II. BIOLOGICAL EFFECTS

X-Ray Sensitivity of Fibroblast Cell Strains Derived from Atomic Bomb Survivors with and without Breast Cancer

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Fibroblasts were established in vitro from skin biopsies obtained from 55 women and 1 man with or without breast cancer and with or without exposure to radiation from the atomic bomb (A-bomb) explosion in Hiroshima. The radiosensitivity of these cells was evaluated by clonogenic assays after exposure to X rays. Dose-response curves were fitted to a multitarget model, \( S/S_0 = A[1-(1-e^{-D/D_0})^N] \). There was no difference in the means or variances of radiosensitivity between exposed and nonexposed groups, or between groups with and without breast cancer.

INTRODUCTION

Epidemiological studies have shown a significant, dose-dependent increase in breast cancer incidence among A-bomb survivors\(^1\). The radiation sensitivity of cells obtained from "normal individuals" shows large individual differences\(^2\text{--}^4\). Hence, it is possible that inborn variations in susceptibility to the lethal effects of radiation could affect the shapes of the ionizing radiation dose-response curves.

To test the hypothesis that skin fibroblasts from A-bomb survivors with radiation-induced cancer are sensitive to the cytotoxic effects of X rays, we have measured the in vitro radiation sensitivity of fibroblast cell strains derived from skin biopsies from individuals with or without breast cancer as well as with or without exposure to A-bomb radiation\(^5\). Breast cancer was chosen, because the low spontaneous incidence of this disease in the past among Japanese women makes it very likely that any given case among the more heavily exposed survivors was in fact radiogenic. As it turned out, we were not as successful as had been hoped in obtaining biopsies from women in the high exposure-breast cancer category. Nevertheless the results reported below
provide at least some tests of the hypothesis.

MATERIALS AND METHODS

Skin fibroblast cells

Fifty six strains were established in vitro (Table 1). The skin tissue of breast cancer patients were obtained from the chest at surgery. Other skin tissues, except SFc1-2, were obtained from the neck, chest or abdomen. From the donor of SFc1-2, normal skin tissue was obtained from the proximity of epidermal inclusion cysts of the right upper arm.

Culture medium

Alpha MEM (GIBCO, NY) with 15% FCS medium was used for the maintenance and subculture for most of the experiments. In some X-ray sensitivity assays, Dulbecco's modified Eagle MEM (GIBCO, NY) with 15% FCS medium was used. 0.25% trypsin with 0.01% EDTA solution was used to harvest the cells.

Chromosome analysis

Cells at 5–8 subcultures after the establishment in vitro were treated colchicine (0.2 μg/ml)

Table 1. Age, DS86 kerma and DS86 organ (breast) dose\(^6\), presence or absence of breast cancer and other cancers of skin tissue donors. Human skin fibroblast (SF) cells were classified into 4 groups (a to d) with or without breast cancer and with or without exposure to A-bomb radiations.

SFb10 was exposed in utero at 3 km or more from hypocenter. Dose for SFb10 was estimated as zero.

| Cell Strain | Donor Age | DS86 (Gy) | Breast Cancer | Other Cancer(s) |
|-------------|-----------|-----------|---------------|----------------|
| SF a-1      | 41        | 0         | 0             | 0              |
| SF c1-1     | 43        | 3         | 1.468         | 1.260          |
| SF c2-4     | 52        | 13        | Unknown       |                |
| SF d1-1     | 52        | 13        | 2.396         | 2.862          |

| Cell Strain | Donor Age | ATB | DS86 (Gy) | Breast Cancer | Other Cancer(s) |
|-------------|-----------|-----|-----------|---------------|----------------|
| SF b-1      | 25        | 0   | 0         | 0             |                |
| SF d2-11    | 48        | 10  | Unknown   |               |                |

1: At Time of Bomb, 2: male, 3: exposed in utero.
for 1.5 hr and then were hypotonically treated in a 0.075 M KCl (2 volumes) and 1% Na citrate buffer solution (1 volume) for 20 min. Cells spread on slides were treated with a 0.1% trypsin for 10–20 seconds at 37°C followed by staining with 2% Giemsa. Metaphases were analyzed on photographs.

**Irradiations**

Two types of X-ray generators were used due to a change in the irradiation facility. One generator was operated at 250 kVp, 30 mA, 0.5 mm Cu plus 1.0 mm Al external filtration and a dose rate of 0.9 Gy/min. The other generator was operated at 220 kVp, 8 mA, 0.3 mm Cu plus 0.5 mm Al and a dose rate of 1.0 Gy/min. The dose rates in air were measured with Victoreen condenser chambers.

**Clonogenic assay for cell survival**

Actively growing cells were harvested and irradiated in growth medium. Immediately after irradiation, appropriate numbers of cells were seeded into 10-cm diameter plastic dishes (6 dishes at each dose point) and cultured for 10–14 days in 95% air + 5% CO₂ at 37°C. The colonies were fixed with ethanol and stained with Giemsa.

**Curve fitting and data analysis**

The dose responses were analyzed using a multitarget model,

\[ \frac{S}{S_0} = A[1-(1-e^{-D/D_0})^N] \]

D is the dose in Gray (Gy) and \( \frac{S}{S_0} \) is the surviving fraction at dose D. \( D_0 \) is the dose that causes the straight-line portion of the survival curve to decrease to 37%. The parameter A is introduced to avoid forcing the survival curve through 1.00 at \( D = 0 \). N and A are the points where the extrapolated straight-line portion of the curve intersects the survival axis. Dose-response curves were computer-generated using the BMDP6D program (BMDP Statistical Software, Inc., California).

**RESULTS**

It is usual that chromosomal karyotype is analyzed on the primary human fibroblasts established in vitro. Chromosome analysis data are given in Table 2.

Only SFcl-2 strain carried a congenitally abnormal karyotype with a 45, XO constitution in 36 of the 38 metaphases analyzed. As was expected many abnormalities were observed in the cells which were obtained from donors exposed to 0.5 Gy or more of A-bomb radiations. Frequent exposures to medical radiations might also account for the increased level of aberration frequency. The medical doses have been estimated for more than 17,000 individuals out of the Life Span Study samples of RERF7–9). Estimate was available for only 10 women in this paper (Table 3). Donors of SFcl-1, SFcl-3 and SFd1-7 had received appreciably more than 0.3 Gy to their skin surfaces. However, no data have been reported for the cytogenetic effects of 0.3 Gy delivered fractionally over about 30 years. The individuals of SFa and SFb groups were also expected to
Table 2. Type and frequency of chromosome aberrations found in skin fibroblast cells at 5-8 subcultures after the establishment in vitro.

ace: acentric fragment, dic: dicentrics, t: reciprocal translocation, inv: inversion, ins: insertion, del: deletion.

Table 3. Cumulative medical doses estimated from the indicated years to the date of skin biopsy.
be exposed to almost the same doses of medical radiations as were groups SFc and SFd. Hence, the high frequency of chromosome aberrations in the SFd group could not be explained simply by the contribution of medical exposures. Although the number of cells scored for each strain is small, chromosome aberration data suggest that the donors of SFd2-11, 12, 13, 16, 17 might have been exposed to large doses of A-bomb radiations.

X-ray experiments on each SF strain were repeated 2–7 times. Figure 1 shows the dose-survival curves among the most extreme in response: SFa4 and SFb8. Curves were fitted to the multitarget model using the best fit values of $D_{10}$, $N$ and $A$. Figure 2 shows the correlation

![Dose-survival curves of two SF strains after the exposures to X rays](image)

**Fig. 1.** Dose-survival curves of two SF strains after the exposures to X rays. Mean survival fraction of several assays was fitted to the multitarget model, $S/S_0 = A[1-(1-e^{-D/D_0})^N]$. Open symbols: SFa4; Closed symbols: SFb8. The different types of symbols indicate experiments carried out independently. The mean survival parameters and standard errors are $D_{10} = 2.18 \pm 0.08$ Gy, $D_0 = 0.88 \pm 0.01$ Gy, $N = 1.2 \pm 0.2$ for SFa4 cell strain, and $D_{10} = 3.40 \pm 0.15$ Gy, $D_0 = 1.22 \pm 0.01$ Gy, $N = 1.7 \pm 0.2$ for SFb8 cell strain.
between Do and D10, the dose necessary to reduce survival to 10%. The relative variation of D10 was about the same as that of D0.

The correlation coefficient and p-value were 0.62 and < 0.001. The D0 value depends upon the slope of dose-survival curve. Since each cell strain has its own N value, it is expected that cell strains with the same D0 value could show the different sensitivity. In this report, the radiosensitivity of each strain was considered with D10 value.

Fig. 2. Correlation between Do and D10.

○ SFa group, ● SFb group, △ SFc group, ▲ SFd group

Fig. 3. Correlations between plating efficiency and donor age (A), between D10 of X rays and donor age (B), and between plating efficiency and D10 of X rays (C). Coefficient values and p values were given in Figures. Symbols are same as Figure 2.
The colony forming ability at zero dose (plating efficiency) ranged from 0.02–0.56 in individuals experiments. Figure 3A shows that there was no strong correlation between average plating efficiency and donor age and Figures 3B and 3C show little effect on average $D_{10}$ of the donor age and the average plating efficiency of a strain.

Figure 4 compares the $D_{10}$ values of 6 groups. Since Tokunaga et al. showed that the cumulative breast cancer risk for women exposed to high doses (0.5 + Gy T65D kerma) was significantly greater than that for women with low doses (0–0.09 Gy), SFd group was classified into three categories; I: breast cancer patients exposed to 0.5 Gy or less, II: patients exposed to more than 0.5 Gy, III: patients exposed to unknown doses. The X-ray sensitivities

![Fig. 4](image)

**Fig. 4.** The distribution of X-ray $D_{10}$ values among the cell strains. Donors of skin tissue were classified as below.

- a: 15 nonexposed individuals without breast cancer (SFa).
- b: 15 nonexposed individuals with breast cancer (SFb).
- c: 9 exposed individuals without breast cancer (SFc).
- d-I: 5 breast cancer patients exposed to 0.5 Gy or less (DS86 kerma).
- d-II: 5 breast cancer patients exposed to more than 0.5 Gy (DS86 kerma).
- d-III: 7 breast cancer patients exposed to unknown dose (DS86 kerma).
of 30 individuals of the nonexposed group (Figure 4a and b) and 9 survivors without breast cancer (Figure 4c) were widely distributed. The D10 values of SF strains ranged from 2.18–3.21 (mean ± S.E.M. = 2.80 ± 0.07) Gy for SFa group, 2.44–3.40 (2.85 ± 0.07) Gy for SFb group and 2.44–3.18 (2.87 ± 0.05) Gy for SFc group. There are a few individuals with high sensitivities in the breast cancer patients receiving a high dose or unknown dose (Figure 4d-II and III). D10 values ranged from 2.21–3.59 (mean ± S.E.M. = 3.01 ± 0.21) Gy in 5 patients exposed to low doses, 2.55–3.11 (2.92 ± 0.09) Gy in 5 patients exposed to high doses and 2.62–3.02 (2.91 ± 0.05) Gy in 7 patients exposed to unknown doses. A weighted least squares analysis* comparing the breast cancer and non-breast cancer groups resulted in an estimated difference in mean D10 of 9.96 ± 7.71 cGy, which is not significantly different from zero.

DISCUSSION AND FUTURE PERSPECTIVE

Chromosomal analysis showed that only one strain, SFc1-2, carried a congenitally abnormal karyotype. SFc1-2 cells did not show an unusual responses to X rays; 2.84 ± 0.06 (S.E.M.) for D10 and 1.19±0.04 (S.E.M.) Gy for D10. Highly frequent chromosome aberrations, mainly reciprocal translocations, were found in cells obtained from A-bomb survivors with breast cancer (Table 2). Most of these residual aberrations presumably were induced by the exposure to A-bomb radiations.

Repeated X-ray survival assays suggested that radiation sensitivity of skin fibroblasts was a genetically stable characteristic (Figure 1). Data on the survival of fibroblasts following X-ray exposure showed large individual differences in D10 values (Figure 2 and 4). The differences in D10 values were relatively independent of the colony forming ability and the donor age (values of r² < 0.2) (Figure 3). For above reasons, the D10 value could be considered as one measure of individual’s radiosensitivity. Repeated X-ray survival assays were conducted using cell strains established from two biopsies which were obtained at different days from each of two patients (SFb2 and SFd1-1)5). There was little difference between the average values of survival parameters (Do, D10 and N) for two strains of each patient, suggesting that the within-individual variation in survival parameters was generally minimal5).

Our results showed no direct correlation between the risk of breast cancer induction after exposure to A-bomb radiation and the D10 values. Our results may be supported by the current data about radiation-induced cell killing in fibroblasts from ataxia-telangiectasia (AT) patients. AT cells are hypersensitive to cell killing by ionizing radiations, but are less mutable by gamma rays than normal cells10,11). These findings suggest that the clonogenic assay of fibroblast cells to ionizing radiations might not be useful for the evaluation of cancer proneness, although a broad variation of radiosensitivity does exist in the 'normal' human population5). According to the new dosimetry system (DS86)6), the estimated neutron dose in Hiroshima has been determined to be about one-tenth of that estimated by T65D12). However, it is well accepted that the lethal effects of neutron exposure are greater than those for X rays. Similar results showing diverse

*Note: weights for each mean D10 were taken as the inverse of (A + B/N), where N is the number of repeat experiments, B the experimental error variance estimated from the repeat experiments, and A the between-individual variance estimates by choosing the value of A which makes the residual mean square error equal to 1.
radiosensitivity were obtained from the dose-survival responses following exposure to fission neutrons generated from $^{252}$Cf. No deviation in sensitivity to fission neutrons was observed between the nonexposed and exposed groups or between individuals with or without breast cancer.

Our results can not deny the possibility that some individuals within the 'normal' population are very radiosensitive and also very susceptible to radiogenic cancer. Different markers for acute radiosensitivity in a large human population may be investigated with the cooperation of other laboratories in future. The registry of those data may provide a consistent association between radiation sensitivity and cancer proneness.

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