Response of Human Tumor Cells of Varying Radiosensitivity and Radiocurability to Fractionated Irradiation

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ABSTRACT

The cytotoxic effects of radiation delivered in daily fractions of 2.0 Gy were examined in plateau phase cultures of human tumor cells of varying in vitro radiosensitivity, derived from tumors of varying radiocurability. Among the eight cell lines examined, three types of responses to fractionated irradiation were observed. In the group composed of tumor cells lines that were radiosensitive in culture (D0 > 2Gy) and derived from known local radiation failures or from tumor histologies associated with radiation failure, a gradual linear reduction in surviving fraction versus total dose was observed. In a second group, composed of cell lines that were radiosensitive in culture (D0 = 1 Gy) but derived from known radiation failures, the surviving fraction initially declined and began to plateau after 6 Gy (three fractions of 2 Gy). In the third group, composed of radiosensitive cell lines derived from tumors associated with high radiocurability, a rapid decline in surviving fraction versus total dose was observed. The in vitro response of human tumor cells to fractionated irradiation delivered at clinically relevant doses appears to be independent of in vitro X-ray sensitivity and p53 status but related to clinical radiocurability, suggesting a possible role in predicting tumor response to radiotherapy.

INTRODUCTION

There has been considerable interest in the development of in vitro techniques for predicting the response of malignant tumors to clinical radiotherapy. On the basis of studies with human tumor cells isolated from surgical specimens, it has been proposed that single-dose graded X-ray survival curves may be useful in predicting patient tumor response because of the wide range of radiobiological parameters that can be measured in vitro. Thus parameters such as SF2, D, and the α/β ratio may correlate with clinical radiosensitivity of the tumor from which the cell lines were derived (1–4). However, studies comparing the survival of tumor cells to single doses of radiation in vitro with the clinical response in vivo have failed to show a correlation when the radiosensitivity of the tumor from which the cells were derived is known (5, 6).

It is likely that the radiosensitivity of human tumors may be dependent on a number of factors other than inherent cellular radiosensitivity that would be operative when solid tumors are irradiated in situ. For example, single-dose in vitro survival curves cannot account for factors such as hypoxic regions in tumor tissue, sublethal and potentially lethal damage repair, redistribution within the cell cycle and repopulation, which occur after fractionated irradiation in vivo. Loss of function of the tumor suppressor gene p53 has also been associated with an increase in both radiation resistance (7–9) and radiation of function of the tumor suppressor gene.

We have shown previously that the radiosensitivity in vitro of skin fibroblasts derived from patients with unusually severe clinical responses to radiotherapy as well as ataxia telangiectasia heterozygotes may be distinguished from that of fibroblasts derived from normal individuals by use of a fractionation assay in confluent, density-inhibited cultures (12). In the present investigation, we examined the effects of X-ray irradiation at clinically relevant daily doses similar to those used in fractionated radiotherapy in plateau phase cultures of human tumor cells of varying radiosensitivity derived from tumors of varying radiocurability. This study was undertaken to determine whether slight differences in the sensitivity to single-dose irradiation in vitro between cell lines from tumors of differing clinical curability and with normal or abnormal p53 may be enhanced by fractionated irradiation in plateau phase cultures. Such cultures contain a large fraction of quiescent or very slowly proliferating cells and thus have significant similarities in proliferation kinetics to human tumors in vivo (13, 14).

MATERIALS AND METHODS

Cells and Culture Conditions. The source and tumor histology for each cell line used in this study are listed in Table 1. The cells were grown in Eagle’s minimal essential medium (Lifs Technologies, Gaithersburg, MD) supplemented with 10–20% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), penicillin (50 units/ml), and streptomycin (50 µg/ml), with or without hydrocortisone (0.4 µg/ml). The cells were maintained at 37°C in a humidified 5% CO2/95% air atmosphere.

Cells used for experiments were grown to plateau phase in 60-mm dishes. The culture medium was then changed daily for 3 days. For the third and final medium change, growth medium was replaced with medium containing 0.2–1.0% fetal bovine serum. At this time, the percentage of cells in S-phase as determined by pulse labeling with [3H]thymidine and autoradiography was less than 8%.

Single-Dose and Fractionated-Dose X-Ray Survival. For the determination of graded single-dose survival curves (0–10 Gy), cultures were irradiated 24 h after the last medium change, subcultured immediately after irradiation, and seeded at low density in complete medium with 15% serum to determine cell survival by a standard colony formation assay. For fractionation experiments, replicate cultures were irradiated (0.5–4.0 Gy fractions) daily beginning 24 h after the last medium change. After irradiation, one dish was subcultured immediately to determine cell survival, and the remaining dishes were returned to the incubator. This fractionation protocol was continued for up to 6 days with 24-h intervals between irradiations. Replicate, nonirradiated cultures of each cell line, grown and maintained in the same manner, were subcultured with each daily fraction to measure cloning efficiencies. The cultures were left in medium containing 0.2–1% serum, and this culture medium was not renewed during the course of the fractionation experiments. The cloning efficiencies of the nonirradiated cultures remained constant. To determine cell survival, cells were subcultured and seeded into 100-mm dishes at two cell densities per dose designed to yield ~50–100 viable colonies. The dishes were returned to the incubator for 14–21 days, after which macroscopic colonies were stained and counted.

Irradiations were performed with a Philips MCN 165 industrial X-ray generator (Philips Electronic Instruments Company, Alpharetta, GA) operated at 160 kVp and 18 mA and delivering a dose-rate to the cells of 0.74 Gy/min. The data from two to four separate experiments were pooled to obtain the survival data presented for each cell line. The survival parameter D0, was obtained by linear regression analysis of the single-dose survival curve data. D was derived by use of a linear quadratic model with α and β values. The SF2 values were taken directly from the survival data.

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Western Analysis. p53 protein expression was assayed by determining the protein level in irradiated and control cultures by Western blotting. Whole-cell protein was extracted 3 h after irradiation (4 Gy) of cells in plateau phase by lysis of the cells in a tridetergent protein lysis buffer. Western blot analysis was performed by standard procedures with the use of anti-p53 (Ab-6), anti-p21WAF1 (Ab-1), and anti-β-tubulin (Ab-1; Oncogene Research Products, Cambridge, MA).

RESULTS

The p53 status of each of the cell lines used in this study, as determined by Western analysis of p53 expression in control and irradiated cells and radiation-induced transactivation of its downstream effector p21WAF1, is shown in Table 2. Representative Western blots of cell lines U1-Mel, Tera-1, Tera-2, SQ-20B, JW-1T, and GL-13 are shown in Fig. 1. Compared with nonirradiated control cells, p53 and p21WAF1 levels were increased after exposure to 4 Gy in cell lines Tera-1, Tera-2, and SCC-61, indicating normal induction of both p53 and p21WAF1. In nonirradiated cultures of GL-13, SQ-20B, and U1-Mel, p53 levels were elevated, and these levels did not increase after exposure to 4 Gy, implying loss of normal p53 function. This was confirmed by the lack of induction of its downstream effector, p21WAF1, in these cell lines. Cell line JW-1T showed no expression of p53 but significant induction of p21WAF1 expression by radiation.

The single-dose survival curves for all of the human tumor cell lines irradiated in plateau phase and immediately subcultured and replated at low cell density are shown in Fig. 2A. The radiobiological survival parameters for these cell lines are shown in Table 2. The D0 (inverse of the slope) values ranged from 1.04 to 2.67 Gy. The D0 values ranged from 1.07 to 4.18 Gy, and the SF2 values ranged from 0.146 to 0.776. The α/β values ranged from 4.2 in the U1-Mel cell line to 131.1 in the Tera-2 cell line.

The response of human tumor cells to fractionated radiation delivered in daily doses of 2 Gy are shown in Fig. 2B. Three general responses to fractionated radiation were observed. In the highly radioresistant (D0 > 2 Gy) cell lines U1-Mel, SQ-20B, and T98G there was a gradual linear reduction in surviving fraction versus total dose. All three of these cell lines were derived from tumors that had local radiation failure. In three radiosensitive cell lines, SCC-61, GL-13, and JW-1T, the surviving fraction declined initially and then began to plateau after ~6 Gy (three fractions of 2 Gy). All three of these cell lines were derived from tumors with local radiation failure. In the radioresistant cell lines Tera-1 and Tera-2, there was a rapid linear decrease in surviving fraction versus total dose. The fractionation curves for these two cell lines were similar to the response observed in the single-dose graded survival curves (Fig. 2A). Interestingly, these two cell lines were derived from tumors that are highly radioresistant (embryonal carcinoma).

Fig. 3 compares the fractionation response of cell lines GL-13 and

![Graph showing fractionation response](image)
Tera-2 given daily doses of 2 Gy. Although the inherent radiosensitivity as determined by single-dose graded X-ray survival curves is quite similar, the response of these two cell lines to fractionated radiation is quite different.

Inherent radiosensitivity (single-dose graded survival curves) and fractionated radiation, delivered in daily fractions of 0.5–4.0 Gy in cell lines GL-13, SCC-61, and Tera-1 are compared in Fig. 4. After 1- or 2-Gy fractions, the fractionation curves in cell lines GL-13 and SCC-61 begin to plateau after three fractions. After a total dose of 6 Gy (six fractions of 1 Gy or three fractions of 2 Gy), the surviving fraction was quite different from single doses of 6 Gy in cell lines GL-13 and SCC-61. With doses of 3 Gy per fraction, the fractionation curves became more linear compared with lower doses per fraction, and the slope of the fractionation curves began to approach that of the single-dose survival curves after 4 Gy per fraction in cell lines GL-13 and SCC-61. However, in cell line Tera-1, the response to fractionated-doses of radiation appears to be independent of fraction size. The response of Tera-1 cells given 0.5-, 1-, or 2-Gy daily fractions was similar to the single-dose graded X-ray survival curve.

Fig. 5 depicts the fractionation response of cell lines SQ-20B, GL-13, and Tera-1 to daily fractions of 2 Gy. The solid lines represent the theoretical fractionation curves that would result if the surviving fraction after the first 2-Gy fraction was raised to the \( n \)th power (\( n \) = number of 2-Gy fractions). As can be seen, the theoretical fractionation curves for cell lines SQ-20B and Tera-1 are similar to the actual fractionation data. In cell line GL-13, the actual fractionation curve began to plateau after three fractions of 2 Gy and was clearly different from the theoretical curve. These data demonstrate that there was equal cell kill by each 2-Gy fraction in cell lines SQ-20B and Tera-1. In cell line GL-13, the surviving fraction versus total dose began to plateau after three fractions (6 Gy total dose), and the cells appeared to be refractory to additional doses of 2 Gy. This phenomenon was also apparent in cell lines SCC-61 and JW-1T (Fig. 2B).

**DISCUSSION**

Fractionated radiation plays an integral role in the management of human cancer. It was determined empirically that radiation delivered in small fractionated doses would produce less damage to normal tissue, providing greater tumor control than radiation delivered in large single doses. A basic assumption was that each dose in fractionated radiation would result in equal cell kill to the tumor cells being irradiated.

It has been proposed recently that inherent radiosensitivity and clinical radioreponse may correlate with the status of the p53 tumor suppressor gene. It is generally accepted that cells from normal tissues expressing mutant p53 are more radiation resistant than cells expressing wild-type p53 (8, 15), a finding consistent with observations in a transplantable tumor system *in vivo* (16). On the other hand, studies with human tumor cells suggest that radiation sensitivity may be independent of p53 status (17–20), and increased radiation sensitivity or prolonged patient survival have been reported in some bladder, head and neck, and glioblastoma tumors that express mutant p53 (10, 11, 21). Contradictory reports of p53 status and radioreponse may be due in part to the techniques (Western analysis and DNA sequencing
versus immunohistochemical detection) used for the determination of p53 status (22).

In the present study, we examined the response of plateau phase cultures of human tumor cells with differing p53 status, varying in vitro radiosensitivity, and clinical radiocurability to fractionated irradiation. The cell lines that were highly radioresistant to single, graded doses of X-ray irradiation (D$_0$ > 2 Gy) were derived from tumors that had local clinical radiation failures and expressed abnormal p53. These cell lines were also resistant to radiation delivered in multiple fractions. Among the tumors that were sensitive to single, graded doses of X-ray irradiation (D$_0$ ≈ 1 Gy), three cell lines (Tera-1, Tera-2, and SCC-61) expressed normal p53, and one cell line (GL-13) expressed abnormal p53. Two of the sensitive cell lines (Tera-1 and Tera-2), which expressed normal p53, demonstrated a rapid decline in surviving fraction versus total dose of fractionated radiation and were derived from tumors that are highly radiocurable (embryonal carcinoma). One sensitive tumor cell line (SCC-61), derived from an unusually aggressive tumor that enlarged during standard fractionation treatment, expressed normal p53, and the surviving fraction versus total dose began to plateau after multiple doses of fractionated radiation. The radiosensitive cell line GL-13, also derived from a tumor that had local radiation failure, expressed abnormal p53, and the surviving fraction versus total dose began to plateau after multiple doses of fractionated radiation.

The cell line JW-1T, derived from a glioblastoma tumor, showed no p53 expression, but p21$^{WAF1}$ expression was enhanced following X-ray irradiation, suggesting an alternate pathway for p21$^{WAF1}$ induction (23, 24). JW-1T demonstrated a refractory response to multiple doses of fractionated radiation, similar to cell line GL-13. The refractory response observed in these radiosensitive cell lines may be overcome by using higher doses per fraction than that used in standard fractionated radiotherapy. When SCC-61 and GL-13 cells were irradiated with 3-Gy fractions, the magnitude of the refractory response decreased versus total dose of radiation. When SCC-61 and GL-13 cells were irradiated with 4-Gy fractions, the slope of the fractionation curve began to approach that of the single-dose survival curves, suggesting that therapeutic radiation should be delivered to the tumor at the highest doses per fraction using multiple radiation fields or intraoperative radiotherapy. The possibility exists that the 4 Gy/fraction curves may also begin to plateau after three fractions of radiation, but the resulting surviving fractions would be at the lower limits of what can be measured using colony-forming assays.

Therapeutic radiation delivered in smaller fractions over a shorter time period (hyperfractionation) than standard fractionation protocols...
may provide increased tumor control with decreased late normal tissue injury (25–27). Haas-Kogan et al. (9) suggested, based on studies with isogenic derivatives of glioblastoma cells, that tumors that express wild-type p53 may benefit from hyperfractionation regimens, whereas tumors that express mutations in p53 should be treated with fewer but larger fractions of radiotherapy, a finding supported by this study.

Our findings indicate that among the eight human tumor cell lines examined, cells that expressed abnormal p53 were generally more resistant to radiation delivered as either single or fractionated doses. Two cell lines, both derived from a tumor histology considered highly resistant to radiation delivered as either single or fractionated doses, were evaluated. Two cell lines, both derived from a tumor histology considered highly resistant to radiation delivered as either single or fractionated doses. Two cell lines, both derived from a tumor histology considered highly resistant to radiation delivered as either single or fractionated doses.

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