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 effect that renders their cells more sensitive to ionizing radiation.

To help explain the unusual radiosensitivity 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% CO₂ humidified 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 × 10⁵ 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 (S₁, S₂,
S₀) who had an unusually severe reaction to radiation therapy showed extrapolation numbers (n) near 1.0, but much reduced values of D₀ (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 (S₁, S₃, S₄), with two of these (S₁, S₂) also showing reduced values of D₀ (Table 1 and Figs. 2-4).

In one experiment, the cells from patient S₂ were irradiated with 200 rad (~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 S₁ and S₃ 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 n of 1.04 and a D₀ of 120 rad. The cells from the three “sensitive” patients had n values of 0.64, 0.69 and 1.27, and D₀ 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 D₀ values of 108 and 101 rad

<table>
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<tr>
<th>Patient</th>
<th>n</th>
<th>D₀ (rad)</th>
<th>Plating efficiency (%)</th>
<th>Clinical sensitivity¹</th>
<th>Complexion</th>
<th>Eyes</th>
<th>Sun sensitivity</th>
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<td>6.4⁵</td>
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<td>11.4⁵</td>
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</table>

¹Skin response to radiation therapy; 0 – normal skin reaction; 1 – most severe; IV – least severe.
²Father’s skin very sun sensitive.
³Has developed skin cancer.
⁴Average values for patients N₁ and N₂, with two experiments on each.
⁵Average values for several experiments.
suggested enhanced radiation sensitivity. This interpretation of the results of Weichselbaum et al.\textsuperscript{1} encouraged us to continue our own studies.

Cells from three of our sensitive patients (S\textsubscript{1}, S\textsubscript{2}, S\textsubscript{3}) 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\textsubscript{1}, S\textsubscript{3}) also showed decreased $D_0$ values (Table 1). The observation that cell populations derived from single cells from two of these patients (S\textsubscript{2}, S\textsubscript{1}) 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\textsubscript{2}, S\textsubscript{4}, S\textsubscript{5}), but each had $D_0$ values that were less than normal (Table 1).

There are two measures of radiation sensitivity \textit{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 \textit{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\textsubscript{4} and S\textsubscript{5} 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.

\textbf{Acknowledgement}—We thank Mr. Douglas P. Strande and Ms. Ine Van Kersen for their excellent technical assistance.

\textbf{REFERENCE}