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● *Brief Communication*

RADIOSENSITIVITY *IN VITRO* OF HUMAN FIBROBLASTS DERIVED FROM PATIENTS WITH A SEVERE SKIN REACTION TO RADIATION THERAPY

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Skin fibroblasts derived from six cancer patients who showed an unusually severe skin reaction to radiation therapy also showed enhanced radiation sensitivity *in vitro*.

Radiosensitivity, Skin fibroblasts, Cancer patients.

INTRODUCTION

Occasionally a course of radiation therapy must be abandoned or protracted because a patient demonstrates an unusually severe skin reaction at the site of therapy. One possible explanation is that these patients have a genetic defect that renders their cells more sensitive to ionizing radiation.

To help explain the unusual radiation sensitivity of the skin of certain radiation therapy patients at Stanford (~ 0.8% of 4100 cancer patients surveyed), we undertook to grow skin fibroblasts from cancer patients who had "normal" and abnormal skin reactions to radiation therapy. We then determined the X-ray sensitivity of these cells *in vitro;* our results are presented in this report.

METHODS AND MATERIALS

Two-millimeter diameter skin biopsies were obtained with informed consent from the buttocks of patients who had a markedly abnormal skin response during the course of a standard scheme of fractionated radiotherapy for malignant disease, and from two patients who had a "normal" skin response.

All cell strains were grown initially as uncloned populations in monolayers in plastic Petri dishes or Falcon flasks at 37°C in a 5% CO_2 humified atmosphere. Tissue culture medium was composed of Eagle's Minimal Essential Medium (MEM)* supplemented with 20 ml MEM essential amino acids (50x)*, 20 ml MEM nonessential amino acids (100x)*, 10 ml MEM vitamins (100x)*, 20 ml MEM sodium pyruvate (100x)*, 2 ml Gentamicin (50 mg/ml) and 25% fetal calf serum. Uncloned cells were used between the 4th and 8th passages (4:1 dilution per passage). Isolation of clones derived from individual cells was performed in the 5th and 7th passages.

Irradiation and assay for survival

Irradiation was carried out prior to subculture to measure survival. After subculturing, cells were counted in a Coulter counter, and appropriate numbers were plated in 60 mm plastic Petri dishes to determine their cloning ability. These dishes were then placed in the incubator with minimum disturbance; 10–12 days later they were fixed, stained, and colonies with 50 or more cells were counted. Experiments were performed with and without feeder layers. For the latter experiments, some of the cells were irradiated with 3000 rad. Enough radiation-killed cells were added to each dilution of experimental cells to yield approximately 2–4 $\times$ 10^5 cells/dish. Heavily irradiated cells by themselves did not give rise to colonies. Survival results with or without feeder layers were within experimental error.

Cells were irradiated with a 85 kVp X-ray machine (9.6 mA, 1.5 mm Al filter) at a dose rate of approximately 130 rad per minute at room temperature on a rotating platform.

RESULTS

Skin fibroblasts from the two patients with neoplastic disease who had a "normal" skin reaction to radiation therapy showed a "normal" response to X-radiation *in vitro* (Fig. 1).

Skin fibroblasts from three of the five patients (S_3, S_4,

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*GIBCO: Grand Island Biological Co.

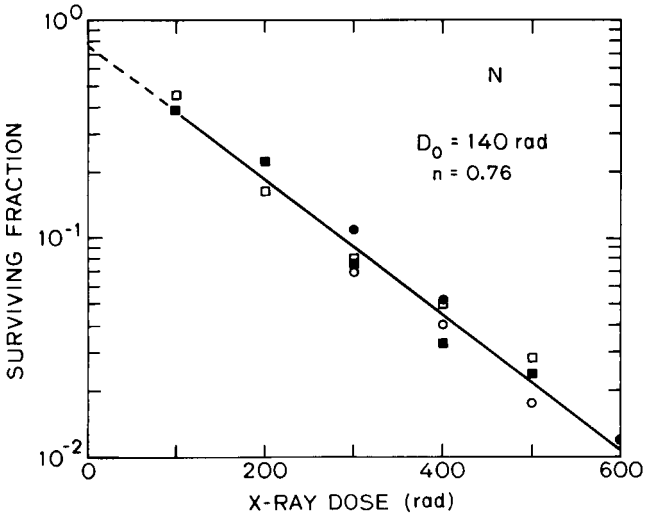

Fig. 1. X-ray survival curves of skin fibroblasts derived from two cancer patients who had a "normal" skin reaction at the site of radiation therapy. The solid symbols (●) (■) indicate results of two experiments on N_1 cells; the open symbols (○) (□) on N_2 cells. The line has been fitted by regression analysis (r^2 = 0.9703).

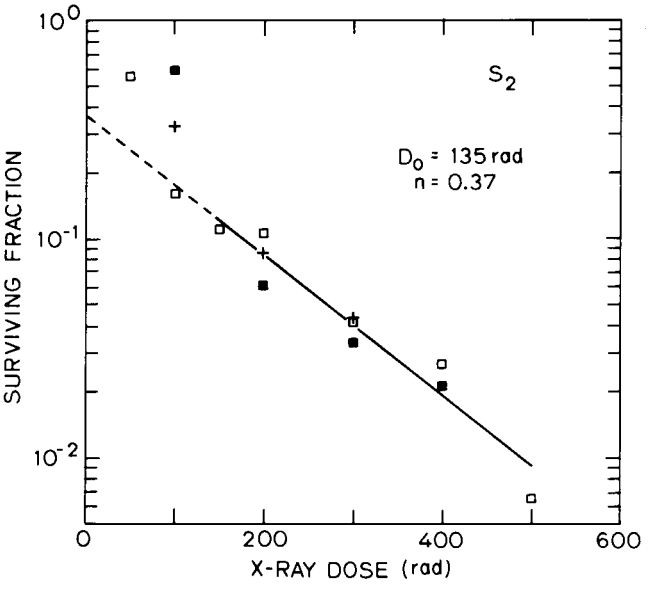

Fig. 2. X-ray survival curves of skin fibroblasts derived from a cancer patient who had an unusually severe skin reaction at the site of radiation therapy (S_2). The curve (□) was run on 7/22/77. The points marked by (■) were run 3/22/78. The sample that had been exposed to 200 rad was regrown and a survival curve (+) was again deterrmined (4/2/78). The line was fitted by regression analysis to all the data points between 150 and 600 rad (r^2 = 0.9320).

S_6) who had an unusually severe reaction to radiation therapy showed extrapolation numbers (n) near 1.0, but much reduced values of D_0 (dose to reduce the survival to 37% on the exponential part of the survival curve) (Table 1), and three showed greatly decreased values of n (S_1, S_2, S_5), with two of these (S_1, S_5) also showing reduced values of D_0 (Table 1 and Figs. 2–4).

In one experiment, the cells from patient S_2 were irradiated with 200 rad ($\sim 10^{-1}$ survival) and regrown; a full survival curve on these cells also showed a biphasic survival curve, indicating that about 60% of the population was quite X-ray sensitive (Fig. 2).

Cells from patients S_2 and S_5 were cloned in an attempt to obtain pure populations of both sensitive and resistant cells. However, the radiation response of cells that were derived from four such clones also showed a biphasic response (data not shown).

DISCUSSION

Weichselbaum *et al.*[1] published results on a study similar to ours, however, they concluded that fibroblasts from their "sensitive" patients showed "normal" survival curves. They studied one "resistant" patient and three "sensitive" patients. The cells from the "resistant" patient had an n of 1.04 and a D_0 of 120 rad. The cells from the three "sensitive" patients had n values of 0.64, 0.69 and 1.27, and D_0 values of 108, 157, and 101 rad, respectively. To us the n values of 0.64 and 0.69 were suggestive of two cell populations with differing radiation sensitivities, and the D_0 values of 108 and 101 rad

Table 1. *In vitro* and clinical data

Patient	n	D_0 (rad)	Plating efficiency (%)	Clinical sensitivity[1]	Complexion	Eyes	Sun sensitivity	Area of origin	Age
N_1	0.76[4]	140[4]	6.8[4]	0	Light	Brown	Normal	England	47
N_2				0	Light	Blue	Normal	Scand.	53
S_2	0.37[5]	135[5]	6.4[5]	IV	Light	Blue	Sensitive	No. Europe	46
S_5	0.39	121	13.3	I	Light	Green	Sensitive	Germany	32
S_1	0.58[5]	118[5]	11.4[5]	I	Light	Blue	Sensitive	Scand.	50
S_3	1.05	128	24.3	III	Average	Brown	Normal	(not available)	68
S_4	1.12	110	19.4	II	Average	Blue	Normal[2]	England	47
S_6	1.1	118	7.6	I	Light	Blue	Sensitive[3]	No. Italy	50

[1]Skin response to radiation therapy; 0 = normal skin reaction; I = most severe; IV = least severe.
[2]Father's skin very sun sensitive.
[3]Has developed skin cancer.
[4]Average values for patients N_1 and N_2, with two experiments on each.
[5]Average values for several experiments.

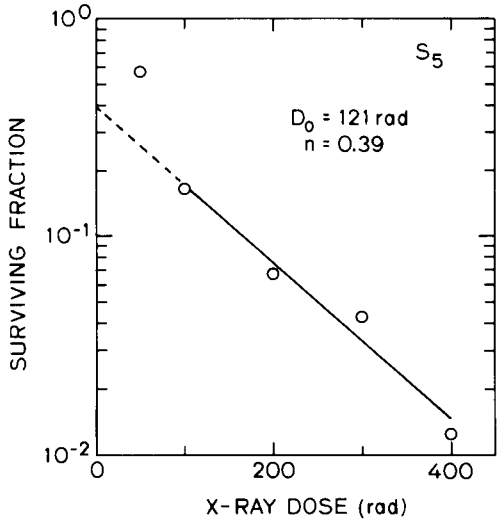

Fig. 3. X-ray survival curve of skin fibroblasts derived from a cancer patient who had an unusually severe skin reaction at the site of radiation therapy (S_5). The line was fitted by regression analysis to the data points between 100 and 400 rad (r^2 = 0.9695).

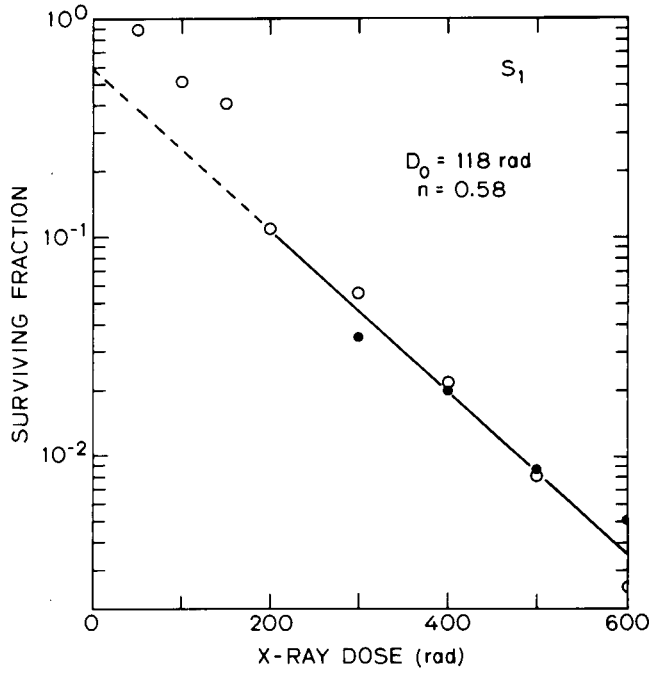

Fig. 4. X-ray survival curve of skin fibroblasts derived from a cancer patient who had an unusually severe skin reaction at the site of radiation therapy (S_1). The points (●) were from an experiment on 3/4/77, the other points (○) are from 6/2/77. The pooled data points between 200 and 600 rad were fitted by regression analysis (r^2 = 0.9496).

suggested enhanced radiation sensitivity. This interpretation of the results of Weichselbaum *et al.*[1] encouraged us to continue our own sudies.

Cells from three of our sensitive patients (S_1, S_2, S_5) showed biphasic survival curves, resulting in *n* values that were much less than 1.0 (Figs. 2–4). Cells from two of these patients (S_1, S_5) also showed decreased D_0 values (Table 1). The observation that cell populations derived from single cells from two of these patients (S_2, S_5) also showed biphasic radiation responses (data not shown) indicates that our biphasic survival results are not artifacts of a mixed cell population. This was also confirmed by a re-irradiation experiment (Fig. 2). Cells from three of our sensitive patients showed normal values of *n* (S_3, S_4, S_6), but each had D_0 values that were less than normal (Table 1).

There are two measures of radiation sensitivity *in vitro:* the fraction of cells showing an unusual response as revealed by *n* values, and the D_0 of the resistant portion of the survival curve. Cells from *all* of our sensitive patients showed either a lower D_0 or a lower extrapolation number than the "normal" controls. Thus, their enhanced radiation sensitivity *in vitro* correlates with their enhanced sensitivity to radiation therapy. It is interesting to note that the survival curves for the cells from patients S_5 and S_1 show a decrease both in *n* and D_0, and they fall into the group showing the most severe skin reaction clinically. We can speculate that different genetic defects influence the radiation sensitivity of these cells, one perhaps being an unusually X-ray sensitive portion of the cell cycle, the other manifesting itself as a low D_0. We have too few patients to draw firm conclusions, but we have exhausted the supply of patients at this hospital.

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REFERENCE

1. Weichselbaum, R.R., Epstein, J., Little, J.B.: *In vitro* radiosensitivity of human diploid fibroblasts derived from patients with unusual clinical responses to radiation. *Radiology* **121:** 479–482, 1976.