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業　績　報　告　書

RERF TR 6-90
研究課題　2-84

乳癌に罹患しているか，又はしていない原爆被爆生存者から得られた皮膚線維芽細胞の放射線感受性§

Radiosensitivity of Skin Fibroblasts from Atomic Bomb Survivors with and without Breast Cancer

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要　約

広島で原爆放射線に被曝したか，もしくはしないか，および乳癌に罹患したか罹患していないかの55人の女性と1人の男性から得られた皮膚生検材料から，培養系で増殖する線維芽細胞を樹立した．これらの細胞にX線，又は^{252}Cf線源からの核分裂中性子を照射し，コロニー形成法によって放射線感受性を評価した．X線線量及び中性子線量に対する生存率曲線は，多標的モデル $S/S_0 = A[1-(1-e^{kD})^N]$ を用いて解析した．中性子線量-生存率関係は単ヒットモデル $S/S_0 = Ae^{kD}$ にも適合した．原爆被爆の有無，あるいは乳癌の有無にかかわらず，放射線感受性の平均値又は分散に差は認められなかった．したがって，サンプルは多くはないが，放射線による試験管内致死効果の高い細胞をもつ女性は，原爆放射線によって乳癌を誘発されやすいという仮説は支持しない．

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Radiosensitivity of Skin Fibroblasts from Atomic Bomb Survivors with and without Breast Cancer[§]

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Summary

Fibroblasts were established in vitro from skin biopsies obtained from 55 women and one man with or without breast cancer and with or without exposure to radiation from the atomic bomb explosion in Hiroshima. The radiosensitivity of these cells was evaluated by clonogenic assays after exposure to X rays or to fission neutrons from a ^{252}Cf source. Data were fitted to a multitarget model, $S/S_0 = A[1 - (1 - e^{kD})^N]$, for both X-ray and neutron dose-survival curves. A single-hit model, $S/S_0 = Ae^{kD}$, fits the neutron dose-survival responses as well. There was no difference in the means or variances of radiosensitivity between exposed and nonexposed groups, or between patients with or without breast cancer. Hence, although the sample is not large, it provides no support for the hypothesis that A-bomb radiation preferentially induces breast cancer in women whose cells in vitro are sensitive to cell killing by radiation.

[§]**Full Japanese text will be available separately.**

A paper based on this report has been accepted for publication by Cancer Research.

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Introduction

Epidemiological studies have shown a significant, dose-dependent increase in breast cancer incidence among survivors exposed to A-bomb radiation.[1] Other studies have also shown that breast tissue is especially sensitive to the carcinogenic effect of exposure to ionizing radiation.[2]

An increased risk of cancer due to radiation exposure may be associated with abnormal in vitro sensitivity of cells to the lethal effect of ionizing radiation. In vitro radiosensitivity has been studied using numerous skin fibroblast strains obtained from normal individuals as well as from patients with cancer-prone hereditary diseases. These studies have demonstrated that the cells of patients with hereditary diseases, such as ataxia telangiectasia (AT)[3] and xeroderma pigmentosum (XP),[4] show unusual sensitivity to X rays or ultraviolet (UV) radiation, and that there is a wide variation in radiosensitivity within normal human populations.[5–8] Weichselbaum et al[9,10] reported that hereditary and D-deletion retinoblastoma cells are significantly sensitive to X rays, which led to the hypothesis that radiosensitivity is linked to neoplastic susceptibility in retinoblastoma cells.[11–13] Based on Weichselbaum et al's data, it could be hypothesized that A-bomb survivors with breast cancer tend to be those whose cells are most susceptible to damage by radiation (the "high susceptibility" hypothesis).

Stewart and Kneale[14,15] have hypothesized that A-bomb survivors may not include radiosensitive persons because they died early of acute infections (the "selection" hypothesis). For this reason, cancer risk among A-bomb survivors may be an underestimate for the general population. If this hypothesis were correct, the A-bomb survivor population would include mainly radioresistant persons who would be less prone to develop radiogenic cancer and therefore may not be representative of the radiosensitivity of the general population.[16]

To test these two hypotheses, 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. 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 would in fact be 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 test of the hypotheses.

Materials and Methods

Skin tissue

Table 1 lists the donors by age at the time of skin biopsy, by A-bomb radiation dose (DS86 kerma and DS86 organ [breast] dose[17,18]), and by the presence or absence of breast (or other) cancer. From 1984 through mid-1987, 56 cell strains were established in vitro: 55 from female donors, and 1 strain from an unexposed male with stomach cancer.

Table 1. Age, dose, presence or absence of breast cancer, other cancer tissues from skin tissue donors, and coefficients of dose-survival responses calculated using equation (2), $S/S_0 = A[1 - (1 - e^{kD})^N]$

Cell strain	Donor age	Age ATB*	DS86 (Gy)		Epilation	Breast cancer	Other cancer(s)	X rays							^{252}Cf		
			Kerma	Breast				No. of repetitions	$D_{10} \pm SE$ (Gy)		$D_0 \pm SE$ (Gy)		$N \pm SE$		D_{10} (Gy)	D_0 (Gy)	N
SF a 1	41		0	0		–		3	2.72	0.13	1.13	0.11	1.3	0.3	1.83	0.81	1.0
2	40		0	0		–		3	2.90	0.08	1.06	0.06	1.7	0.4	1.41	0.56	1.2
3	54		0	0		–		7	2.27	0.14	1.03	0.10	1.0	0.1	1.24	0.51	1.2
4	14		0	0		–		6	2.18	0.07	0.88	0.01	1.2	0.1	1.19	0.52	1.0
5	33		0	0		–		2	2.75	0.16	1.20	0.04	1.0	0.2	1.21	0.54	0.9
6	42		0	0		–		4	2.97	0.13	1.13	0.06	1.4	0.1	1.78	0.86	0.8
7	27		0	0		–		2	2.85	0.05	1.16	0.01	1.2	0.1			
8	54		0	0		–	Chest	3	3.19	0.29	1.20	0.07	1.5	0.2	1.49	0.64	1.0
9	55		0	0		–		5	2.82	0.19	1.04	0.10	1.6	0.2	1.49	0.57	1.4
10	60		0	0		–	Uterus	2	2.50	0.14	0.83	0.00	2.1	0.3	1.20	0.56	0.9
11	51		0	0		–		4	3.21	0.16	1.04	0.06	2.2	0.1	1.93	0.82	1.0
12	73		0	0		–		2	2.86	0.21	1.19	0.04	1.2	0.3	1.77	0.76	1.0
13	58		0	0		–	Thyroid, colon	4	2.86	0.17	0.97	0.06	2.0	0.2	1.94	0.88	0.9
14**	63		0	0		–	Stomach	2	3.00	0.01	1.21	0.02	1.2	0.0	1.65	0.60	1.6
15	38		0	0		–		2	2.85	0.14	1.00	0.01	1.8	0.3	1.60	0.76	0.8
								Average	2.80	0.07	1.07	0.03	1.5	0.1	1.55	0.67	1.1

(Continued)

Human skin fibroblast (SF) cells were derived from nonexposed individuals without breast cancer (SFa), nonexposed individuals with breast cancer (SFb), exposed individuals without breast cancer (SFc), and exposed individuals with breast cancer (SFd).

SF10 was exposed in utero at 3 km or more from the hypocenter. Kerma cannot be calculated for the SFc2 and SFd2 groups. Dose is unknown for SFc2-7, SFc2-8, SFd2-11, SFd2-12, and SFd2-17, although these donors are registered in RERF's master file.

RERF's records show that the donor of SFd2-12 was exposed at 467 m from the hypocenter, although the shielding circumstances are unclear.

Epilation: The plus sign (+) means that epilation on more than a quarter of the head occurred within two months of the explosion. Use of the minus sign (–) means no epilation occurred.

*At the time of the bombing **Male †Exposed in utero

Table 1 (Continued)

Cell strain	Donor age	Age ATB*	DS86 (Gy)		Epilation	Breast cancer	Other cancer(s)	X rays						^{252}Cf		
			Kerma	Breast				No. of repetitions	$D_{10} \pm SE$ (Gy)		$D_0 \pm SE$ (Gy)		$N \pm SE$		D_{10} (Gy)	D_0 (Gy)	N
SF b 1	25		0	0		+		2	2.75	0.09	1.09	0.03	1.3	0.2	1.93	0.82	1.0
2	55		0	0		+		4	2.97	0.04	0.99	0.03	2.1	0.2	1.82	0.80	0.9
3	53		0	0		+		5	2.46	0.24	0.90	0.04	2.2	0.1	1.45	0.63	1.0
4	50		0	0		+		2	2.84	0.00	1.17	0.03	1.2	0.1			
5	55		0	0		+		4	2.44	0.25	1.02	0.08	1.1	0.1	1.84	0.75	1.2
6	38		0	0		+		4	2.82	0.12	1.11	0.05	1.3	0.2	1.71	0.60	1.8
7	40		0	0		+		2	2.92	0.06	1.20	0.01	1.1	0.1	1.57	0.66	1.1
8	53		0	0		+		4	3.40	0.15	1.22	0.01	1.7	0.2	1.63	0.75	0.9
9	70		0	0		+		3	3.02	0.06	1.13	0.03	1.5	0.2	1.62	0.56	1.8
10†	40		0	0		+		4	3.00	0.19	1.23	0.06	1.2	0.1	1.29	0.65	0.7
11	57		0	0		+		3	2.69	0.22	0.81	0.04	3.1	0.8	1.78	0.93	0.7
12	55		0	0		+		2	2.64	0.08	0.77	0.09	3.9	1.9	1.50	0.59	1.3
13	70		0	0		+		4	3.17	0.07	1.03	0.07	2.5	0.6	1.22	0.49	1.2
14	41		0	0		+		2	2.63	0.18	1.19	0.13	0.9	0.1	1.22	0.47	1.3
15	59		0	0		+		5	2.95	0.08	1.16	0.03	1.3	0.1	1.68	0.76	0.9
								Average	2.85	0.07	1.07	0.04	1.8	0.2	1.59	0.68	1.1

(Continued)

Table 1 (Continued)

Cell strain	Donor age	Age ATB*	DS86 (Gy)		Epilation	Breast cancer	Other cancer(s)	X rays							^{252}Cf		
			Kerma	Breast				No. of repetitions	$D_{10} \pm SE$ (Gy)		$D_0 \pm SE$ (Gy)		$N \pm SE$		D_{10} (Gy)	D_0 (Gy)	N
SF c1–1	43	3	1.488	1.260	+	–		5	2.44	0.17	0.92	0.08	1.8	0.5	1.74	0.76	1.0
2	50	12	0.958	0.824	–	–		2	2.84	0.06	1.19	0.04	1.1	0.0	1.23	0.49	1.2
3	57	18	0.320	0.290	–	–		2	3.18	0.04	1.21	0.02	1.4	0.1			
4	56	18	0.023	0.019	–	–		3	3.11	0.27	1.26	0.07	1.2	0.1	1.30	0.56	1.0
5	46	6	0.099	0.094	–	–		2	3.10	0.21	1.28	0.05	1.1	0.1	1.80	0.81	0.9
								Average	2.93	0.14	1.17	0.07	1.3	0.1	1.52	0.66	1.0
SF c2–6	52	13	unknown			–		2	2.99	0.04	1.27	0.04	1.1	0.0	1.20	0.47	1.3
7	70	30	unknown			–		7	2.79	0.13	0.93	0.04	2.1	0.2	1.52	0.70	0.9
8	52	11	unknown			–	Thyroid	2	2.77	0.21	0.98	0.01	1.8	0.4	1.50	0.62	1.1
9	62	21	unknown			–		2	2.91	0.03	1.12	0.01	1.4	0.0	1.59	0.72	0.9
								Average	2.87	0.05	1.08	0.08	1.6	0.2	1.45	0.63	1.1
							Average of SF c		2.90	0.08	1.13	0.05	1.4	0.1	1.49	0.64	1.0

(Continued)

Table 1 (Continued)

Cell strain	Donor age	Age ATB*	DS86 (Gy) Kerma	DS86 (Gy) Breast	Epilation	Breast cancer	Other cancer(s)	X rays No. of repetitions	X rays $D_{10} \pm SE$ (Gy)		X rays $D_0 \pm SE$ (Gy)		X rays $N \pm SE$		^{252}Cf D_{10} (Gy)	^{252}Cf D_0 (Gy)	^{252}Cf N
SF d1–1	52	13	3.286	2.882	+	+		5	3.05	0.11	1.15	0.06	1.5	0.1	1.46	0.63	1.0
2†	38		0.054	0.038		+		2	2.21	0.11	0.81	0.13	1.8	0.5	1.90	0.93	0.8
3	76	36	0.234	0.220	–	+		2	2.90	0.01	1.23	0.01	1.1	0.0			
4	63	25	0.057	0.054	–	+		6	3.59	0.14	1.23	0.05	2.2	0.4	1.64	0.76	0.9
5	60	19	0.067	0.057	–	+		4	3.10	0.17	0.97	0.08	2.6	0.4	1.37	0.62	0.9
6	47	6	0.625	0.595	–	+		4	3.04	0.14	1.02	0.05	2.2	0.4	1.56	0.79	0.7
7	57	16	0.852	0.596	+	+		4	2.55	0.22	0.92	0.05	1.9	0.4	1.80	0.91	0.7
8	58	17	2.047	1.547	+	+		7	3.11	0.08	1.05	0.05	2.3	0.4	1.84	0.89	0.8
9	45	4	0.508	0.482	–	+		2	2.83	0.14	1.10	0.06	1.3	0.0	1.48	0.72	0.8
10	89	47	0.180	0.183	–	+		2	3.27	0.02	1.25	0.06	1.4	0.2			
								Average	2.97	0.12	1.07	0.05	1.8	0.2	1.63	0.78	0.8
SF d2–11	48	10	unknown		+	+		2	2.85	0.04	1.08	0.12	1.6	0.5	1.78	0.83	0.9
12	54	15	unknown		+	+		3	2.99	0.22	0.99	0.08	2.1	0.2	1.97	0.87	1.0
13	72	33	unknown			+		3	3.02	0.11	1.18	0.09	1.3	0.2	1.35	0.60	0.9
14	69	29	unknown			+		4	2.93	0.42	1.04	0.07	1.8	0.5	1.54	0.73	0.8
15	60	19	unknown			+		5	2.94	0.19	1.15	0.06	1.3	0.1	1.59	0.79	0.7
16	55	12	unknown			+		2	2.62	0.08	0.84	0.01	2.4	0.3	1.47	0.67	0.9
17	53	11	unknown			+		3	3.00	0.07	1.31	0.02	1.0	0.1	1.51	0.67	0.9
								Average	2.91	0.05	1.08	0.06	1.6	0.2	1.60	0.74	0.8
							Average of SF d		2.94	0.07	1.08	0.03	1.8	0.1	1.62	0.76	0.9
							Average of groups		2.87	0.03	1.09	0.01	1.6	0.1	1.56	0.69	1.0

The skin tissue of breast cancer patients was obtained from the chest at the time of surgery. Other skin tissue, except sample SFc1-2, was obtained from the neck, chest or abdomen. From the donor of SFc1-2, normal skin tissue in the proximity of epidermal inclusion cysts of the right upper arm was obtained.

Establishment of primary culture cells

Skin tissues were washed thoroughly in phosphate-buffered saline supplemented with antibiotics (penicillin and streptomycin) after confirming the absence of keloids and moles. Tissues were minced into 1 mm^3 or smaller pieces and put into alpha MEM culture solution (GIBCO, Grand Island, NY) containing approximately 30% fetal calf serum (FCS). After 3–5 days, the culture solution was replaced with alpha MEM supplemented with 15% FCS. After 10–14 days, cells proliferating and expanding from the tissue pieces were harvested and collected for subsequent subculture. Primary cell strains are denoted by the prefix SF (skin fibroblasts).

AT skin fibroblasts

AT cell strains with the prefix GM were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). AT3BI and AT5BI used in the survival assay for ^{252}Cf radiation were obtained from Dr. O. Nikaido of Kanazawa University and were cryopreserved in liquid N_2.[19]

Culture medium

Alpha MEM with 15% FCS medium was used for maintenance and subculture for most of the experiments. In some X-ray cytotoxicity assays, Dulbecco's modified Eagle MEM (GIBCO, Grand Island, NY) culture solution supplemented with 15% FCS was used. Alpha MEM with 5% dimethylsulfoxide (DMSO) plus 20% FCS was used in the cryopreservation of cells. To harvest the cells, 0.25% trypsin with 0.01% EDTA solution was used.

X-ray irradiation

Actively growing cells were harvested and suspended in growth medium, after which 1×10^5 cells (1 mL) (estimated by using a Coulter counter) were put into a plastic test tube (Corning Co., Corning, NY) and irradiated at room temperature. Two types of X-ray generators were used due to a change in the irradiation facility. Both generators gave similar results. One generator was operated at 250 kVp, 30 mA, 0.5 mm Cu plus 1.0 mm Al external filtration, and at 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 at a dose rate of 1.0 Gy/min. The dose rates in air were measured with a Victoreen condenser chamber at room temperature. AT cells received doses of 0, 0.25, 0.5, 1.0, 2.0, and 3.0 Gy, and SF cells received doses of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 Gy. Each X-ray experiment on SF cells was repeated 2–7 times (Table 1).

Neutron irradiation

Actively growing cells (3×10^5 or 1 mL) were put into a test tube (Pyrex glass, inner diameter 15 mm, length 125 mm) and irradiated at the Research Institute for Nuclear Medicine and Biology of Hiroshima University. Each tube was set up in one of eight aluminum tubes (inner diameter 18 mm, length 120 mm, 1 mm thick) located 8 cm from the center of a continuously rotating table. Neutron irradiations were carried out at room temperature using a stainless steel encapsulated ^{252}Cf source (length 17 mm, diameter 9.5 mm, Type X-35, Amersham International p.l.c., Buckinghamshire, UK) located at the central axis of the radiation device.[20] The dose rate, as determined using a Three-Terminal Ionization Chamber (Types IC-17 and IC-17G, Far-West Technology, Inc., Goleta, Calif) and a Fricke dosimeter, was calculated at 1.18–1.34 cGy/min. The neutron to gamma-ray dose ratio was 2.0. Following irradiation at 0, 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 Gy, cells attached to the glass tube wall were harvested with a trypsin-EDTA solution.

Clonogenic assay for cell survival

Immediately after irradiation, appropriate known numbers of cells were seeded into 10-cm-diameter plastic culture dishes (six dishes at each dose point) (Corning Co., Corning, NY) and cultured for 10–14 days in 95% air plus 5% CO_2 at 37°C. During this period, the culture solution was changed once or twice. The colonies were fixed with ethanol and stained with Giemsa. Colonies composed of 50 or more cells were scored, and plating efficiencies and percentage survivals were calculated.

Curve fitting and data analysis

The dose responses were analyzed using a single-hit model,

$$S/S_0 = Ae^{kD} \quad , \tag{1}$$

and a multitarget model,

$$S/S_0 = A[1 - (1 - e^{kD})^N] \quad , \tag{2}$$

where D is the dose in gray and S/S_0 is the surviving fraction at dose D. D_0, the dose that causes the straight-line portion of the survival curve to decrease to 37%, is –1/k. N·A is the point where the extrapolated straight-line portion of the curve intersects the survival axis. The slope, k, and intercept, N, of each survival curve are estimated by least-squares regression analysis as the parameters of a nonlinear function, which avoids the assumption that all observed dose-response points are on the exponential portion of the survival curve. For equation (2), the logarithm of the surviving fraction is represented by the survival model,

$$\ln(S/S_0) = \ln(A) + \ln[1 - (1 - e^{kD})^N] \quad , \tag{2'}$$

where ln(A) is the intercept of the equation, ln(N) + ln(A) is the extrapolation intercept of the linear part of the equation, and k is the slope of the linear part of the equation. All of the data points, including the control, are used to estimate the parameters of the equation. The parameter A is introduced to avoid the restriction of forcing equation (2') through zero, ln(1), at zero dose. This recognizes the fact that survival at zero dose is measured and thus has an error, and it permits the estimation of k and N independently of this one dose point. A nonlinear least-squares program was used to estimate the parameters of the model. D_{10}, the dose necessary to reduce survival to 10%, was calculated from the appropriate equation using the best fit values of k, N, and A. Dose-response curves were computer-generated using the BMDP6D program (BMDP Statistical Software, Inc., Los Angeles, Calif).

Results

The average X-ray survival curve values for the SF cell strains are given in Table 1. Those for parallel experiments with AT "positive control" cell strains are given in Table 2. The average survival curves for the four groups of SF cell strains and for the AT cell strains are depicted graphically in Figure 1. For the AT strains, the curves were best fitted by equation (2), with D_0 (= –1/k) values in the range of 0.49–0.66 Gy. Equation (2) was also fitted to the data for the SF strains. D_0 values for the SF cells ranged from 0.77–1.31 Gy in these analysis. There was little effect of the donors' age on in vitro radiosensitivity (Table 1). The correlation coefficients and p-values between age and average D_{10} for X rays were, respectively: SFa, 0.34 and 0.22; SFb, 0.23 and 0.40; SFc, 0.22 and 0.56; and SFd, 0.50 and 0.04. Moreover, although colony-forming ability at zero dose (plating efficiency) ranged from 0.02–0.56 in individual experiments, radiosensitivity was not significantly associated with plating efficiency. The correlation coefficients and p-values between plating efficiency and the average D_{10} for X rays were, respectively: SFa, 0.29 and 0.29; SFb, –0.11 and 0.70; SFc, –0.09 and 0.82; and SFd, 0.16 and 0.53. A one-way analysis of variance was performed to test whether the mean D_{10} differed among the 56 individuals. The result was significant at $p < 0.001$, with $F_{55,132} = 2.98$.

Table 2. Survival curve parameters of ataxia telangiectasia cell strains

Cell strain	X rays			^{252}Cf		
	D_{10} (Gy)	D_0 (Gy)	N	D_{10} (Gy)	D_0 (Gy)	N
AT3B1	1.52	0.65	1.1	0.93	0.40	1.0
AT5B1	1.59	0.66	1.1	1.06	0.46	1.0
GM2052	1.07	0.50	0.9			
GM2530	1.49	0.62	1.1			
GM3395	1.20	0.49	1.2			
GM3487	1.59	0.57	1.7			
GM648	1.36	0.60	1.0			
Average	1.40	0.58	1.2	1.00	0.43	1.0

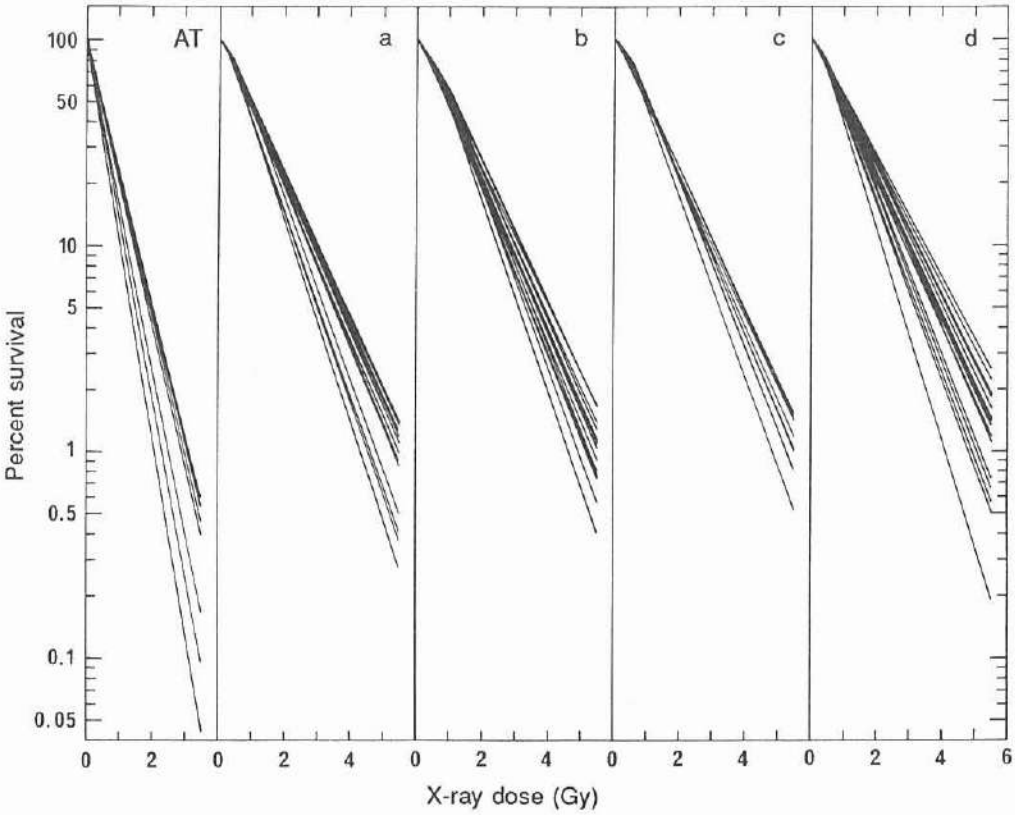

Figure 1. Survival curves following X irradiation of ataxia telangiectasia (AT) cells and skin fibroblast (SF) cells. AT cells received doses of 0, 0.25, 0.5, 1.0, 2.0, and 3.0 Gy, whereas SF cells received doses of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 Gy (dose rate: 0.9 or 1.0 Gy/min). Lane AT: cell strains AT3BI, AT5BI, GM648AT, GM2052AT, GM2530AT, GM3395AT, GM3487AT. Lane a: skin cells derived from 15 nonexposed individuals without breast cancer. Lane b: skin cells derived from 15 nonexposed individuals with breast cancer. Lane c: skin cells derived from 9 exposed individuals without breast cancer. Lane d: skin cells derived from 17 exposed individuals with breast cancer.

Repeated X-ray survival assays were conducted using cell strains established from new biopsies of two subjects (Table 3). There was little difference between the average values of three parameters for two strains of each patient, suggesting that radiation sensitivity of skin fibroblasts was a genetically stable characteristic.

According to the new dosimetry system (DS86), the estimated neutron dose in Hiroshima is about one-tenth of the T65D estimate. However, it is well accepted that the lethal effects of neutron exposure are greater than those for X rays. Figure 2 shows the dose-survival responses from two tests of four SF cell strains exposed to ^{252}Cf radiation. The dose responses were best fitted by equation (2). The N and D_0 values obtained are listed in Table 1 for SF cell strains and in

Table 3. Comparison of survival-curve parameters of cell strains established from the repeated biopsy samples of two women. Average: the mean ± SE

Strain	Date of biopsy	Experiment number	D_{10} (Gy)	D_0 (Gy)	N
SFb 2	23 January 1984	20	3.09	1.05	1.9
		596	2.96	0.90	2.8
		2114	2.93	1.01	1.9
		Average	2.99 ± 0.05	0.99 ± 0.04	2.2 ± 0.3
	2 February 1984	583	291	1.01	1.8
SFd 1-1	4 June 1984	7	3.40	1.25	1.5
		643	2.73	0.94	1.8
		667	3.07	1.27	1.1
		Average	3.07 ± 0.19	1.15 ± 0.11	1.5 ± 0.2
	10 July 1984	645	3.07	1.12	1.6
		646	2.99	1.15	1.4
		Average	3.03 ± 0.04	1.14 ± 0.02	1.5 ± 0.1

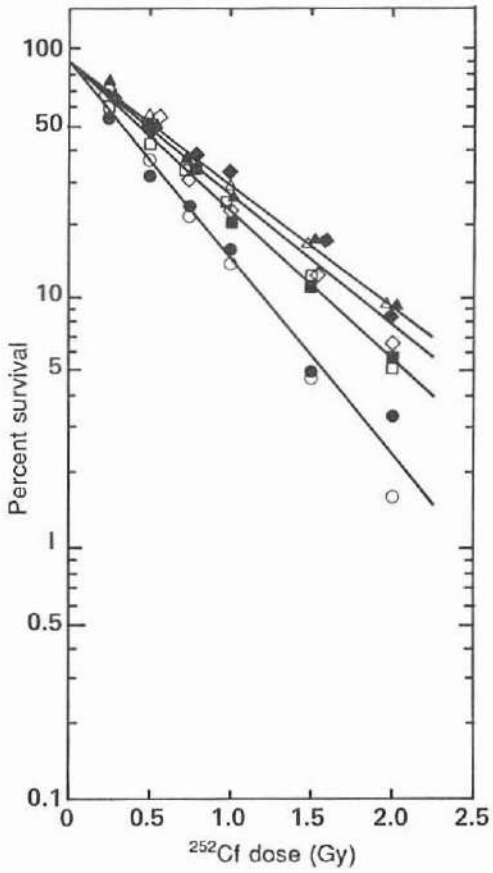

Figure 2. Survival curves for irradiation of four skin fibroblast strains with ^{252}Cf radiation. Different symbols represent data from two independent experiments for each strain. Cells received doses of 0, 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 Gy. Dose responses were fitted to equation (2), $S/S_0 = A[1 - (1 - e^{kD})^N]$. Dose rates: 1.178–1.337 cGy/min. Ratio of the neutron dose to gamma-ray dose: 2.0.

○ ●: SFa10, △ ▲: SFa13,
◇ ◆: SFb2, □ ■: SFc9.

Table 2 for two AT cell strains. D_0 values for SF cells ranged from 0.47–0.93 Gy. Because the N values of the dose-response curves of the cell strains differed, radiosensitivity was compared by means of the D_{10} values (Table 4).

Table 4. Comparison of D_{10} values and RBE values (± SE). Data were fitted to equation (2) for X-ray survival curves, and to equations (1) and (2) for survival responses after exposures to ^{252}Cf radiations. RBE values were calculated using D_{10} values obtained from equation (2)

Group	$S/S_0 = Ae^{kD}$	$S/S_0 = A[1 - (1 - e^{kD})^N]$		RBE
	^{252}Cf (cGy)	^{252}Cf (cGy)	X rays (cGy)	
SF a	155 ± 27	155 ± 26	280 ± 28	1.83 ± 0.23
SF b	158 ± 23	159 ± 22	285 ± 25	1.83 ± 0.34
SF c	148 ± 23	149 ± 21	290 ± 21	1.99 ± 0.35
SF d	163 ± 18	162 ± 19	294 ± 29	1.84 ± 0.30
Mean ± SE	156.0 ± 3.1	156.3 ± 2.8	287.3 ± 3.0	1.87 ± 0.04

The mean D_{10} value of 56 SF strains after X-ray irradiation was 287.3 ± 3.1 (SEM) cGy. The mean D_{10} value of 51 SF strains after exposure to ^{252}Cf radiation was similar for both models: 156.0 ± 3.1 (SEM) cGy for equation (1) and 156.3 ± 2.8 (SEM) cGy for equation (2). This correspondence is expected because the values of N were very close to 1 using equation (2). Although the dose rate of X rays was higher than that of ^{252}Cf radiation, the mean value of the relative biological effectiveness (RBE) was 1.87. If the assumption is made that the gamma-ray component and the neutron (n) component act independently, the RBE of ^{252}Cf neutrons (RBE_n) can be calculated as follows:

$$RBE_n = \frac{(RBE_{n+\gamma} - f_\gamma)}{f_n} \quad ,$$

where f represents the fraction of the dose due to neutrons or gamma radiation. The RBE_n of ^{252}Cf radiation to high-dose-rate X rays was calculated as 2.29.

Figure 3 compares the D_{10} values for X ray and ^{252}Cf radiation, using equation (2). The two AT strains showed the lowest values for both types of radiation. There was no correlation between X-ray sensitivity and neutron sensitivity among 51 SF cell strains (correlation coefficient, 0.092; p-value, 0.52).

To examine the selection theory of Stewart, SF cells were grouped by dose (DS86) (Figure 4). D_{10} values for X-irradiated AT strains ranged from 1.07–1.59 Gy. D_{10} values of SF strains ranged from 2.18–3.40 (mean ± SEM = 2.82 ± 0.05) Gy in 30 individuals of the nonexposed group, 2.21–3.59 (2.98 ± 0.10) Gy in 12

individuals of the group exposed to 0.01–0.99 Gy, 2.44–3.11 (2.87 ± 0.21) Gy in three individuals of the group exposed to doses above 1 Gy, and 2.62–3.02 (2.89 ± 0.04) Gy in 11 individuals exposed to unknown doses. No cell strains exhibited X-ray sensitivity comparable to AT cells among the 56 SF cell strains studied. The X-ray sensitivity of 30 individuals of the nonexposed group (Figure 4a) and 26 individuals of the exposed group (Figure 4b–d) was widely distributed, but there was little difference in distribution between the two groups. Moreover, the group exposed to a high dose (1 Gy or greater) was not skewed towards X-ray resistance.

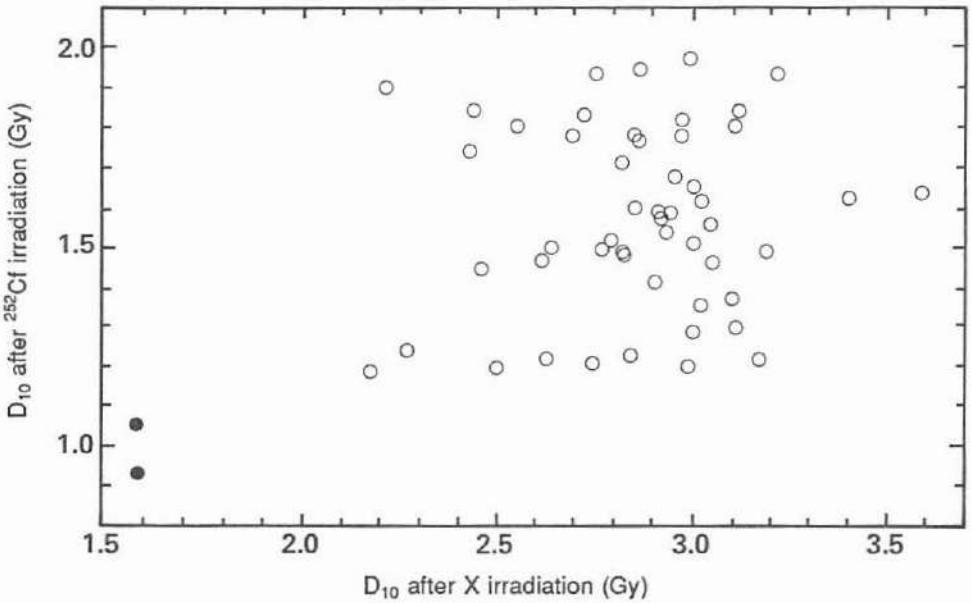

Figure 3. Comparison of the D_{10} values for 51 SF strains (○) and two AT strains (●) following X irradiation and ^{252}Cf irradiation. D_{10} values were calculated from the survival curves fitted to equation (2).

The D_{10} values for breast cancer patients of the nonexposed group and the exposed group (Figure 4) were uniformly distributed and displayed a range of sensitivity comparable to the group without breast cancer. A weighted least-squares analysis* comparing the breast cancer and non-breast-cancer groups resulted in an estimated difference in mean D_{10} of 9.96 ± 7.71 cGy, which is not significantly different from zero.

** Weights for each mean D_{10} 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 estimated by choosing the value of A which makes the residual mean square error equal to 1.*

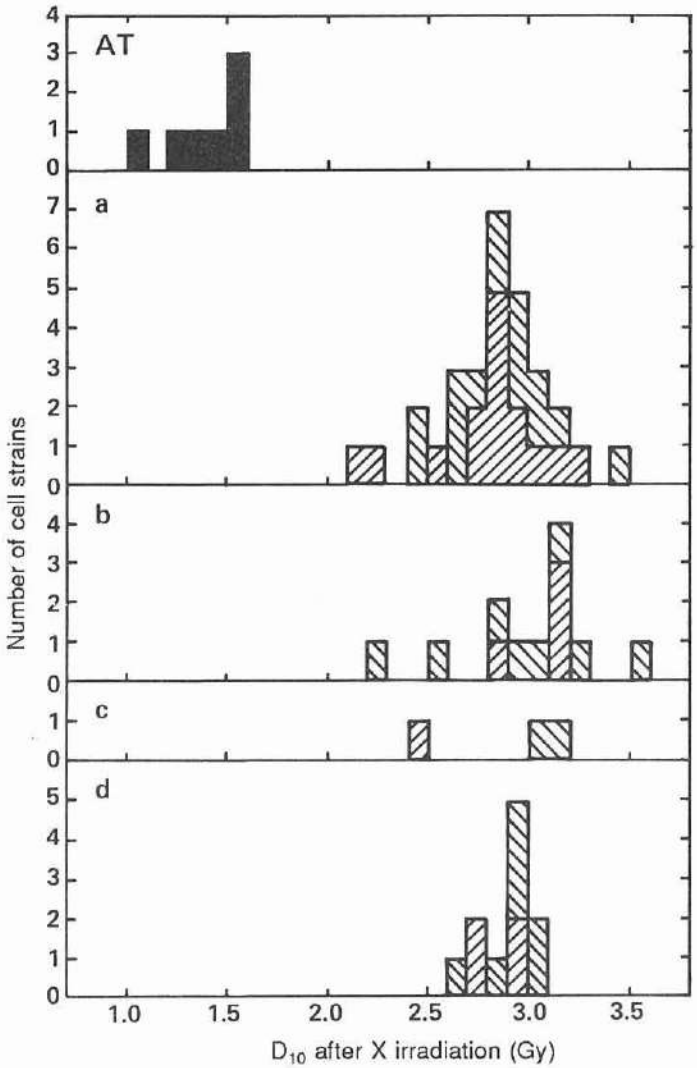

Figure 4. The distribution of X irradiation D_{10} values (Table 1) among the cell strains. AT: Seven AT cell strains. a: Nonexposed individuals. b: Individuals exposed to 0.01–0.99 Gy. c: Individuals exposed to 1.00 Gy or more. d: Individuals whose doses are unknown.

▨ : without breast cancer
▧ : with breast cancer

D_{10} values following ^{252}Cf irradiation were also compared for four groups classified by A-bomb dose (DS86) (Figure 5). D_{10} values of AT cells were between 0.93–1.06 Gy. D_{10} values of SF cells (mean ± SEM) were 1.19–1.94 (1.57 ± 0.05) Gy in 28 individuals of the nonexposed group, 1.23–1.90 (1.56 ± 0.08) Gy in nine individuals of the group exposed to 0.01–0.99 Gy, 1.46–1.84 (1.68

± 0.11) Gy in three individuals of the group exposed to doses above 1 Gy, and 1.20–1.97 (1.55 ± 0.06) Gy in 11 individuals who received unknown doses. No deviation in sensitivity to fission neutrons was observed between the nonexposed and exposed groups or between individuals with or without breast cancer. These results suggest that radiogenic cancer does not develop specifically in radiation-sensitive women.

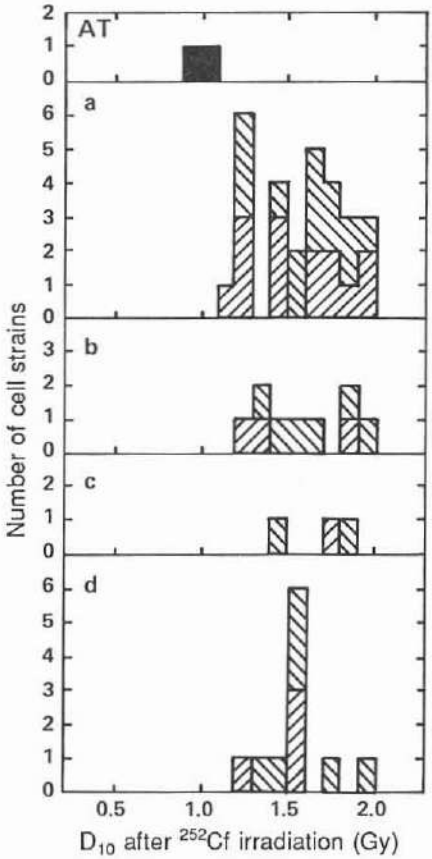

Figure 5. The distribution of ^{252}Cf D_{10} values among the cell strains (Table 1). AT: Cell strains AT3BI and AT5BI. a: Nonexposed individuals. b: Individuals exposed to 0.01–0.99 Gy. c: Individuals exposed to 1.00 Gy or more. d: Individuals whose doses are unknown.

▨ : without breast cancer
▧ : with breast cancer

Discussion

The radiosensitivity of many skin fibroblasts obtained from normal individuals and hereditary disease patients have been studied, but no cell strains have been found that are more sensitive to ionizing radiation than AT cells. Cells obtained from bilateral retinoblastoma patients, trisomy-13 patients, and AT heterozygote patients, or those with progeria, Werner's syndrome, and Fanconi's anemia showed slightly elevated sensitivity,[6,7] whereas the sensitivity of cells obtained from "normal individuals" exhibited great individual differences.[5–7]

The high susceptibility hypothesis assumes that persons with a high risk of cancer or other diseases due to A-bomb radiation exposure genetically have a high sensitivity to radiation damage.[9–13] Tokunaga et al[1] confirmed that breast cancer is one of the most typical late effects induced by A-bomb radiation. Breast cancer is a useful example of the multi-step theory of carcinogenesis. Breast cells that have sustained hereditary damage (a tumor initiator) are believed to develop into a clinical cancer as a subsequent effect of hormones such as prolactin (a tumor promotor).[21,22] Although we do not know the exact process of carcinogenesis in the breast tissue cells of A-bomb survivors, if the high susceptibility hypotheses were correct, the radiosensitivity of A-bomb survivors with breast cancer would be expected to be greater than that of survivors without breast cancer.

Our data on the survival of fibroblasts following X-ray exposure revealed great individual differences in X-ray sensitivity in normal unexposed human populations (Figures 1 and 4). These results are consistent with prior reports of differences in the clinical symptoms of acute radiation damage observed in survivors who had been exposed to similar doses.[23] In addition, of those whose radiation dose from the A-bomb was 4 Gy (T65D) or more, no more than 2.4% developed leukemia, a malignant neoplasm with the highest relative risk attributable to radiation.[23] Similar results showing diverse radiosensitivity were obtained from the dose-survival responses following exposure to fission neutrons generated from ^{252}Cf (Figures 2 and 5).

Our results showed no direct correlation between the risk of breast cancer induction after A-bomb radiation exposure and the radiosensitivity of cells measured by clonogenic assay. Our results may be supported by the current data about radiation-induced cell killing in fibroblasts obtained from patients with hereditary disorders. Weichselbaum et al[9,10] reported that skin fibroblasts derived from patients with D-deletion type (13q–) retinoblastoma are highly sensitive to X rays. However, results contradicting their report have also appeared.[24–26] Wang et al[27,28] showed that fibroblasts from hereditary retinoblastoma exhibited normal sensitivity to the mutagenic effects and neoplastic-transforming effects of ^{60}Co gamma rays. AT cells are hypersensitive to cell killing by ionizing radiations, but are not more mutable by gamma rays than normal cells.[29,30] Recent work of Little et al[31] concluded that the greater radiosensitivity of retinoblastoma cells occurred in isolated cases, and that the sensitivities were still within the envelope of normal variation. A consistent association between radiation sensitivity and cancer proneness has not yet been found in studies employing ionizing radiations.

It has not been possible to obtain direct evidence either in support of the selection hypothesis for A-bomb survivors or opposed to it. In particular, it is impossible to know whether A-bomb survivors who died early of acute infections were more radiosensitive. Although only a limited number of cases were

examined for their in vitro radiosensitivity, our results provided no indication that the A-bomb population includes a relatively large number of radioresistant (or radiosensitive) persons, or that the radioresistant (or radiosensitive) individuals are less or more prone to the development of cancer than is the general population.

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乳癌罹患の又は罹患していない原爆被爆生存者から得られた皮膚線維芽細胞の放射線感受性§

Radiosensitivity of Skin Fibroblasts from Atomic Bomb Survivors with and without Breast Cancer

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要　約

広島での原爆放射線に被曝したか，もしくはしないか，及び乳癌に罹患したか罹患しないかの4グループの55人の女性と1人の男性から得られた皮膚生検材料から，培養系で増殖する線維芽細胞を樹立した．これらの細胞にX線，又は ^{252}Cf 線源からの核分裂中性子を照射し，コロニー形成法によって放射線感受性を評価した．X線線量及び中性子線量に対する生存率曲線は，多標的モデル $S/S_0 = A\,[1-(1-e^{kD})^N]$ を用いて解析した．中性子線量－生存率関係は単ヒットモデル $S/S_0 = Ae^{kD}$ にも適合させた．原爆被爆の有無，あるいは乳癌の有無にかかわらず，放射線感受性の平均値又は分散に差は認められなかった．したがって，サンプルは多くはないが，放射線による試験管内細胞致死効果の高い細胞をもつ女性は，原爆放射線によって乳癌を誘発されやすいという仮説は支持しない．

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緒　言

疫学調査で，原爆放射線被曝者に線量に依存する有意な乳癌発生率の増加が認められた.[1] 他の調査でも，乳房組織は電離放射線被曝の発癌効果に対し特に感受性が高いことが認められた.[2]

放射線被曝による癌のリスクの増加は，細胞の電離放射線の致死効果に対する試験管内感受性異常と関連があるかもしれない．正常人及び癌になりやすい遺伝性疾患患者から得られた多数の皮膚線維芽細胞株を用いて試験管内放射線感受性を調べた．これらの調査で，毛細血管拡張性運動失調症(AT)[3] 及び色素性乾皮症(XP)[4]のような遺伝性疾患患者の細胞にはX線又は紫外線(UV)放射線に対する感受性異常が認められ，また正常なヒト集団内でも放射線感受性に大きな変動があることが認められた.[5-8] Weichselbaum ら[9,10] は，遺伝性D欠失網膜芽細胞腫細胞はX線感受性が有意に高く，その結果，網膜芽細胞腫細胞においては放射線感受性は新生物感受性と関連があるという仮説が導かれることを報告した.[11-13] Weishselbaum のデータに基づけば，乳癌をもつ原爆被爆者は傾向として放射線損傷に対する感受性が最も高い細胞をもつ人であるという仮説が立てられる(「高感受性」仮説).

Stewart 及び Kneale[14,15] は，放射線感受性の高い人は急性感染症により早死にしたので，そのような人は原爆被爆生存者には含まれていないかもしれないと推論した(「選択」仮説)．このため，被爆生存者の癌リスクの推定値を一般集団に適用した場合，低すぎるかもしれない．この仮説が正しければ，被爆生存者集団は主として放射線に対して抵抗性があり，放射線による癌を発現しにくい人から成っているので，一般集団の放射線感受性を代表するものではない.[16]

これら二つの仮説を検証するために，乳癌をもっている人ともっていない人及び原爆放射線に被曝した人としなかった人の皮膚生検材料から得た線維芽細胞株の試験管内放射線感受性を測定した．日本人女性のこれまでの乳癌の自然発生率は低かったので，高線量被曝者における乳癌は放射線誘発であった可能性が大きいと思われるという理由で乳癌が調査対象に選ばれた．しかし，結果的には高線量被曝し乳癌をもつ女性から期待したほど生検材料を得ることはできなかった．しかしながら，以下に報告する結果によって，少なくとも若干の仮説検証が行われる．

材料及び方法

皮膚組織

表1は，提供者を皮膚生検提供時の年齢，原爆放射線量（DS86 カーマ及び DS86 臓器［乳癌］線量[17,18]）及び乳房（又は他の）癌の有無別に記載したものである．1984年から1987年半ばまで，女性由来の55細胞株及び胃癌をもった非被曝男性由来の1細胞株の計56細胞株を試験管内で樹立した．

乳癌患者の皮膚組織は手術時に胸部から得た．サンプル SFc1-2 を除いて他の皮膚組織は頚部，胸部及び腹部から得た．SFc1-2 の提供者からは右上腕部の表皮封入体嚢胞付近の正常皮膚組織を得た．

初代培養細胞の樹立

皮膚組織はケロイド及び色素性母斑が無いことを確認した後，抗生物質（ペニシリン及びストレプトマイシン）を含むリン酸緩衝生理食塩水中で入念に洗浄した．組織は1 mm^3 以下の切片にして，約30％胎児仔ウシ血清（FCS）を含む α MEM 培養液（New York 州 Grand Island, GIBCO 社）に入れた．3～5日後，培養液を15％ FCS を含むα MEM と交換した．10～14日後，組織片から拡大増殖する細胞を採取した後に継代培養した．初代細胞株は接頭辞 SF（皮膚線維芽細胞）で示す．

AT皮膚線維芽細胞

接頭辞 GM の AT 細胞株は NIGMS Human Genetic Mutant Cell Repository（New Jersey 州 Camden）から得た．^{252}Cf 放射線の生存率測定に用いた AT3BI 及び AT5BI は金沢大学の二階堂教授から得て，液体 N_2 に凍結保存してあった．[19]

培養液

大部分の実験において細胞株の維持及び継代培養に15％ FCS を含むα MEM 培養液を使用した．X線細胞毒性測定で，15％ FCS を含む Dulbecco の調製 Eagle MEM（New York 州 Gland Island, GIBCO 社）を使用したこともある．5％ジメチルスルホキシド（DMSO）と20％ FCS を含むα MEM を細胞の凍結保存に使用した．細胞剥離には0.01％ EDTA を含む0.25％トリプシン溶液を使用した．

表1. 皮膚組織提供者の年齢，線量，乳癌の有無，他の癌組織，及び方程式(2)，$S/S_0 = A[1-(1-e^{kD})^N]$を用いて算定された線量生存率の反応係数

細胞株	提供者年齢	原爆時年齢	DS86 (Gy) カーマ	DS86 (Gy) 乳房	脱毛	乳癌	その他の癌	X線 繰返し検査数	X線 D_{10}±SE (Gy)		X線 D_0±SE (Gy)		X線 N±SE		^{252}Cf D_{10} (Gy)	^{252}Cf D_0 (Gy)	^{252}Cf N
SF a 1	41		0	0		–		3	2.72	0.13	1.13	0.11	1.3	0.3	1.83	0.81	1.0
2	40		0	0		–		3	2.90	0.08	1.06	0.06	1.7	0.4	1.41	0.56	1.2
3	54		0	0		–		7	2.27	0.14	1.03	0.10	1.0	0.1	1.24	0.51	1.2
4	14		0	0		–		6	2.18	0.07	0.88	0.01	1.2	0.1	1.19	0.52	1.0
5	33		0	0		–		2	2.75	0.16	1.20	0.04	1.0	0.2	1.21	0.54	0.9
6	42		0	0		–		4	2.97	0.13	1.13	0.06	1.4	0.1	1.78	0.86	0.8
7	27		0	0		–		2	2.85	0.05	1.16	0.01	1.2	0.1			
8	54		0	0		–	胸部	3	3.19	0.29	1.20	0.07	1.5	0.2	1.49	0.64	1.0
9	55		0	0		–		5	2.82	0.19	1.04	0.10	1.6	0.2	1.49	0.57	1.4
10	60		0	0		–	子宮	2	2.50	0.14	0.83	0.00	2.1	0.3	1.20	0.56	0.9
11	51		0	0		–		4	3.21	0.16	1.04	0.06	2.2	0.1	1.93	0.82	1.0
12	73		0	0		–		2	2.86	0.21	1.19	0.04	1.2	0.3	1.77	0.76	1.0
13	58		0	0		–	甲状腺，結腸	4	2.86	0.17	0.97	0.06	2.0	0.2	1.94	0.88	0.9
14**	63		0	0		–	胃癌	2	3.00	0.01	1.21	0.02	1.2	0.0	1.65	0.60	1.6
15	38		0	0		–		2	2.85	0.14	1.00	0.01	1.8	0.3	1.60	0.76	0.8
								平均	2.80	0.07	1.07	0.03	1.5	0.1	1.55	0.67	1.1

(続く)

ヒト皮膚線維芽細胞（SF）は，乳癌をもたない非被曝者（SFa），乳癌をもつ非被曝者（SFb），乳癌をもたない被曝者（SFc）及び乳癌をもつ被曝者（SFd）から得た．

SFb10 は爆心地から 3km 以上で胎内被爆した．SFc2 及び SFd2 群はカーマを算定できない．SFc2-7，SFc2-8，SFd2－11，SFd2-12 及び SFd2-17 の提供者は放影研の原簿記録に登録されているが，それらの線量は不明である．

放影研の記録によれば，SFd2-12 の提供者は爆心地から 467 m で被爆したが，遮蔽状況は不明である．

脱毛：＋は，被爆2か月以内に頭部4分の1以上に脱毛が起こったことを意味する．－は脱毛が起こらなかったことを意味する．

†原爆時胎内．　　** 男性．

表 1 （続き）

細胞株	提供者年齢	原爆時年齢	DS86 (Gy) カーマ	DS86 (Gy) 乳房	脱毛	乳癌	その他の癌	X線 繰返し検査数	X線 D_{10} ± SE (Gy)		X線 D_0 ± SE (Gy)		X線 N ± SE		^{252}Cf D_{10} (Gy)	^{252}Cf D_0 (Gy)	^{252}Cf N
SF b 1	25		0	0		+		2	2.75	0.09	1.09	0.03	1.3	0.2	1.93	0.82	1.0
2	55		0	0		+		4	2.97	0.04	0.99	0.03	2.1	0.2	1.82	0.80	0.9
3	53		0	0		+		5	2.46	0.24	0.90	0.04	2.2	0.1	1.45	0.63	1.0
4	50		0	0		+		2	2.84	0.00	1.17	0.03	1.2	0.1			
5	55		0	0		+		4	2.44	0.25	1.02	0.08	1.1	0.1	1.84	0.75	1.2
6	38		0	0		+		4	2.82	0.12	1.11	0.05	1.3	0.2	1.71	0.60	1.8
7	40		0	0		+			2.92	0.06	1.20	0.01	1.1	0.1	1.57	0.66	1.1
8	53		0	0		+			3.40	0.15	1.22	0.01	1.7	0.2	1.63	0.75	0.9
9	70		0	0		+		3	3.02	0.06	1.13	0.03	1.5	0.2	1.62	0.56	1.8
10†	40		0	0		+		4	3.00	0.19	1.23	0.06	1.2	0.1	1.29	0.65	0.7
11	57		0	0		+		3	2.69	0.22	0.81	0.04	3.1	0.8	1.78	0.93	0.7
12	55		0	0		+		2	2.64	0.08	0.77	0.09	3.9	1.9	1.50	0.59	1.3
13	70		0	0		+		4	3.17	0.07	1.03	0.07	2.5	0.6	1.22	0.49	1.2
14	41		0	0		+		2	2.63	0.18	1.19	0.13	0.9	0.1	1.22	0.47	1.3
15	59		0	0		+		5	2.95	0.08	1.16	0.03	1.3	0.1	1.68	0.76	0.9
								平均	2.85	0.07	1.07	0.04	1.8	0.2	1.59	0.68	1.1

（続く）

表 1（続き）

細胞株	提供者年齢	原爆時年齢	DS86 (Gy) カーマ	DS86 (Gy) 乳房	脱毛	乳癌	その他の癌	X線 繰返し検査数	X線 D_{10}±SE (Gy)		X線 D_0±SE (Gy)		X線 N±SE		^{252}Cf D_{10} (Gy)	^{252}Cf D_0 (Gy)	^{252}Cf N
SF c1-1	43	3	1.488	1.260	+	−		5	2.44	0.17	0.92	0.08	1.8	0.5	1.74	0.76	1.0
2	50	12	0.958	0.824	−	−		2	2.84	0.06	1.19	0.04	1.1	0.0	1.23	0.49	1.2
3	57	18	0.320	0.290	−	−		2	3.18	0.04	1.21	0.02	1.4	0.1			
4	56	18	0.023	0.019	−	−		3	3.11	0.27	1.26	0.07	1.2	0.1	1.30	0.56	1.0
5	46	6	0.099	0.094	−	−		2	3.10	0.21	1.28	0.05	1.1	0.1	1.80	0.81	0.9
								平均	2.93	0.14	1.17	0.07	1.3	0.1	1.52	0.66	1.0
SF c2-6	52	13	不明			−		2	2.99	0.04	1.27	0.04	1.1	0.0	1.20	0.47	1.3
7	70	30	不明			−		7	2.79	0.13	0.93	0.04	2.1	0.2	1.52	0.70	0.9
8	52	11	不明			−	甲状腺	2	2.77	0.21	0.98	0.01	1.8	0.4	1.50	0.62	1.1
9	62	21	不明			−		2	2.91	0.03	1.12	0.01	1.4	0.0	1.59	0.72	0.9
								平均	2.87	0.05	1.08	0.08	1.6	0.2	1.45	0.63	1.1
							SFb の平均値		2.90	0.08	1.13	0.05	1.4	0.1	1.49	0.64	1.0

（続く）

表 1（続き）

細胞株	提供者年齢	原爆時年齢	DS86 (Gy) カーマ	DS86 (Gy) 乳房	脱毛	乳癌	その他の癌	X線 繰返し検査数	D_{10}±SE (Gy)		D_0±SE (Gy)		N±SE		^{252}Cf D_{10} (Gy)	^{252}Cf D_0 (Gy)	^{252}Cf N
SF d1-1	52	13	3.286	2.882	+	+		5	3.05	0.11	1.15	0.06	1.5	0.1	1.46	0.63	1.0
2†	38		0.054	0.038		+		2	2.21	0.11	0.81	0.13	1.8	0.5	1.90	0.93	0.8
3	76	36	0.234	0.220		+		2	2.90	0.01	1.23	0.01	1.1	0.0			
4	63	25	0.057	0.054	−	+		6	3.59	0.14	1.23	0.05	2.2	0.4	1.64	0.76	0.9
5	60	19	0.067	0.057	−	+		4	3.10	0.17	0.97	0.08	2.6	0.4	1.37	0.62	0.9
6	47	6	0.625	0.595	−	+		4	3.04	0.14	1.02	0.05	2.2	0.4	1.56	0.79	0.7
7	57	16	0.852	0.596	+	+		4	2.55	0.22	0.92	0.05	1.9	0.4	1.80	0.91	0.7
8	58	17	2.047	1.547	+	+		7	3.11	0.08	1.05	0.05	2.3	0.4	1.84	0.89	0.8
9	45	4	0.508	0.482	−	+		2	2.83	0.14	1.10	0.06	1.3	0.0	1.48	0.72	0.8
10	89	47	0.180	0.183	−	+		2	3.27	0.02	1.25	0.06	1.4	0.2			
							平均		2.97	0.12	1.07	0.05	1.8	0.2	1.63	0.78	0.8
SF d2-11	48	10	不明		+	+		2	2.85	0.04	1.08	0.12	1.6	0.5	1.78	0.83	0.9
12	54	15	不明		+	+		3	2.99	0.22	0.99	0.08	2.1	0.2	1.97	0.87	1.0
13	72	33	不明			+		3	3.02	0.11	1.18	0.09	1.3	0.2	1.35	0.60	0.9
14	69	29	不明			+		4	2.93	0.42	1.04	0.07	1.8	0.5	1.54	0.73	0.8
15	60	19	不明			+		5	2.94	0.19	1.15	0.06	1.3	0.1	1.59	0.79	0.7
16	55	12	不明			+		2	2.62	0.08	0.84	0.01	2.4	0.3	1.47	0.67	0.9
17	53	11	不明			+		3	3.00	0.07	1.31	0.02	1.0	0.1	1.51	0.67	0.9
							平均		2.91	0.05	1.08	0.06	1.6	0.2	1.60	0.74	0.8
							SFdの平均値		2.94	0.07	1.08	0.03	1.8	0.1	1.62	0.76	0.9
							各グループ平均値		2.87	0.03	1.09	0.01	1.6	0.1	1.56	0.69	1.0

X 線照射

活発に増殖している細胞を増殖培養液中に懸濁した後，(Coulter 計数器を用いて推定した) 1×10^5 個の細胞(1 mL)をプラスチック試験管(New York 州 Corning, Corning 社)に入れ，室温で照射した．照射施設を変更したため2種類のX線発生装置を用いた．両装置から得られた結果は同じであった．一つの装置は 250 kVp, 30 mA, 0.5 mm Cu + 1.0 mm Al 外部フィルター，線量率 0.9 Gy／分で作動させ，他の一つは220 kVp, 8 mA, 0.3 mm Cu +0.5 mm Al 外部フィルター，線量率 1.0 Gy／分で作動させた．空中線量率は Victoreen コンデンサー器を用いて室温で測定した．AT 細胞には0, 0.25, 0.5, 1.0, 2.0, 3.0 Gy, SF 細胞には線量は0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 Gy の線量を照射した．SF 細胞のX線実験は二つの装置でそれぞれ2～7回繰り返し行った(表1)．

中性子照射

活発に増殖している細胞(3×10個又は1 mL)を試験管(パイレックスガラス，内径15 mm，長さ125 mm)に入れ，広島大学原爆放射能医学研究所で照射した．各試験管は連続回転テーブルの中心から8 cmのところにある八つのアルミニウム管(内径18 mm，長さ120 mm，厚さ1 mm)に入れた．中性子照射装置の中央軸にあるステンレススチールに包まれた ^{252}Cf 線源(英国 Buckinghamshire, Amersham International 社製，X-35型，長さ17 mm，直径9.5 mm)を用いて室温で中性子照射を行った.[20] Three-Terminal電離箱(California 州 Goleta, Far-West Technology 社製 IC-17 型及び IC-17G 型)及び Fricke 線量計を用いて測定した線量率は1.18～1.34 cGy／分であった．ガンマ線に対する中性子の比率は2.0であった．0, 0.25, 0.50, 0.75, 1.0, 1.5 及び2.0 Gy 照射後，ガラス管に付着した細胞をトリプシン EDTA 溶液で剥離した．

クローン形成法による細胞生存率

照射直後，適当な数の細胞を直径10 cmのプラスチック培養皿(各線量点に6枚のシャーレ) (New York 州 Corning, Corning 社)に播種し，空気 95% + CO_2 5%，37℃中で10～14日間培養した．この期間中培養液を1,2回交換した．コロニーはエタノールで固定し，ギムザ染色した．50個以上の細胞から成るコロニーを調べて，コロニー形成率及び生存率を算定した．

曲線適合及びデータ解析

線量反応は単ヒットモデル

$$S/S_0 = Ae^{kD} \tag{1}$$

及び多標的モデル

$$S/S_0 = A\ [1-(1-e^{kD})^N] \tag{2}$$

を用いて解析した．この場合線量Dは gray で示し，S/S_0 は線量Dにおける生存率である．生存曲線の直線部分を37％に減少させる線量 D_0は $-1/k$ で表される．N・A は，曲線の補外直線部が生存軸と交差する点である．各生存曲線の勾配k及び切片Nは，観察された線量反応点はすべて生存曲線の指数部分にあるという仮定を排除する最小二乗回帰解析により非直線関数のパラメータとして推定した．方程式(2)については，生存率の対数は次の生存モデルにより示される．

$$\ln(S/S_0) = \ln(A) + \ln\ [1-(1-e^{kD})^N] \tag{2'}$$

この場合 ln(A) は方程式の切片であり，ln(N) + ln(A) は方程式の直線部分の補外切片で，kは方程式の直線部分の勾配である．対照を含めすべてのデータを用いて方程式のパラメータを推定した．パラメータAを導入して，方程式(2′)が0線量で ln(1) = 0 に到達することを回避した．このことは0線量時の生存率が測定され，その結果誤差があることを認めており，またそのことによりこの一つの線量点とは無関係にk及びNの推定が可能となる．非線形最小二乗法を用いてモデルのパラメータを推定した．k，N及びAの最適合値を用いた適切な方程式から，生存率を10％に減少させるのに必要な線量 D_{10} を算定した．BMDP6D プログラム(California 州 Los Angeles, BMDP Statistical Software 社)を用いて線量反応曲線をコンピュータで作った．

結　果

SF 細胞株のX線生存率曲線平均値を表1に示した．AT「正対照」細胞株を用いた並行実験で得た平均値を表2に示した．SF 細胞株4グループ及び AT 細胞株の平均生存率曲線を図1に示した．AT 細胞株では曲線は方程式(2)に最もよく適合し，0.49～0.66 Gy の範囲の D_0(= $-1/k$) 値が得られた．方程式(2)はSF

表2. 毛細血管拡張性運動失調症細胞株の生存率曲線パラメータ

細胞株	X線			^{252}Cf		
	D_{10} (Gy)	D_0 (Gy)	N	D_{10} (Gy)	D_0 (Gy)	N
AT3B1	1.52	0.65	1.1	0.93	0.40	1.0
AT5B1	1.59	0.66	1.1	1.06	0.46	1.0
GM2052	1.07	0.50	0.9			
GM2530	1.49	0.62	1.1			
GM3395	1.20	0.49	1.2			
GM3487	1.59	0.57	1.7			
GM648	1.36	0.60	1.0			
平均	1.40	0.58	1.2	1.00	0.43	1.0

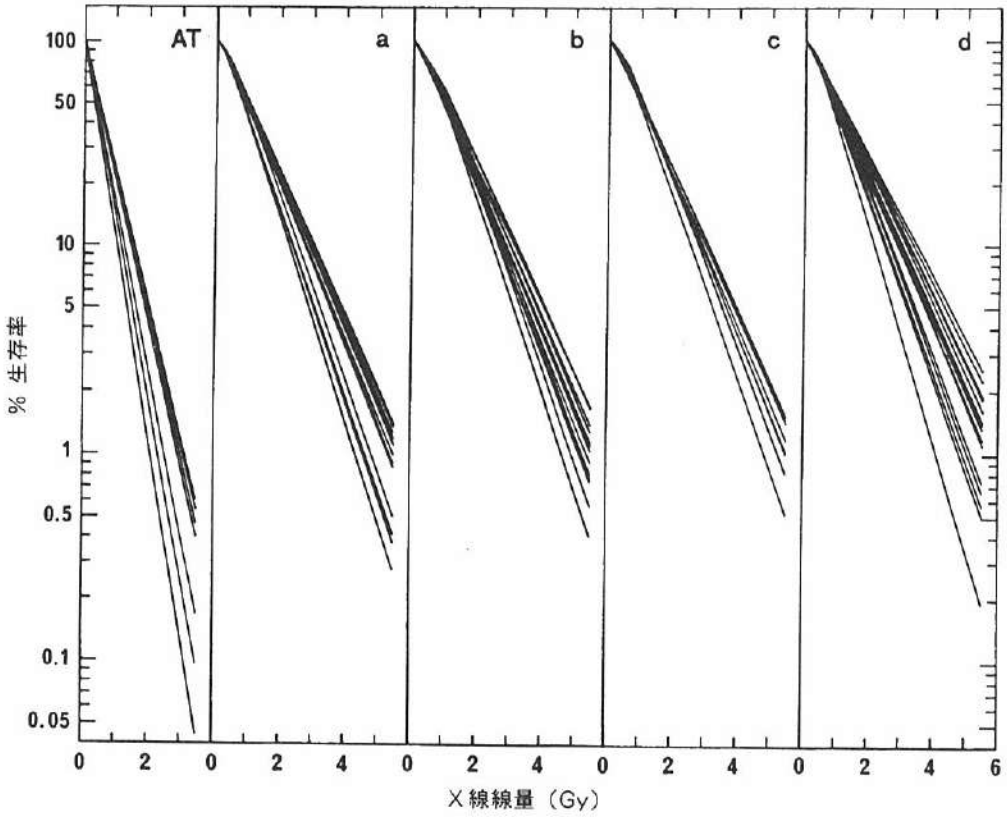

図1. 毛細血管拡張性運動失調症(AT)細胞及び皮膚線維芽細胞(SF)のX線照射後の生存率曲線. AT細胞には0, 0.25, 0.5, 1.0, 2.0及び3.0 Gyの, SF細胞には0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0及び8.0 Gy(線量率0.9又は1.0 Gy/分)の線量を照射した. レーンAT：細胞株 AT3BI, AT5BI, GM648AT, GM2052AT, GM2530AT, GM3395AT, GM3487AT. レーンa：乳癌をもたない非被曝者15人から得た皮膚細胞. レーンb：乳癌をもつ非被曝者15 人から得た皮膚細胞. レーンc：乳癌をもたない被曝者9人から得た皮膚細胞. レーンd：乳癌をもつ被曝者17人から得た皮膚細胞.

細胞株のデータにも適合した．SF 細胞の D_0 値はこれらの解析では 0.77～1.31 Gy であった．試験管内放射線感受性に及ぼす提供者の年齢の影響はほとんどなかった(表1)．年齢とX線の平均 D_{10} 値間の相関係数とp値はそれぞれ SFa, 0.34及び0.22；SFb, 0.23及び0.40；SFc, 0.22及び0.56；並びに SFd, 0.50及び0.04であった．更に，0線量でのコロニー形成能(コロニー形成率)は各実験で0.02～0.56の範囲であったが，放射性感受性とコロニー形成率との関連は有意ではなかった．コロニー形成率とX線の平均 D_{10} 値間の相関係数及びp値はそれぞれ SFa, 0.29及び0.29；SFb, －0.11及び0.70；SFc, －0.09及び0.82；並びに SFd, 0.16及び0.53であった．単向分散解析を行って56人の間で平均 D_{10} 値に差があるかどうか調べた．結果は $p < 0.001$ で有意であり，$F_{55,132} = 2.98$ であった．

対象者2人の再生検から樹立された細胞株を用いて，繰り返しX線生存測定を行った(表3)．この2人の患者のそれぞれから得た二つの細胞株の平均値は三つのパラメータについてほとんど差がなく，これは皮膚線維芽細胞の放射線感受性は遺伝学的に安定していることを示唆している．

表3．女性2人の反復生検標本から樹立された細胞株の生存率曲線パラメータの比較

株	生検日	実験番号	D_{10} (Gy)	D_0 (Gy)	N
SFb 2	1984年1月23日	20	3.09	1.05	1.9
		596	2.96	0.90	2.8
		2114	2.93	1.01	1.9
	1984年2月2日	平均	2.99 ± 0.05	0.99 ± 0.04	2.2 ± 0.3
		583	291	1.01	1.8
SFd 1-1	1984年6月4日	7	3.40	1.25	1.5
		643	2.73	0.94	1.8
		667	3.07	1.27	1.1
		平均	3.07 ± 0.19	1.15 ± 0.11	1.5 ± 0.2
	1984年7月10日	645	3.07	1.12	1.6
		646	2.99	1.15	1.4
		平均	3.03 ± 0.04	1.14 ± 0.02	1.5 ± 0.1

平均値： 平均±SE

新線量推定方式(DS86)によれば，広島の推定中性子線量は T65D 推定値の約10分の1である．しかし，中性子被曝の致死効果はX線の致死効果よりも大きいことが認められている．図2は，^{252}Cf 放射線に被曝した四つの SF 細胞株の2回の検定から得た線量生存率反応を示している．線量反応は方程式(2)に最もよく適合した．細胞株ごとに線量反応曲線のN値は異なっていたので，放射線感受性は D_{10} 値の平均を使って比較した(表4)．

X線照射後の56の SF 細胞株の平均 D_{10} 値は287.3±3.0(SEM) cGyであった．^{252}Cf 放射線照射後の51の SF 細胞株の平均 D_{10} 値は両モデルで類似している．すなわち，方程式(1)では156.0±3.1(SEM)，方程式(2)では156.3±2.8(SEM) cGy であった．方程式(2)のN値は1に非常に近づいたのでこの一致

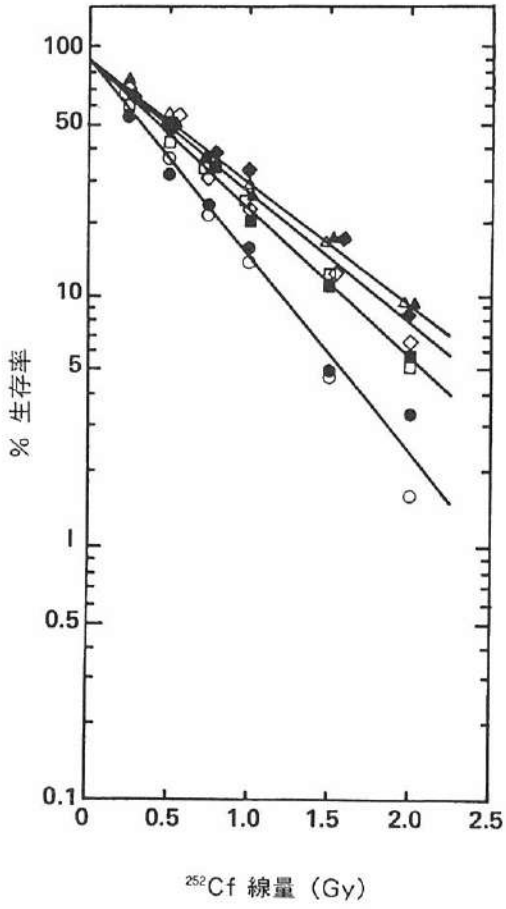

図2．皮膚線維芽細胞4株を ^{252}Cf 放射線で照射した場合の生存率曲線．各細胞株について行った2回の実験から得たデータはそれぞれ異なる記号で示している．細胞に照射された線量は0, 0.25, 0.50, 0.75, 1.0, 1.5及び2.0 Gyである．線量反応は方程式(2)の $S/S_0 = A[1-(1-e^{kD})^N]$ に適合させた．線量率1.178～1.337 cGy／分．中性子線量対ガンマ線線量比 2.0．

○● SFa10, △▲ SFa13,
◇◆ SFb2, □■ SFc9

表4. D_{10} 値と RBE 値(± SE)の比較

グループ	$S/S_0 = Ae^{kD}$	$S/S_0 = A[1-(1-e^{kD})^N]$		RBE
	^{252}Cf (cGy)	^{252}Cf (cGy)	X rays (cGy)	
SF a	155 ± 27	155 ± 26	280 ± 28	1.83 ± 0.23
SF b	158 ± 23	159 ± 22	285 ± 25	1.83 ± 0.34
SF c	148 ± 23	149 ± 21	290 ± 21	1.99 ± 0.35
SF d	163 ± 18	162 ± 19	294 ± 29	1.84 ± 0.30
平均 ± SE	156.0 ± 3.1	156.3 ± 2.8	287.3 ± 3.0	1.87 ± 0.04

データは，X線生存率曲線について方程式(2)に，^{252}Cf 放射線被曝後の生存率反応については方程式(1)及び(2)に適合させた．方程式(2)から得られた D_{10} 値を用いてREB 値を算定した．

は当然である．X線の線量率は ^{252}Cf 放射線の線量率よりも高かったが，生物学的効果比(RBE)の平均値は1.87であった．ガンマ線成分と中性子(n)成分はそれぞれ独自に作用すると仮定すれば，^{252}Cf 中性子の RBE(RBE_n)は次のように算定できる．

$$RBE_n = \frac{(RBE_{n+\gamma} - f_\gamma)}{f_n}$$

この場合fは中性子又はガンマ放射線による線量の割合を示す．高線量率X線に対する ^{252}Cf 放射線の RBE_n は2.29と算定された．

図3は，方程式(2)を使ってX線と ^{252}Cf 放射線の D_{10} 値を比較したものである．二つの AT 細胞株は2種類の放射線について最低値を示した．SF 細胞51株においてX線感受性と中性子感受性との間には相関関係はなかった(相関係数0.092；p値0.52)．

Stewart の選択説を検証するために SF 細胞を線量(DS86)別にグループ分けした(図4)．X線照射した AT 細胞株の D_{10}値は1.07～1.59 Gy であった．SF 細胞株の D_{10} 値は，非被曝群30人で2.18～3.40(平均値 ± SEM = 2.82 ± 0.05) Gy，0.01～0.99 Gy 被曝群12人で2.21～3.59(2.98 ± 0.10) Gy，1 Gy以上被曝群3人で2.44～3.11(2.87 ± 0.21) Gy 及び被曝線量不明者11人で2.62～3.02(2.89 ±

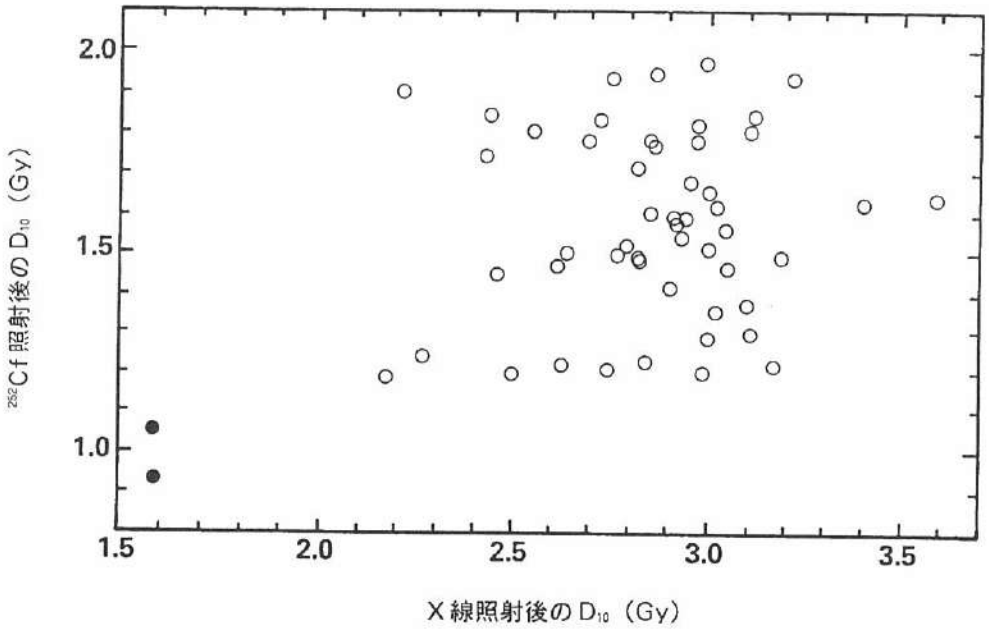

図3. X線照射及び ^{252}Cf 照射後の SF 細胞51株(○)及び AT 細胞2株(●)の D_{10} 値の比較. D_{10} 値は方程式(2)に適合させた生存率曲線から算定した.

0.04) Gy であった. 調べた SF 細胞56株の中で AT 細胞に匹敵するX線感受性を示した細胞株はなかった. 非被曝群30人(図4a)と被曝群26人(図4b－d)のX線感受性は広く分布していたが, 両群間での分布差はほとんどなかった. 更に, 高線量(1 Gy以上)被曝群がX線抵抗性側に偏向していることはなかった.

非被曝群及び被曝群の乳癌患者の D_{10} 値(図4)は分布が一様で, 乳癌をもたないグループと同程度の感受性を示した. 加重最小二乗解析*を行って, 乳癌群と非乳癌群を比較したところ, 9.96 ± 7.71 cGy の差が推定平均 D_{10} 値に見られたが, これは0に比べ有意差はない.

^{252}Cf 放射線照射後の D_{10} 値も原爆線量(DS86)別に分類した四つのグループで比較した(図5). AT 細胞の D_{10} 値は0.93～1.06 Gy であった. SF 細胞の D_{10} 値(平均値± SEM)は, 非被曝群28人で1.19～1.94 (1.57 ± 0.05) Gy, 0.01～0.99 Gy

*各平均 D_{10} 値の加重値は(A + B／N)の逆数とみなした. この場合Nは繰り返し実験数, Bは繰り返し実験から推定された実験誤差変量, Aは残留平均平方誤差が1に等しくなるA値を選んで推定した個人間変量である.

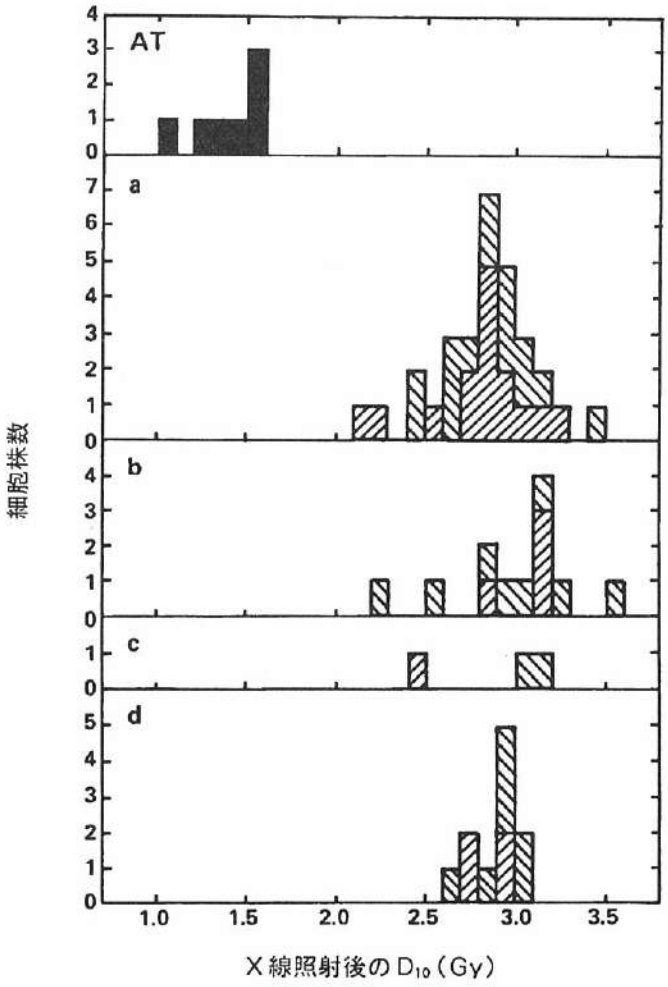

図4. 細胞株間におけるX線照射 D_{10} 値（表1）の分布.
AT：AT細胞7株. a：非被曝者. b：0.01～0.99 Gy被曝者.
c：1.00 Gy以上の被曝者. d：被曝線量不明者.

▨ 乳癌もたない　　▧ 乳癌もつ

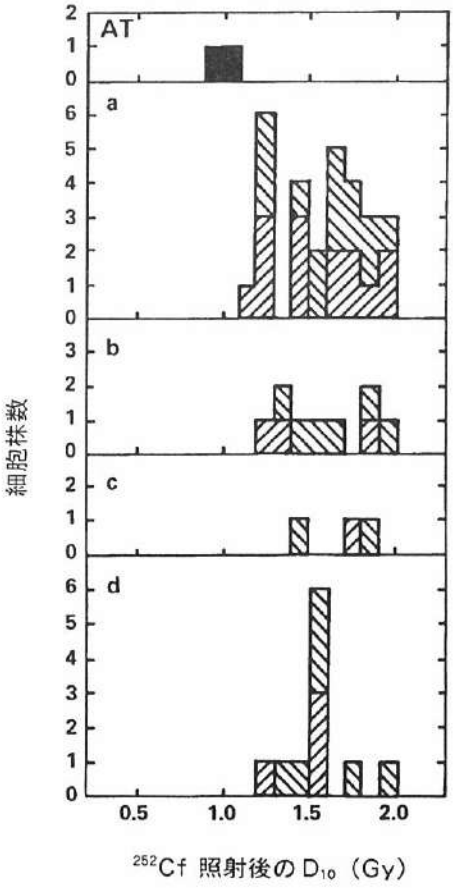

図5. 細胞株間における ^{252}Cf 照射 D_{10} 値(表1)の分布. AT：細胞株AT3BI 及び AT5BI. a：非被曝者. b：0.01～0.99 Gy被曝者. c：1.00 Gy以上被曝者. d：被曝線量不明者.

▨ 乳癌もたない
▧ 乳癌もつ

被曝群9人で1.23～1.90(1.56±0.08) Gy, 1 Gy以上被曝群3人で1.46～1.84(1.68±0.11) Gy, 及び被曝線量不明者11人で1.20～1.97(1.55±0.06) Gyであった. 非被曝群と被曝群又は乳癌のある人とない人の間で核分裂中性子感受性の偏りは認められなかった. これらの結果は, 放射線誘発癌は放射線感受性の高い女性に特異的に発生するものではないことを示唆している.

考 察

正常人及び遺伝性疾患患者から得られた多数の皮膚線維芽細胞の放射線感受性が調べられているが, 電離放射線感受性が AT 細胞よりも高い細胞株は見つかっていない. 両側性網膜芽腫患者, トリソミー13患者及び AT 異種接合体患者又は早老症患者, ウェルナー症候群患者及びファンコニー貧血患者から得られた細胞の感受性

は若干高いことが示されているが,[6,7]「正常人」から得られた細胞の感受性にも大きな個人差がある.[5-7]

高感受性仮説は,原爆放射線被曝による癌又は他の疾患のリスクが高い人は遺伝的に放射線損傷に対する感受性が高いことを想定している.[9-13] 徳永ら[1] は,乳癌は原爆放射線により誘発される最も典型的な晩発効果の一つであることを確認した.乳癌は発癌の多段階説を裏付ける有用な1例である.遺伝的損傷(腫瘍初発因子)を受けた乳房細胞はプロラクチン(腫瘍促進因子)のようなホルモンの続発効果として臨床腫瘍に進展するものと考えられている.[21,22] 原爆生存者の乳房組織細胞の正確な発癌過程はわからないが,高感受性説が正しいとすれば,乳癌をもった原爆被爆者の放射線感受性は乳癌をもたない被爆者よりも高いことが予想される.

X線照射された線維芽細胞の生存率に関する我々のデータでは,正常かつ非被曝集団のX線感受性には大きな個人差があることが認められた(図1及び4).これらの結果は,同程度の線量に被曝した生存者に観察された急性放射線損傷の臨床症状に差があるという過去の報告と一致している.[23] 加うるに,原爆放射線量が4 Gy(T65D)以上の被爆者のうち放射線による相対リスクが最高の悪性新生物である白血病になった者はわずか2.4%であった.[23] ^{252}Cf から発生する核分裂中性子を照射した後の線量生存率反応からも,広域の放射線感受性を示す同様の結果が得られた(図2及び5).

我々が得た結果は,原爆放射線被曝後の乳癌誘発リスクとクローン形成法により測定された細胞の放射線感受性との間には直接の相関関係がないことを示した.遺伝性疾患患者から得られた線維芽細胞の放射線誘発細胞死に関する最近のデータは我々の結果を支持するかもしれない.Weichselbaum ら[9,10] は,D欠失型(13q−)網膜芽細胞腫患者から得られた皮膚線維芽細胞はX線に対する感受性が高いことを報告した.しかし,彼らの報告と矛盾する結果も現れた.[24-26] Wang ら[27,28] は,遺伝性網膜芽細胞腫の線維芽細胞は ^{60}Co ガンマ線の突然変異原性効果及び試験管内発癌効果に対して正常細胞と同じ感受性を示すことを明らかにした.AT 細胞は電離放射線の致死効果に高感受性であるが,ガンマ線誘発突然変異が正常細胞よりも起こりやすいことはなかった.[29,30] Little ら[31] の最近の研究は,網膜芽細胞腫の高い放射線感受性は特例であるが,その感受性は正常変域の範囲内にあるという結論

を示した．放射線感受性と癌罹患性との間に一貫した関連性があることは電離放射線を用いた調査ではまだ認められていない．

原爆被爆者に関する選択仮説を支持又はそれに反対する直接的証拠を得ることはできなかった．特に，急性感染症のため早く亡くなった原爆被爆者の放射線感受性が高いかどうかを知ることは不可能である．試験管内放射線感受性を調べたのはごく少数例であったが，我々が得た結果は，原爆生存者集団には放射線抵抗性の（又は放射線高感受性の）人が比較的多い，あるいは放射線抵抗性の（又は放射線高感受性の）人が一般集団より癌になりにくい又はなりやすいということを示さなかった．

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