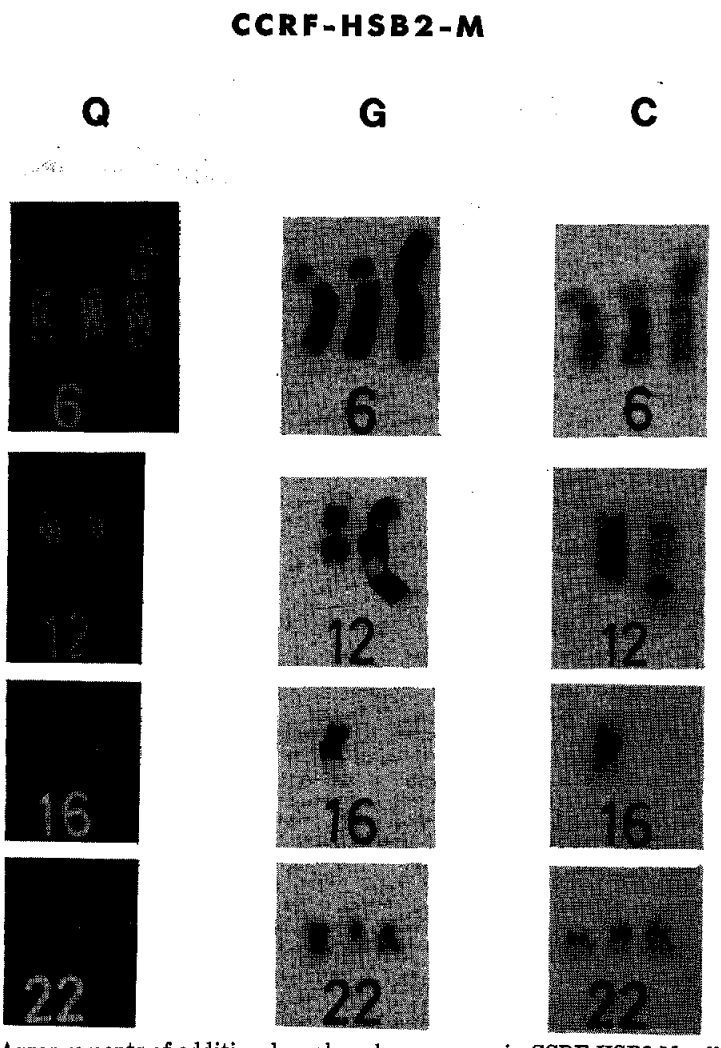

Fig. 3. Arrangements of additional marker chromosomes in CCRF-HSB2-M cells with Q-, G- and C-banding patterns.

ing the karyotypes of HSB2 and HSB2-M cells, it was found that though the HSB2-M cells gained some additional changes, i.e., +t(6;6)(p11;q13), t(12;16)(q24;q11), −16p and +22, these two cell lines had the same original translocation, t(1;7)(p32;q32). This finding strongly supports the idea that HSB2-M cells were derived from the HSB2 cell line. A normal B-cell line (KS 64) had a normal karyotype, 46, XY, was used as a control.

Cell growth

The growth curves of these three cell lines were shown in Fig. 4. Growth

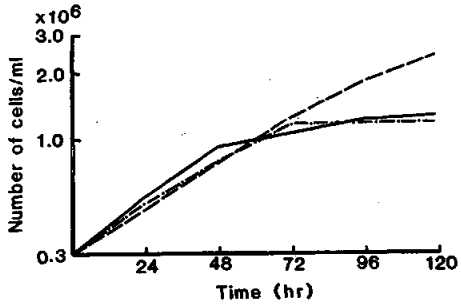

Fig. 4. Growth curves of the T-cell (CCRF-HSB2 and CCRF-HSB2-M) lines and normal B-cell (KS 64) lines. Each point of data shows an average number. CCRF-HSB2:——, CCRF-HSB2-M:— — —, KS 64:—·—·—.

patterns of these cell lines were very similar at the exponential growth phase with their doubling times ranging from 30 to 36 hr (Fig. 4), though HSB2 and KS 64 cells reached the stationary growth phase earlier than HSB2-M cells. The generation times (cell cycle time) of these cell lines were about 24 hr. The HSB2-M cells grew exponentially throughout the 5 day culture periods, whereas the other two cell lines (HSB2 and KS 64) with cell aggregates usually stopped growing after 48 hr of exponential cell growth, possibly because of limited cell proliferation within a cell clot.

Radiation effect on cell killing

The *in vitro* radiation effect of T-cell lines (HSB2 and HSB2-M) and a normal control B-cell line (KS 64) was studied by a cell viability test using the trypan blue dye exclusion test. As previously reported (Shiraishi *et al.* 1976, 1978), HSB2 cells exhibited a significantly higher radiosensitivity than the normal B-cell line. In contrast, HSB2-M cells were radioresistant in relation to the survival at various doses (50, 100, 200, 300, 400 and 500 rads) of X-irradiation (Fig. 5). Fig. 5 shows the survival curves at various doses of irradiation after 72 hr. The slants of the curves of the three cell lines show the differences of radiosensitivity. The order of high radiosensitivity was HSB2, HSB2-M and KS 64, though HSB2-M cells were radioresistant and were not significantly different from their radiosensitivity of KS 64 cells. Fig. 6 exhibits the percent survival with 100 rad of X-ray at various intervals after irradiation. 100 rad of X-ray killed approximately 70% of HSB2 cells after 5 days of culture, though HSB2-M and normal cells were radioresistant and there was no significant difference of percent survival between both of them. The effect of cell growth on cells irradiated with 100 rad of X-ray was evaluated in relation to cell number at various periods following irradiation (Fig. 7). The number of HSB2-M cells increased with time and reached to the

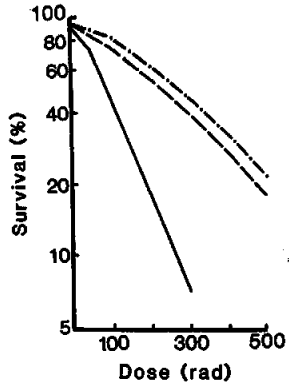

Fig. 5. Survival curves for the T- and B-cell lines at 72 hr of incubation following irradiation with 50, 100, 200, 300, 400 and 500 rads of X-ray. CCRF-HSB2:——, CCRF-HSB2-M:— — —, KS 64:—·—·—.

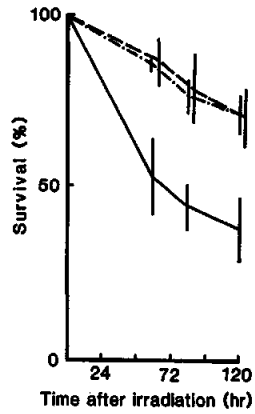

Fig. 6. Effect of X-ray (100 rad) on cell growth in T- and B-cell lines at various periods following irradiation. The cross points of the error bar represent the mean values from five experiments and the error bars represent the standard deviations. CCRF-HSB2:——, CCRF-HSB2-M:— — —, KS 64:—·—·—.

same level as normal cells (KS 64) in a few days after irradiation. In contrast, no cell growth was observed following 100 rad of irradiation of the HSB2 cells.

Chromosome damages

Chromosomal aberrations were analysed in HSB2, HSB2-M and normal (KS 64) cells following irradiation at 100 rad. Chromosomal aberrations were analysed at 0, 12, 24, 48, 72 and 96 hrs after irradiation. In all tests and control cultures, 100–200 metaphases (randomly chosen) were examined by scoring the frequency of chromosomal aberrations, including chromatid and chromo-

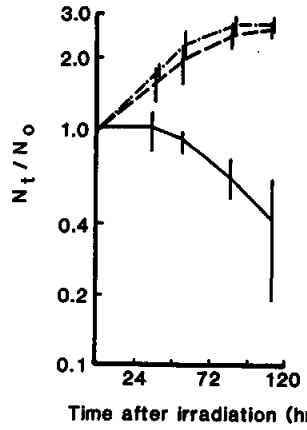

Fig. 7. Effect of X-irradiation (100 rad) on cell viability in three cell lines (CCRF-HSB2, CCRF-HSB2-M and KS 64). It was noticed no cell growth was seen in CCRF-HSB2 cell line. The cross points of the error bar represent the mean values from five experiments and the error bars represent the standard deviations. N_0: viable cell number at 0, N_t: viable cell number at given time. CCRF-HSB2: ——, CCRF-HSB2-M: — — —, KS 64: —·—·—.

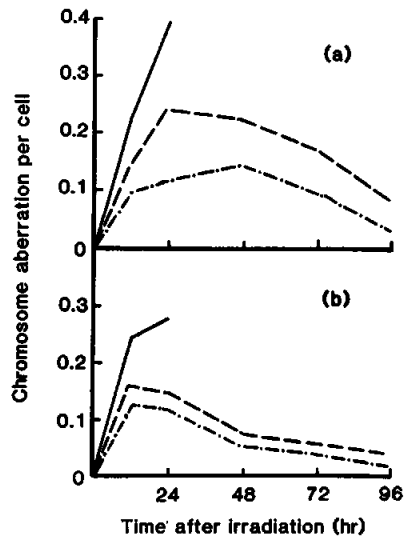

Fig. 8. The changes of incidence of chromosomal aberration (chromatid and chromosome deletions) with time course (12, 24, 48, 72 and 96 hrs) following 100 rad of X-irradiation. Chromosome deletions (a) Chromatid deletions (b). CCRF-HSB2: ——, CCRF-HSB2-M: — — —, KS 64: —·—·—.

some deletions, chromatid exchanges, translocations, dicentrics, rings and acentric fragments. Special attention was taken to identify the chromatid and chromosome deletions, since their frequencies of the aberrations were

extremely high in HSB2 cells and other types of aberrations were similar level among these three cell lines. Extremely high frequencies of chromatid and chromosome deletions were detected at 12 and 24 hrs after 100 rad of irradiation in HSB2 cells (Fig. 8). However, chromosome analysis was difficult in HSB2 cells after 48 hr of irradiation because of cell death. In HSB2-M and normal (KS 64) cells, the frequencies of chromatid deletions got to the peak at 12 hr after irradiation and gradually decreased with time (24, 48, 72 and 96 hrs). On the other hand, the frequencies of chromosome deletions reached to the peak at 24 (HSB2-M) and 48 (KS 64) hrs and gradually decreased, though their aberration frequencies of chromatid and chromosome deletions in HSB2-M cells were slightly higher than those in normal cells.

4. DISCUSSION

The present study describes chromosomal changes and radiosensitivity in human T-cell lines, originated from acute lymphoblastic leukemia (ALL). A human leukemic T-cell line (HSB2) was extremely radiosensitive with regard to certain chromosomal aberrations (chromatid and chromosome deletions) and cell survival. However, interestingly in the course of *in vitro* long term culture, the radioresistant cell clone (HSB2-M) separated spontaneously from the HSB2 cell line. HSB2 cells grew forming cell aggregates but HSB2-M cells proliferated without forming cell aggregates as a suspension of free cells, so they were easily distinguished from the cell aggregates. Dose-survival curves clearly showed that HSB2-M cells were as radioresistant as normal (KS 64) cells. The finding that HSB2 and HSB2-M cells have an identical balanced translocation, i.e., t(1;7)(p32;q32), strongly indicates the common origin of these two cell lines, though HSB_2-M cells gained additional chromosomal changes. With respect to X-ray induced chromosomal aberrations, a high frequency of chromosomal aberrations was noted at 24 hr after irradiation in HSB2 cells. However, HSB2 cells could not be analysed after 48 hr of irradiation because of rare mitotic cells due to high radiosensitivity. Even though HSB2-M cells exhibited more increase in chromosomal abberrations following irradiation as compared to normal (KS 64) cells, their aberrations decreased with time, as normal (KS 64) cells did. The decrease of chromosomal aberrations and cell survival recovery following irradiation with time indicated that HSB2-M cells may be more repair-proficient than HSB2 cells. It is interesting to attempt to relate the radiation survival character with the change in the DNA content of the cell, such as certain additional chromosomal changes, i.e., +iso6q and +22. Some other investigators (Szumiel 1981; Leith 1982) have tried to correlate the difference of radiation sensitivity with a cell DNA content of ploidy. It is considered that the fate of DNA damages is influenced by such factors as number of chromosomes (Szumiel 1981) and chromatin organization (Chiu

1982). Szumiel (1981) proposed that such factors contributing to intrinsic radiosensitivity as repair enzymes, chromatin condensation and energy supply may depend on the function of membranes. This proposal may be important to our T-cell lines since the ability of forming cell aggregates may be related to the character of each cell membrane and may be reflected in their different radiosensitivities.

In conclusion, an increased radiosensitivity of HSB2 cells, consisting of remarkable chromosomal aberrations and a high percentage of nonviable cells, suggested that HSB2 cells were defective in their DNA-repair mechanisms necessary to overcome the lesions produced by X-ray. Considering the finding that the radiation survival character of HSB2-M cells changes to the normal level of control cells (KS 64), there is a strong possibility that HSB2-M cells may be more repair-proficient and that the additional chromosomes (+iso6q and +22) may play an important role in ameliolating the low repair ability due to gene-deficiency in HSB2 cells. The changes of radiation sensitivity might have been seen in *in vivo* malignant cells in which radiation therapy had been carried out. Thus, these data may shed light on solving the mechanism of emergence of radioresistant cells during the course of clinical treatment.

REFERENCES

BROMER, R. H. MITCHELL, L. B. and SOARES, N. (1982) Response of human hematopoietic precursor cells (CFUc) to hyperthermia and radiation. *Cancer Res.* **42**, 1261–1265.

CASPERSSON, T., JOHANSSON, C. and MODEST, E. J. (1970) Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma (Berl.)* **30**, 215–227.

CHEN, C. P., LAVIN, F. M. and KIDSON, C. (1978) Identification of ataxia telangiectasia heterozygotes, a cancer prone population. *Nature* **274**, 484–486.

CHIU, S., OLEINICK, N. L., FRIEDMAN, L. R. and STAMBROOK, P. J. (1982) Hypersensitivity of DNA in transcriptionally active chromatin to ionizing radiation. *Biochem. Biophys. Acta.* **699**, 15–21.

CLEAVER, J. E., CHAR, D., CHARLES, W. C. and RAND, N. (1982) Repair and replication of DNA in hereditary (bilateral) retinoblastoma cells after X-irradiation. *Cancer Res.* **42**, 1343–1347.

GOODWIN, G. and BAYLIN, B. S. (1982) Relationships between neuroendocrine differentiation and sensitivity to X-radiation in culture line OH-1 of human small cell lung carcinoma. *Cancer Res.* **42**, 1361–1367.

KNOX, J. S., WILSON, D. F., GREENBERG, B. R., SHIFRINE, M., ROSENBLATT, L. S., REEVES. J. D. and MISRA, H. (1981) Increased radiosensitivity of subpopulation of T-lymphocyte progenitors from patients with Fanconi's anemia. *Blood* **57**, 1043–1048.

KOHN, H. P., KRAEMER, H. K. and BUCHANAN, K. J. (1982) Influence of ataxia telangiectasia gene dosage on bleomycin-induced chromosome breakage and inhibition of replication in human lymphoblastoid cell lines. *Exp. Cell Res.* **137**, 387–395.

LEITH, T. J., DEXTER, L. D., DEWYNGART, K. J., ZEMAN, M. E. CHU, Y. M., CALABREST, P. and GLICHSMAN, S. A. (1982) Differential responses to X-irradiation of subpopulations of two heterogenous human carcinomas *in vitro*. *Cancer Res.* **42**, 2556–2561.

PAINTER, R. B. and YOUNG, B. R. (1980) Radiosensitivity in ataxia telangiectasia: A new explanation. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7315–7317.

RAHMSDORF, J. H., PONTA, H., BACHILE, M., MALLICH, U., WEISSEZAHN, F. K. and HERRLICH,

P. (1981) Differential cells from Balb/c mice differ in their radiosensitivity. *Exp. Cell Res.* **136**, 111–117.

SHIRAISHI, Y. and YOSHIDA, T. H. (1973) Comparative banding pattern analysis in human chromosomes induced by urea and trypsin treatments. *Jpn. J. Genet.* **48**, 11–17.

SHIRAISHI, Y., MINOWADA, J. and SANDBERG, A. A. (1976) Differential sensitivity to X-ray of chromosomes of blood T-lymphocytes and B- and T-cell lines. *In Vitro* **12**, 495–509.

SHIRAISHI, Y., HOLDWORTH, N. R., MINOWADA, J. and SANDBERG, A. A. (1978) Specificity of chromosomal changes induced with X-rays in a human T-cell line. *Radiat. Res.* **73**, 452–463.

SUMNER, A. T. (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **75**, 304–306.

SZUMIEL, I. (1980) Intrinsic radiosensitivity of proliferating mammalian cells. *Adv. Radiat. Biol.* **9**, 281–323.