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Research Project 3-86

Technical Report Series

Absence of Correlations between the Radiosensitivity of Human T-lymphocytes at G_0 and Skin Fibroblasts at Log Phase from the Same Individuals

Jun-ichi Kushiro, M.D.; Nori Nakamura, Ph.D.; Seishi Kyoizumi, Ph.D.; Masayuki Nishiki, M.D.; Kiyohiko Dohi, M.D.; Mitoshi Akiyama, M.D.

放影研 RERF **Radiation Effects Research Foundation**
A Cooperative Japan–United States Research Organization

RERF Technical Report Series

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In this way, the Foundation will be able to more expeditiously report recent findings on the late biological effects of exposure of man to ionizing radiation resulting from the atomic bombings of Hiroshima and Nagasaki.

1989年から，放射線影響研究所の業績報告書は，従来の日英両文を併記した方式では発行しない．主要な報告書については，今後も日英両文で印刷するが，それぞれ別に発行する．内容が高度に専門的であり，一般の関心が少ないと思われる報告書については英文のみとし，日本文の要約を添付する．

これにより，広島・長崎の原爆電離放射線被曝の人体に及ぼす晩発性生物学的影響に関する最近の知見を今までよりも速やかにお知らせできることと思う．

The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

財団法人　放射線影響研究所
業　績　報　告　書

RERF TR 17-89
研究課題　3-86

同一人由来のリンパ球と皮膚線維芽細胞の放射線感受性§

Absence of Correlations between the Radiosensitivity of Human T-lymphocytes at G_0 and Skin Fibroblasts at Log Phase from the Same Individuals

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要　約

マウスにおける研究によると，系統によって放射線による種々の影響に差異が認められる．純系のマウスに対して，ヒトはその遺伝的背景が不均一であるから，年齢や性だけでなく，遺伝子構成の違いによっても放射線に対する感受性に個人差があるのではないかという考えがある．もしも，そのようなことが事実であれば，原爆被爆者の疫学調査に依存するところが大きい放射線防護基準の設定のためには，高線量被爆者の平均放射線感受性が，一般集団と同じであるかどうか，もし偏りがあるとしたらその程度はどのくらいであるかという大変難しい問題が生じる．

従来，ヒト細胞を用いた放射線感受性の個人差に関する調査には，皮膚由来の線維芽細胞に，試験管内で既知の量の放射線を照射してコロニー形成頻度を調べる方法が用いられてきた．その結果によれば，恐らくは未知の遺伝的要因に起因すると思われる差異が示唆されている．最近になって，インターロイキン2が発見されたことにより，ヒト末梢血Tリンパ球のコロニー形成が可能になった．ところがリンパ球を用いた我々の予備調査の結果は，皮膚線維芽細胞で報告されているほどの個人差は認められなかった．これは，たまたま調査したリンパ球提供者が日本人集団を代表するものでなく，偏っていたのかもしれないし，あるいはリンパ球と皮膚線維芽細胞という異なった細胞に付随した固有の変動の反映であるのかもしれない．そこで，本調査ではこのどちらに原因があるのかを明らかにする目的で，外科手術をうけた22名の人について皮膚線維芽細胞とリンパ球の両方の放射線感受性を調査した．感受性の指標としては，細胞のコロニー形成能の喪失を用いた．

§本報告にはこの要約以外に訳文はない．

承認1989年9月21日　　印刷1990年1月

その結果は，D_{10}値（90％の細胞がコロニー形成能力を喪失するのに必要なX線の線量）は，G_0期照射Tリンパ球では3.58±0.21 Gy（平均±標準偏差），対数増殖期照射の皮膚線維芽細胞では3.19±0.37 Gy であった．変動係数（CV）は各々6％と11％であった．

同一人由来のリンパ球の反復調査の結果もCV 6％であったので，少なくともリンパ球に関しては，放射線感受性に関する個人差は大変少なく，変動の大半は実験誤差に起因するものであると考えられた．したがって，同一提供者由来の2種類の細胞のD_{10}値の間に統計学的に有意な相関が認められなかったのも，納得のいくことである．皮膚線維芽細胞において観察されたCV 11％は，従来の報告に見られる値とよく一致している．線維芽細胞を用いた調査のCV値がリンパ球のそれよりも約2倍も大きいということは，放射線照射によるDNA傷害の修復能力が細胞の種類により異なっているのか，あるいは皮膚線維芽細胞は見かけは同じでも質的に異なる集団から成り立っている可能性を示唆していると思われる．

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Absence of Correlations between the Radiosensitivity of Human T-lymphocytes at G_0 and Skin Fibroblasts at Log Phase from the Same Individuals§

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Summary

Matched samples of peripheral T-lymphocytes and skin fibroblasts from a total of 22 patients who underwent various surgical procedures were tested for a dose-survival study using loss of colony-forming ability as the end point. The results showed that the mean D_{10} (the dose required to kill 90% of the cells) ±SD was 3.58 ± 0.21 Gy for T-lymphocytes irradiated at G_0 and 3.19 ± 0.37 Gy for skin fibroblasts irradiated at log phase. The coefficient of variation was found to be 6% and 11%, respectively. Contrary to expectation, regression analysis of the D_{10} values for the two cell types revealed no significant correlations.

The absence of correlation is most probably derived from the fact that the apparent interindividual variability of dose-survival curves is largely caused by random experimental fluctuations, at least for lymphocytes. Possible reasons for the greater variability observed in the fibroblast assay are discussed.

Introduction

In assessing the risks of low-level ionizing radiation exposure in humans, individual variation in radiosensitivity based on genetic heterogeneity has not been considered,[1] because the genes which affect repair processes after radiation exposure are not well understood. Acknowledging this, the BEIR III report states that "If population subsets can be identified as being at substantially greater risk of radiation carcinogenesis, their risk will require separate estimation."[1]

§**The complete text of this report will not be available in Japanese.**

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In the past decades, studies of human cells derived from cancer-prone repair-deficient genetic disorders, such as xeroderma pigmentosum, ataxia telangiectasia (AT), and Fanconi's anemia, have revealed certain aspects of various kinds of DNA damage and its repair. However, cultured cells from AT patients, though hypersensitive to the killing effect of ionizing radiation,[2,3] do not seem to be hypermutable in regard to the induction of somatic mutations,[4–6] thus providing no evidence that AT patients are at a higher risk of radiation-induced cancer than normal individuals.

Skin fibroblasts have long been used for experimental studies of individual variation mainly to the killing effect of ionizing radiation.[7–11] Results of these studies suggest the presence of significant interindividual variation among cells derived from apparently normal people, which probably can be attributed to some unidentified genetic factors.[7,11]

Recently, use of the lymphocyte colony assay as an efficient alternative to skin fibroblasts became possible due to the discovery of the T-cell growth factor, interleukin-2, which permits the growth of T-lymphocyte colonies in vitro.[12–15]

Our preliminary results using the lymphocyte assay suggest that interindividual variation in radiosensitivity is quite small when compared to some published data for skin fibroblasts obtained from Caucasians.[7,9,11] But, the laboratory volunteers who provided our blood samples may not have been representative of the general population or may have exhibited what appears to be a greater homogeneity among Japanese donors than exists among Caucasians, as suggested by the allele frequency at the HLA locus.[16]

So, to further examine the differences between our results and those of previous studies, T-lymphocytes and skin fibroblasts from the same Japanese individuals were used in dose-survival experiments. Thus, we hoped to be able to distinguish the existence of any inherent biases either in the assay methods themselves or in the donors.

Materials and Methods

Donors

A total of 22 Japanese patients, who had undergone surgery at the Second Department of Surgery, Hiroshima University School of Medicine, were the subjects of this study. Among these were six cases of breast cancer, three of hepatoma, three of hyperthyroidism, two each of thyroid cancer, gastric cancer, and lung cancer, and one each of esophageal varix, Zollinger-Ellison syndrome, parathyroid tumor, and thymoma. The age distribution of the 10 men and 12 women ranged from 20 to 72 years old (see also Table 4).

Lymphocytes

Peripheral blood was defibrinated by glass beads, and mononuclear cells were isolated by Ficoll/Hypaque density gradient centrifugation.[17] The cells were suspended with Earle's balanced salt solution containing 2.5% fetal calf serum (FCS) and 1% penicillin and streptomycin, were washed three times by centrifugation, and were used for the experiments.

Skin fibroblasts

A strip of surgically removed skin was minced by scalpel and placed on a collagen gel matrix (primary culture).[18] After 7–10 days of incubation with medium consisting of alpha-MEM, 15% FCS, and 1% penicillin and streptomycin, the cells were digested along with the collagen gel using trypsin and collagenase, after which the freed cells were washed twice by centrifugation and seeded in a 10-cm plastic dish (passage 1). Before cultures attained confluency, a 1:3 subculture was made (passage 2). At this stage, cells were stored under liquid nitrogen.

Colony formation of lymphocytes

Allogeneic human lymphocytes and a B-cell line (OKIB) were used as feeder cells.[15] Lymphocytes from more than three donors, previously separated and stored under liquid nitrogen, were thawed and washed twice by centrifugation, and then were used after exposure to 50 Gy of X irradiation. OKIB, a B-cell line spontaneously established from a leukemia patient, was also used after exposure to 50 Gy of X irradiation. OKIB was verified to be mycoplasma-free.

In each well of a 96-well microplate (round bottom, Costar #3799, Cambridge, Mass), 2×10^4 allogeneic lymphocytes and 1.5×10^4 OKIB cells were seeded in 0.1 mL of growth medium. The medium is made of MEM supplemented with 1% nonessential amino acids, 24 mM HEPES, 1% L-glutamine, 9% FCS, 1% pooled human serum of any ABO type, 1:2000 PHA-P, and 0.2 ng/mL recombinant interleukin-2 (rIL-2), which was kindly provided by Takeda Pharmacy Ltd., Tokyo.[15]

On day –1, feeder cells were plated. On day 0, the lymphocytes to be tested were isolated, counted after staining of mononuclear cells by Türk's solution, diluted with medium devoid of PHA, and added to each well in 0.1 mL of medium. The average number of cells seeded per well was: two cells/well for the 0-Gy and 1-Gy plates, three cells/well for the 2-Gy, five cells/well for the 3-Gy, 20 cells/well for the 4-Gy and 70 cells/well for the 5-Gy. Immediately after the seeding, the plates were exposed to X irradiation. Our previous study showed that lymphocytes irradiated immediately before or soon after adding PHA did not show a significant difference in cloning efficiency.[15] In the present study, cells were X irradiated within one hour after seeding in medium containing PHA. We consider the dose-survival results representative of G_0 lymphocytes. After incubation for one week under humidified conditions with 5% CO_2 and 95% air at 37°C, 0.1 mL of the medium was removed from each well and fresh medium containing a double strength of rIL-2 was added. Two weeks after irradiation, the presence

or absence of lymphocyte colonies was determined for each well by means of an inverted microscope. Cloning efficiency (CE) was calculated using the formula

$$CE = \frac{-\ln \text{ (fraction of wells without a colony)}}{\text{average number of lymphocytes seeded per well}}$$

Survival curves were obtained by fitting the data to a linear-quadratic equation, $-\ln (CE) = aD + bD^2 + c$, using an unweighted least squares method. Based on a and b, the dose required to kill 90% of the cells (D_{10}) was calculated. For the summary figures of the dose-survival curves, the CE at zero dose calculated from the fitted curve was taken as 100% of the surviving fraction, since there was no reason to expect that the zero-dose CE observed was better estimated than that at any other dose.[9,12] More than 80% of the colonies consisted of lymphocytes bearing T helper/inducer surface markers.[19]

Colony formation of skin fibroblasts

Skin fibroblasts stored under liquid nitrogen at passage 2 were thawed and incubated for 2–3 days, and a single cell suspension was prepared by trypsinization before the cells attained confluency. After washing once, the cells were diluted to 1.0×10^5/mL and were exposed to graded doses of X rays at ice-water temperature. After irradiation, cells were serially diluted and plated in three 10-cm dishes per each dose point (100 cells/dish for 0 Gy, 150 cells for 1 Gy, 300 cells for 2 Gy, 600 cells for 3 Gy, 1,500 cells for 4 Gy, and 3,000 cells for 5 Gy). After two weeks of incubation, the dishes were air-dried and Giemsa-stained for colony counting.

X-ray irradiation

A Shimadzu 250-S machine (Shimadzu Co., Kyoto) was operated at 220 kVp, 8 mA with a filter of 0.5-mm Al and 0.3-mm Cu. The dose rate was about 0.25 Gy/min. The total dose was measured for each experiment by a Victoreen 500 dosimeter (Victoreen Inc., Cleveland, Ohio) which had been calibrated at the Research Institute for Nuclear Medicine and Biology, Hiroshima University.

Lymphocyte subset measurement

Isolated mononuclear cells were also subjected to lymphocyte subset measurements.[20] A series of monoclonal antibodies (Becton Dickinson Immunocytometry Systems [BD], Sunnyvale, Calif, and Coulter Immunology, Hialeah, Fla) were employed to measure the various subset frequencies as summarized in Table 1. The helper-to-suppressor ratio (H/S) was determined by Simul-test (BD). A FACS analyzer (BD) was used for these measurements.

Evaluating dispersions of dose-survival data

To compare the dispersion of dose-survival data between the lymphocytes and skin fibroblasts, the absolute value of the difference in the natural log-transformed CE between the observed and the computer fit was calculated for

Table 1. Monoclonal antibodies used for measuring lymphocyte subset frequencies and their corresponding lymphocyte functions

Monoclonal antibodies	Lymphocyte surface antigens	Assigned major lymphocyte subset
$Leu5^+$	$CD2^+$	pan T
$Leu4^+$	$CD3^+$	mature T
$T4^+$	$CD4^+$	helper and inducer T
$T4^+$ $2H4^+$	$CD4^+$ $CD45RA^+$	suppressor inducer T
$T4^+$ $2H4^-$	$CD4^+$ $CD45RA^-$	helper inducer T
$Leu2a^+$	$CD8^+$	suppressor and cytotoxic T
$Leu2a^+$ $Leu15^+$	$CD8^+$ $CD11^+$	suppressor T
$Leu2a^+$ $Leu15^-$	$CD8^+$ $CD11^-$	cytotoxic T
$Leu7^+$	$CD16^+$	natural killer
$Leu11^+$	$CD16^+$	natural killer
$Leu12^+$	$CD12^+$	B

each CE, viz., $y = |\ln CE_{observed} - \ln CE_{fitted}|$. Subsequently, the y values at the six dose levels were summed up for each survival curve giving $Y = \sum_{i=0}^{5} y_i$ where i denotes each dose level (0–5 Gy). For example, when the observed CEs differed by 10% and 20%, either greater or less than the fitted CE at each dose level, Y = 0.57 and 1.09, respectively.

Results

Dose-survival curves of lymphocytes and skin fibroblasts

All of the original CE data is presented in Tables 2 and 3 for lymphocytes and skin fibroblasts, respectively. Examples of the dose-survival curves are shown in Figure 1 with the original data points. These are the cases in which the Y value, the sum of $|\ln CE_{observed} - \ln CE_{fitted}|$ at the six dose levels (see the Materials and Methods section), was smallest (viz., 0.213 in Figure 1A and 0.144 in Figure 1C) and largest (viz., 1.006 in Figure 1B and 0.853 in Figure 1D) among each type of cell examined. Thus, the fit of the remaining 20 curves is between these two extreme cases. The 22 computer-calculated survival curves of the lymphocytes and the fibroblasts are shown in Figures 2A and 2B, respectively. Typical shouldered survival curves were observed for the lymphocytes as previously reported,[14,15] whereas it was less pronounced for the fibroblasts. The calculated D_{10} values of each survival curve are presented in the last columns of Tables 2 and 3 and the distributions are shown in Figure 3. It varied from 3.18–3.98 Gy for the lymphocytes and from 2.53–4.04 Gy for the fibroblasts. The mean ±SD was 3.58 ± 0.21 Gy (coefficient of variation [CV] = 6%) and 3.19 ± 0.37 Gy (CV = 11%), respectively. As is apparent, the variability of the survival curves was greater in the fibroblasts than in the lymphocytes, as seen from Figures 2 and 3.

Table 2. Cloning efficiency and D_{10} value of lymphocytes irradiated at the G_0 stage

Donor code	X-ray dose (Gy)						D_{10} (Gy)
	0	1	2	3	4	5	
Lym #63	0.41	0.35	0.17	0.052	0.014	0.0054	3.18
#64	0.29	0.24	0.11	0.044	0.017	0.0051	3.44
#65	0.53	0.45	0.23	0.11	0.043	0.014	3.76
#67	0.37	0.24	0.17	0.078	0.018	0.0060	3.61
#68	0.40	0.36	0.19	0.058	0.032	0.0074	3.58
#69	0.49	0.38	0.22	0.066	0.021	0.0070	3.33
#70	0.41	0.41	0.15	0.075	0.031	0.0085	3.58
#71	0.43	0.41	0.19	0.11	0.022	0.0096	3.64
#72	0.58	0.45	0.23	0.12	0.041	0.011	3.67
#73	0.46	0.38	0.28	0.087	0.053	0.011	3.92
#74	0.50	0.45	0.27	0.091	0.044	0.012	3.76
#75	0.35	0.35	0.11	0.055	0.014	0.0024	3.26
#76	0.37	0.25	0.17	0.081	0.023	0.0072	3.71
#77	0.44	0.28	0.16	0.072	0.015	0.0072	3.31
#78	0.49	0.43	0.26	0.104	0.032	0.012	3.72
#79	0.41	0.29	0.15	0.072	0.023	0.0088	3.41
#80	0.57	0.44	0.33	0.104	0.040	0.013	3.72
#81	0.55	0.41	0.20	0.099	0.031	0.0072	3.50
#82	0.38	0.30	0.21	0.097	0.034	0.012	3.98
#83	0.49	0.35	0.28	0.094	0.031	0.013	3.73
#84	0.53	0.46	0.20	0.104	0.025	0.011	3.47
#85	0.65	0.35	0.21	0.087	0.033	0.016	3.35

Table 3. Cloning efficiency and D_{10} value of skin fibroblasts irradiated at the log phase

Donor code	X-ray dose (Gy)						D_{10} (Gy)
	0	1	2	3	4	5	
SF #63	0.73	0.49	0.25	0.12	0.053	0.016	3.59
#64	0.79	0.56	0.28	0.14	0.045	0.018	3.52
#65	0.55	0.35	0.15	0.073	0.020	0.0092	3.12
#67	0.60	0.34	0.18	0.076	0.018	0.0071	3.11
#68	0.55	0.28	0.11	0.032	0.0089	0.0023	2.55
#69	0.74	0.45	0.18	0.092	0.029	0.012	3.09
#70	0.70	0.42	0.22	0.11	0.037	0.015	3.43
#71	0.67	0.55	0.31	0.15	0.068	0.033	4.04
#72	0.55	0.33	0.14	0.058	0.023	0.0059	3.09
#73	0.42	0.22	0.089	0.044	0.0098	0.0033	2.87
#74	0.55	0.42	0.22	0.093	0.044	0.017	3.68
#75	0.60	0.32	0.14	0.068	0.022	0.0062	3.07
#76	0.63	0.32	0.16	0.082	0.038	0.014	3.34
#77	0.46	0.23	0.104	0.043	0.014	0.0058	2.86
#78	0.71	0.39	0.24	0.12	0.046	0.020	3.61
#79	0.43	0.18	0.078	0.023	0.011	0.0032	2.53
#80	0.63	0.38	0.22	0.098	0.039	0.015	3.32
#81	0.71	0.31	0.19	0.074	0.032	0.0081	3.17
#82	0.45	0.29	0.14	0.042	0.015	0.0052	2.98
#83	0.23	0.089	0.067	0.025	0.0080	0.0041	3.11
#84	0.59	0.44	0.22	0.079	0.023	0.0096	3.24
#85	0.59	0.35	0.15	0.049	0.015	0.0042	2.82

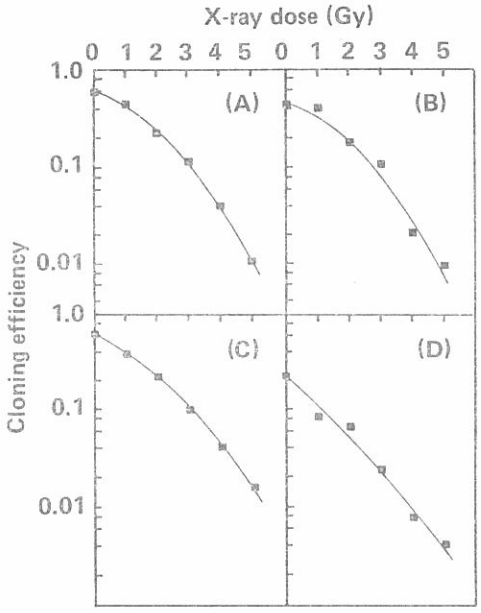

Figure 1. Dose-survival curves of unstimulated lymphocytes (A, B) and skin fibroblasts at the log phase (C, D). (A) and (C) are the best fitted cases and (B) and (D) the least among the lymphocytes (Lym) and skin fibroblasts (SF), respectively, namely Y = 0.213 (A), 1.006 (B), 0.144 (C), and 0.853 (D). (For details, see the text.) The cell strains are Lym 72 (A), Lym 71 (B), SF 80 (C) and SF 83 (D). Original data are presented in Tables 2 and 3.

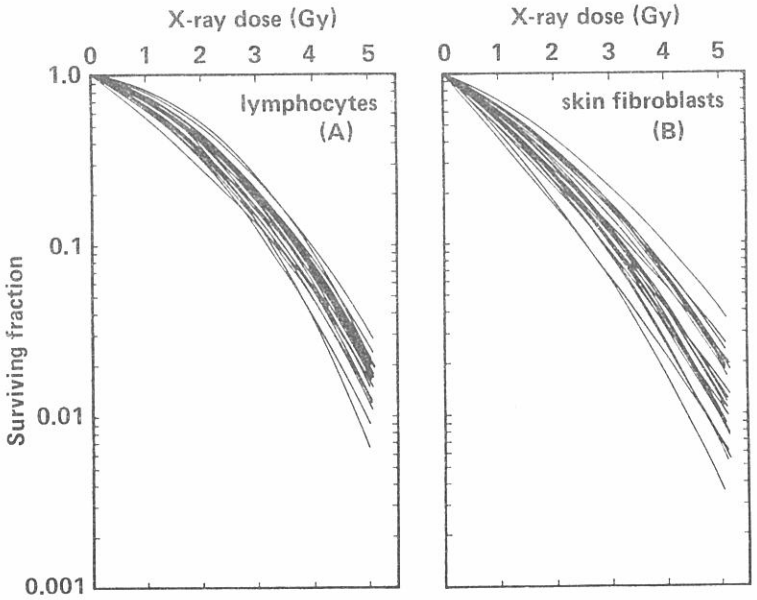

Figure 2. Fitted dose-survival curves of T-lymphocytes (A) and skin fibroblasts (B) derived from 22 individuals irradiated at the G_0 and log phases, respectively.

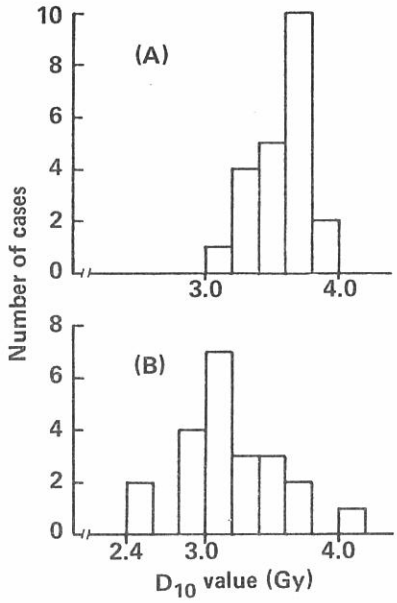

Figure 3. Distribution of D_{10} values for lymphocytes (A) and skin fibroblasts (B).

Comparing dispersions of the survival data for lymphocytes and skin fibroblasts

To clarify whether the apparent greater variability of fibroblast survival curves is due to greater dispersion of the observed CE, deviations of observed CE from the fitted curve were examined. For this purpose, each observed CE was compared to the corresponding fitted value and the ratio provided a measure of how close the two values agreed (Figure 4). The values of this ratio varied from 0.71–1.32 for the lymphocytes and from 0.78–1.26 for the fibroblasts. The mean ±SD was 1.01 ± 0.13 (CV = 12%) for the lymphocytes and was 1.01 ± 0.09 (CV = 9%) for the fibroblasts. Contrary to expectation, the distribution was narrower in fibroblasts than in lymphocytes. For example, only 60% of the lymphocyte CE values fell in the range 0.90–1.10 of the ratio value, whereas 79% did so for the fibroblasts. Likewise, 77% and 93% of the observed CE values were within the range of the ratio of 0.85–1.15 for the lymphocytes and fibroblasts, respectively. A scatter diagram of the value of the ratio by dose did not show any evidence that the ratio varied in any consistent way as a function of the X-ray dose (data not shown).

The better distribution along the fitted curve of the actually observed CE for the fibroblasts than for the lymphocytes is also apparent from the distribution of the Y value for each dose-survival curve (Figure 5). The mean ±SD of the Y value was 0.59 ± 0.25 for the lymphocytes and was 0.42 ± 0.18 for the fibroblasts. This difference was statistically significant (t-test, $p < 0.05$). Therefore, the apparent

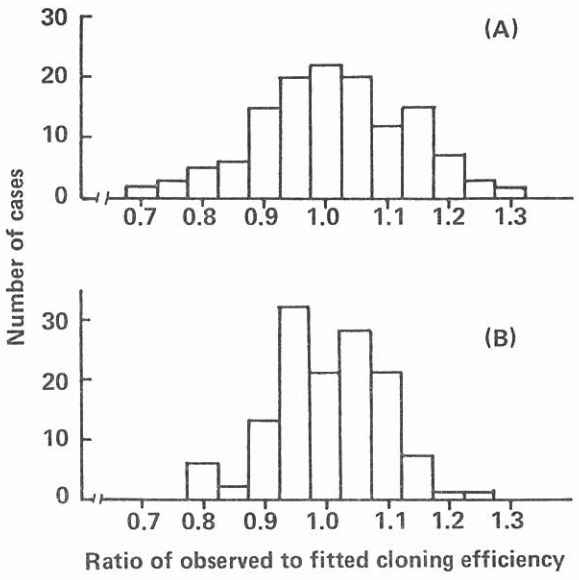

Figure 4. The ratio of the observed to computer-fitted values of CE for lymphocytes (A) and skin fibroblasts (B).

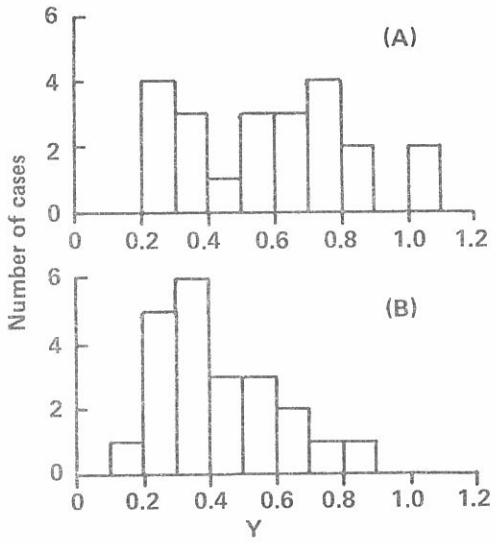

Figure 5. The Y value, the sum of $|\ln CE_{observed} - \ln CE_{fitted}|$ at the six dose levels for each dose-survival curve of lymphocytes (A) and skin fibroblasts (B).

greater variation of the survival curves or of the D_{10} values observed for the fibroblasts was not caused by the greater dispersions of the observed CE in the fibroblast assay itself.

Relationship between the D_{10} value and other factors

To examine possible confounding factors related to the apparent variation in dose-survival curves, the relationship between the D_{10} values and other factors were analyzed. Substantial evidence suggests that suppressor T-lymphocytes are radiosensitive.[21,22] Although it is not yet well understood to which lymphocyte subset such suppressor T cells belong, we suspect that the difference in subset frequency among individuals might affect the dose-survival curve of the lymphocytes. In Table 4, the frequency of the 11 subsets, the helper-to-suppressor ratio, donor age, and sex are shown. No significant correlation was observed between any of the particular lymphocyte subset frequencies and the D_{10} value. No correlation was detected between the D_{10} value and donor age for either lymphocytes and skin fibroblasts, nor between the CE at zero dose and the D_{10} value for lymphocytes. However, for fibroblasts, the regression analysis revealed a positive correlation between the CE at zero dose and the D_{10} value ($p < 0.02$). That is, fibroblast strains with a lower CE at zero dose tended to show smaller D_{10} values.

Table 4. Lymphocyte subset frequency (%) in the 22 patients

Donor code	Subset*											H/S ratio	Age (years)	Sex
	Pan T	Mature T	Th/i	Tsi	Thi	Ts/c	Ts	Tc	NK (Leu7+)	NK (Leu11+)	B			
#63	83.1	74.2	44.7	11.0	33.7	33.1	8.4	24.7	12.8	8.5	14.8	1.52	31	F
#64	83.4	68.5	46.7	2.9	43.8	18.5	1.0	17.5	16.7	11.5	13.7	2.98	62	M
#65	82.9	67.7	49.4	14.5	34.9	25.1	6.9	18.2	15.1	16.3	10.4	2.10	38	F
#67	87.2	65.4	21.9	7.3	14.6	37.2	6.5	30.7	22.3	12.3	10.3	0.56	47	F
#68	75.4	64.9	39.3	17.2	22.1	23.7	7.1	16.6	17.1	10.1	22.9	1.64	24	F
#69	87.2	57.6	40.0	6.3	33.7	17.0	4.9	12.1	32.0	19.6	6.9	3.45	53	F
#70	80.0	62.2	38.3	8.3	30.5	31.3	15.5	15.8	36.6	18.1	6.3	1.35	66	M
#71	83.7	74.2	51.2	18.2	33.0	20.1	8.8	11.3	18.1	6.9	11.5	3.96	20	M
#72	-	62.7	52.9	18.6	34.3	16.9	7.1	9.8	15.6	9.4	11.0	3.63	55	M
#73	-	71.6	56.8	23.7	33.1	13.3	3.8	9.5	8.4	4.2	11.8	5.10	44	F
#74	-	67.2	36.3	11.0	25.3	38.7	12.4	26.3	28.5	7.1	3.7	0.98	52	F
#75	75.6	56.7	42.8	16.1	26.7	14.4	6.3	8.1	27.0	9.9	11.0	3.44	54	F
#76	77.4	68.2	41.1	4.3	36.8	25.6	10.2	15.4	21.5	2.9	7.5	1.65	52	M
#77	71.7	59.3	40.4	7.5	32.9	19.4	5.7	13.7	23.4	11.9	3.4	2.32	70	M
#78	88.3	73.4	52.6	17.5	35.1	21.6	7.7	13.9	21.5	8.7	8.6	2.83	51	F
#79	90.7	84.1	52.6	3.6	49.0	32.7	11.3	21.4	31.4	3.6	8.5	1.66	47	M
#80	78.6	73.4	48.6	11.0	37.6	18.2	7.7	5.7	21.8	13.6	4.7	3.06	72	F
#81	84.1	65.9	45.7	6.6	39.1	21.4	10.1	11.3	19.0	8.0	12.0	2.69	67	F
#82	72.6	62.8	31.2	7.7	23.5	29.2	11.5	17.7	28.0	13.3	13.0	1.10	35	M
#83	82.6	66.9	43.1	6.7	36.4	17.8	3.2	14.6	17.1	7.8	8.3	2.64	43	F
#84	85.1	76.4	44.4	2.6	41.8	35.4	3.8	31.6	14.4	4.2	10.7	1.27	58	M
#85	78.6	69.6	42.7	13.1	29.6	23.8	10.9	12.9	26.5	10.0	8.1	1.98	66	M

*Th/i = helper and inducer T, Tsi = suppressor inducer T, Thi = helper inducer T, Ts/c = suppressor and cytotoxic T, Ts = suppressor T, Tc = cytotoxic T, NK = natural killer, H/S ratio = helper-to-suppressor ratio.

Correlation of D_{10} values for lymphocytes and skin fibroblasts

The D_{10} values of lymphocytes were plotted against the D_{10} values of skin fibroblasts for the 22 individuals (Figure 6). Regression analysis of the D_{10} values for the two cell types did not show a significant correlation ($p > 0.10$).

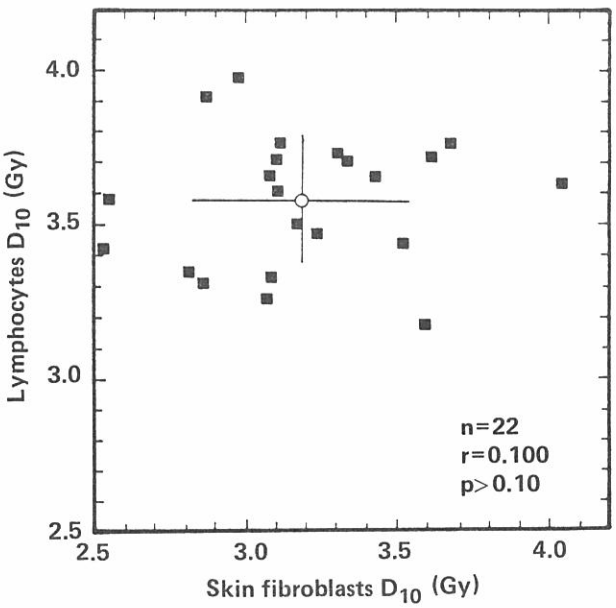

Figure 6. Distribution of the D_{10} values of lymphocytes and skin fibroblasts from 22 individuals (■). The open circle represents the means of each set of D_{10} values and the bars represent the standard deviations of the means.

Discussion

To evaluate the host variation in radiosensitivity using human somatic cells, it is quite important to know the extent of interexperimental variations associated with the assay. If the variations are considerably greater than the real individual variation, it is expected that detecting interindividual variations would be difficult because interexperimental variations are inevitably associated with each examination along with the interindividual variations. Also, if the extent of interexperimental variations were not properly taken into account, the results for individual variations would be overestimated, especially when only a limited number of tests is possible for the screening of a large number of individuals. Repeated examinations of the same cells are very important in this context. Results for the fibroblast strains tested 10 or more times showed that the CV for the mean of the D_0 values (the mean inactivation dose) was 11% for GM495 ($n = 10$),[11] 15% for GM1652 ($n = 10$),[11] 11% for 1BR.2 ($n = 20$),[9] 9% for 1BR.3 ($n = 24$),[9] 17% for 1BR ($n = 15$),[7] and 18% for 2BI ($n = 14$),[7] for an average CV value of 14%.

What is the CV of the D_0 values among the different fibroblast strains? Cox and Masson[23] examined a total of 44 strains and reported that the D_0 value ranged from 0.98–1.60 Gy with a mean ±SD of 1.22 ± 0.17 Gy (CV = 14%) based on at least two sets of dose-survival data for each strain. They also mentioned that "repeat experiments with normal cultures from the same biopsy within and between laboratories and with cultures from two separate biopsies of the same subject may give D_0 variations as great as 25 rad" and suggested that the significance of some outlying D_0 values based on one or two experimental observations was uncertain. In the paper of Arlett and Harcourt,[7] the mean D_0 value ±SD of the cell strains whose survival curves were not significantly different from that of the reference control strain 2BI or 1BR was 1.24 ± 0.16 Gy (n = 26) or 1.39 ± 0.18 Gy (n = 19), and the CV was about 13% for both. Weichselbaum et al[8] reported that the average D_0 value for 22 cell strains including six normal strains was 1.45 ± 0.09 Gy, and the CV was only 6%. Recently, a more extensive report from the same laboratory, however, showed a CV of 19% for the mean of the D_0 values of 24 cell strains from presumably normal individuals.[11] Compared with a CV of 9%–18% for the mean of the D_0 values obtained from repeated examination of several cell strains mentioned above, these results do not unequivocally demonstrate the presence of heterogeneity in individual radiosensitivity. In the present study, we used a different parameter, viz., D_{10}, and the CV was 11% for the mean of the D_{10} values of 22 fibroblast strains. Since only one test was carried out for each cell strain, it is conceivable that the apparent interstrain variation is due largely to random experimental fluctuations.

The lymphocyte results, on the other hand, showed less D_{10} variability (a CV of 6% for the mean), as compared to that for skin fibroblasts. Because CE values observed for the lymphocytes are *not* better distributed along the fitted curves than those for fibroblasts, the different variability between the two cell types cannot be attributed to the difference in the dispersion of survival data. Cole et al[14] also reported survival data for G_0 lymphocytes from nine normal individuals using different methods from ours. Their D_{10} values were distributed between 3.0 and 4.2 Gy, very similar to our present results. However, the CV for the mean of the D_0 values was greater, viz., about 15%, than our present results. This discrepancy may be derived either from the relatively small number of individuals tested by Cole et al or possibly from greater genetic heterogeneity in Caucasians than in Japanese. Recently in our laboratory, more than 100 Japanese were tested for G_0 lymphocyte survival curves and a CV of 7% for the mean of the D_{10} values was observed (Nakamura et al, unpublished observation). Moreover, our recent results for the repeated examination of freshly isolated lymphocytes from a single donor revealed that the mean ±SD of the D_{10} value was 3.66 ± 0.21 Gy (n = 28), and the CV was 6%.[15] Such similar CV values for the mean D_{10} values observed either after repeated assay of lymphocytes from a single donor or after a single assay from many different individuals can be most simply interpreted as showing that individual variation is quite small. Thus, the apparent variation of the lymphocyte survival curves for the present 22 individuals is also mostly

derived from random interexperimental fluctuations. Consequently, it would not be surprising that no significant correlation was observed between the D_{10} values of two types of cells from the same donor.

What is the meaning of the different distribution of D_{10} values for lymphocytes and fibroblasts? Do these values reflect the true variation in human radiosensitivity? Because the mechanisms of tissue-dependent differences in cellular radiosensitivity are not understood, the possibility that the expression of repair function may vary between lymphocytes and skin fibroblasts cannot be excluded at the present time. However, it is worth examining here the possible reasons for different variability in the dose-survival response between the two cell types tested in the present study.

Less interindividual variation observed in the lymphocyte assay might be related to irradiating the cells at the G_0 stage, because recovery from potentially lethal damage would take place to a greater extent in these cells than in those irradiated at log phase. Consequently, interindividual variation might have been diminished. A recent study by Cole et al[14] showed that the variation of D_0 values for G_0 T-lymphocytes from nine individuals and for T-lymphocyte lines at log phase from nine different normal individuals was quite similar. Thus, it appears that variability of radiosensitivity is not greatly affected by the specific stage of the cell cycle at the time of irradiation. However, because the two types of lymphocytes reported by Cole et al[14] were not derived from the same individuals, a comparative study of cells from the same individual is required. Such a study is now being planned in our laboratory.

It is unlikely that G_0 lymphocytes in our assay die via interphase death, thus masking interindividual variation. This is assumed primarily because the surviving fraction of G_0 lymphocytes irradiated and incubated for up to two days before PHA stimulation remained essentially unchanged (Nakamura, unpublished observation). In addition, G_0 lymphocytes from AT patients are as sensitive as skin fibroblasts,[13–15] and the RBE for ^{252}Cf neutrons was 3.0 or greater for lymphocyte killing (Nakamura, unpublished observation)—similar to the values reported for other cultured cells[24] for which mitotic death is the common mechanism.

As for skin fibroblasts, the greater variation in D_{10} values in the present study might be related to their use at a very early passage. We used a collagen gel for the primary culture of skin pieces and keratinocyte sheets were frequently observed. Usually, these cells hardly grow after dispersion into single cells on plastic dishes, whereas some may have remained in the culture at passage 2, as used in the present study. Therefore, different degrees of keratinocyte contamination might have increased the interstrain variation. This might also explain the continuously bending nature of some survival curves for the present fibroblast strains, because fresh keratinocytes are reported to show a shouldered curve[25] whereas many survival curves reported for the fibroblasts at

more advanced passages are close to linear.[7–9,11] If this were the case, we would expect that the present fibroblast strains would show survival curves closer to linear, as well as less interstrain variation as the passage level increases; such a study is currently in progress. Other researchers have suggested that the CE[26] and passage level[27] affect fibroblast radiosensitivity. However, no systematic effect of passage level, CE, serum lot, age, or sex of the donor on dose response was recently demonstrated.[11] The positive correlation between CE and D_{10} value observed in our 22 strains of fibroblasts is not yet understood.

Apart from interexperimental fluctuations of the fibroblast assay, several cell strains are reported to show significantly different dose-survival curves after repeated tests, e.g., strains 1BR and 2BI.[7] This may be due to unidentified genetic factors, as suggested,[11] or to biases introduced during an early passage of the cell strains. In addition, the radiosensitivity of skin fibroblasts derived from different sites may not be identical. Jacobs et al[28] compared the sensitivity of lung- and skin-derived fibroblasts from the same fetal material obtained from therapeutic abortions using N-methyl-N'-nitro-N-nitrosoguanidine as a mutagen. The dose-survival curves for the six matched strains showed that variation of the D_{10} value is considerably greater in skin fibroblasts than in lung fibroblasts (a CV of about 23% and 12%, respectively). Even more pronounced differences were observed for the induction of 6-thioguanine-resistant mutations. Clearly, greater refinement in fibroblast characterization is needed. Lymphocytes, however, can be defined at the molecular level by a series of monoclonal antibodies specific to different surface markers and thus may provide a more precise evaluation of possible interindividual variation in human radiosensitivity.

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