In Vitro Radiation Response of Cells from Four Human Tumors Propagated in Immune-suppressed Mice

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ABSTRACT

Two recently developed clonogenic assays for human tumor cells have been used to measure the in vitro radiation cell survival of four human tumors, a pancreatic carcinoma, a colonic carcinoma, an oat cell carcinoma of the lung, and a melanoma, propagated as xenografts in immune-suppressed mice. The slopes and shoulders of the survival curves for the first three tumors were all similar with D50's, respectively, of 94, 100, and 131 rads and with D50's, respectively, of 8, 44, and 41 rads. However, melanoma cells from the fourth tumor had a survival curve that differed from those of the other three, both in having a wider shoulder with a D50 of 216 rads and in having a shallower slope with a D50 value of 183 rads. It is suggested that the wide shoulder to the melanoma cell survival curve may in part explain the poor response to small fractionated doses of radiotherapy usually observed clinically for this tumor type. However, the data from the other three tumors suggest that differences in radiotherapeutic response seen in the clinic for these tumors cannot be attributed to differences in intrinsic radiosensitivity of the tumor cells.

INTRODUCTION

Human tumors show a wide range of clinical response to radiotherapy, but the underlying biological basis for this remains uncertain. The in vitro radiosensitivities of cells from many different experimental animal tumors and tumor cell lines under aerobic conditions have been found nearly always to lie within a fairly narrow range (2, 20, 21). This suggests that differences in the intrinsic radiosensitivity of tumor cells as measured in these in vitro studies are not a major factor in determining differences in therapeutic response and that instead extrinsic factors including, for example, tumor cell hypoxia are probably of much greater importance (6, 10).

Until now clonogenic assay techniques for cells taken directly from human solid tumors have not been available, and the radiobiological data on which the interpretation of clinical response to radiotherapy is based have of necessity been extrapolated from cell lines established from human tumor tissue grown in culture (1, 13, 19, 20) or from experimental animal tumor models (2, 8, 16, 18). Recently, we have developed 2 clonogenic assay methods for cells from human tumors propagated as xenografts in immune-suppressed mice (4, 17). We have used these assays to study the in vitro radiosensitivities of cells from 4 human tumors of types that show considerable differences clinically in their response to radiotherapy and to assess whether the similarities in intrinsic radiosensitivity already established for other mammalian cell types are also found for cells from human tumors.

MATERIALS AND METHODS

Human Tumor Xenografts. Three of the xenografts used in these experiments (HX18, HX32, and HX33) were grown from human biopsy material obtained at surgery and immediately inoculated s.c. in the flank of syngeneic CBA/Lac mice, immune-suppressed by thymectomy, whole-body irradiation, and marrow transplantation, as described by Pickard et al. (12). The fourth, melanoma HX34, was grown first in short-term culture to obtain sufficient cells for inoculation and then passaged continuously as a tumor in similarly prepared mice. Details of the origin of these tumors, their histology, and requirements for cell suspension are described in Table 1, and further data on the histology, growth rate, and kinetics of 1 of the xenografts (HX18) have been reported previously (12).

Cell Suspensions. The technique of preparing tumor cell suspensions was determined empirically for each tumor and has already been reported elsewhere (17); details are summarized in the table.

Assay Techniques. Two clonogenic assays were used for these experiments. The first used an in vitro agar technique with a replenishable liquid phase and is being reported in detail elsewhere (15); the second, also described elsewhere, was used as an agar in diffusion chamber technique (17). Only the main points will be summarized here.

In the in vitro assay, washed rat erythrocytes were mixed with the tumor cell suspension in 0.3% agar in Ham's F12 medium containing 15% fetal calf serum. One-ml aliquots were allowed to set in 170-mm test tubes (Falcon Plastics, Oxnard, Calif.) (5). The cultures were gassed with 5% oxygen, 90% nitrogen, and 5% carbon dioxide and were incubated at 37°C. After 5 days, liquid culture medium was pipetted on top of the agar and replaced at weekly intervals. Colonies were scored after 3 weeks for HX32 and HX34 and after 4 weeks for HX18 and HX33 by decanting the agar onto a microscope slide, covering it with a 25- x 50-mm coverglass, and counting colonies of more than 50 cells at ×40.

In the diffusion chamber assay, tumor cell suspensions in Ham's F12 medium, 15% calf serum, and a final 0.3% concentration of agar were loaded into modified diffusion chambers subsequently implanted under ether anesthesia.
into the peritoneal cavity of C57BL mice pretreated with whole-body irradiation to promote colony growth. Twenty-one days later for HX32 and HX34 and 28 days later for HX18 and HX33, the chambers were removed, and colonies of 50 cells or more were counted.

Irradiation. Single-cell suspensions were prepared from 5- to 8-mm-diameter tumors, and measured aliquots were irradiated in vitro at room temperature in polystyrene test tubes with $^{60}$Co γ-radiation at a dose rate of 500 rads/min. During irradiation the tubes were suspended in water to ensure adequate buildup of radiation dose.

The surviving fraction of colony-forming cells after each dose of radiation was calculated as the number of colonies produced per 100 irradiated cells divided by the number produced per 100 unirradiated controls.

Chromosomal Analysis. Cell suspensions from the 4 tumors were prepared for chromosomal analysis, and all were shown to be of human karyotype with typical acrocentric and metacentric chromosomes that are lacking in mouse karyotypes.

RESULTS

The in vitro radiation survival curves for the 4 human tumors are shown in Chart 1, and the survival parameters derived by linear regression analysis of the exponential part of the curve for each tumor are given in Table 1. From Chart 1, it was considered that surviving fractions for HX34 cells treated with less than 400 rads did not lie on the exponential portion of the cell survival curve, and these were therefore excluded from the analysis. No significant difference was found between the results of the in vitro assay and the agar diffusion chamber assay analyzed independently of each other.

The radiation survival curves of 3 of the tumors, colonic carcinoma HX18, pancreatic carcinoma HX32, and oat cell carcinoma of lung HX33, were all very similar with $D_s$ of, respectively, 94 (+14, -10), 100 (+9, -8), and 131 (+23, -18) rads, where the figures in parentheses represent 67% fiducial limits. The respective $D_s$ for the 3 tumor cell types were 8 (+27, -34), 44 (+25, -30), and 41 (+36, -47)
rads. There was no significant difference between any of these results.

The shoulder of the radiation survival curve of the fourth tumor, melanoma HX34, was wider than that of the other 3 tumors, with a $D_s$ of 216 (+48, -57) rads, and this difference reached statistical significance when compared with the $D_s$ for HX18 cells ($p < 0.05$). The $D_s$ for HX34 of 183 (+21, -17) rads was also higher than that of the other 3 tumors.

![Chart 1](chart1.png)

**Chart 1.** Survival curves of cells from 4 human tumor xenografts irradiated in vitro. •, O, melanoma; A, Δ, colonic carcinoma; ■, □, pancreatic carcinoma; ▲, ◀, oat cell carcinoma of lung; •, A, □, ▲, agar diffusion chamber technique; O, Δ, □, ◀, in vitro technique.

<table>
<thead>
<tr>
<th>Code</th>
<th>Histology</th>
<th>Cell suspension technique</th>
<th>% plating efficiency</th>
<th>$D_s$ (rads)</th>
<th>$D_a$ (rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HX18</td>
<td>Colonic adenocarcinoma, poorly differentiated</td>
<td>Trypsin</td>
<td>0.5-2.5</td>
<td>94 (+14, -10)$^a$</td>
<td>8 (+27, -34)$^a$</td>
</tr>
<tr>
<td>HX32</td>
<td>Pancreatic carcinoma anaplastic (Met)$^b$</td>
<td>Collagenase + trypsin</td>
<td>25-35</td>
<td>100 (+9, -8)</td>
<td>44 (+25, -30)</td>
</tr>
<tr>
<td>HX33</td>
<td>Oat cell carcinoma of lung (Met)</td>
<td>PBS</td>
<td>0.3-1.5</td>
<td>131 (+24, -18)</td>
<td>41 (+36, -47)</td>
</tr>
<tr>
<td>HX34</td>
<td>Melanoma (Met)$^c$</td>
<td>PBS</td>
<td>15-25</td>
<td>183 (+21, -17)</td>
<td>216 (+48, -57)</td>
</tr>
</tbody>
</table>

$^a$ With 67% fiducial limits.

$^b$ The abbreviations used are: Met, metastasis biopsy; PBS, phosphate-buffered saline (KCl, 200 mg/liter; NaCl, 8000 mg/liter, KH$_2$PO$_4$, 200 mg/liter, and Na$_2$HPO$_4$, 1150 mg/liter).

$^c$ After short-term monolayer culture.
and this difference was significant compared with HX32 ($p < 0.01$) and HX18 ($p < 0.002$).

**DISCUSSION**

The 2 colony assay techniques used in these studies have enabled us for the first time to culture tumor cells taken directly from human tumor xenografts without previous adaptation to culture (except for initial short-term culture of melanoma HX34) and have made it possible to carry out on human tumors investigations that were previously limited to experimental animal tumors or to established cell lines. The 2 assays produce results that are reproducible over a series of experiments and that do not differ significantly, whichever assay is used.

A comparison of the results obtained with the 4 tumors shows differences between the melanoma and the other tumors. In particular, the survival curve for the melanoma has a larger shoulder ($D_0$, 216 rads) than those of the other 3 tumors ($D_0$, 8, 41, and 44 rads). Similar wide shoulders were reported by Barranco et al. (1) and Thomson et al. (19) with the use of established cell lines of human melanoma cells irradiated in vitro. On the basis of these results, the melanoma appears to be remarkably resistant to small doses of radiation of the size commonly used in fractionated radiotherapy, and this finding is in keeping with the clinical observation that melanomas usually respond relatively poorly to radiotherapy in conventional dosage (15).

Of the remaining tumors studied, oat cell carcinomas of the lung have a clinical response to radiotherapy of over 80% (3), and the probability of local control is high with doses of about 4500 rads fractionated over 4 or 5 weeks (14). In contrast, rectal carcinomas are difficult to control with external radiotherapy, and local control can usually be achieved only with high-dose (10,000 to 15,000 rads) intracavitary irradiation of small tumors (11). The clinical radioresponsiveness of pancreatic carcinoma is difficult to assess: high-dose split-course irradiation appears to prolong survival and may perhaps eradicate tumors in a minority of patients; (7), but again failure to achieve local control with radiotherapy is common (9). In view of these differences in clinical response between oat cell carcinomas and the other 2 tumor types, it is of interest that we have found the in vitro radiosensitivities of cells from these tumor types to be similar to one another. Furthermore, the $D_0$ values lie within the same fairly narrow range as was previously established for experimental animal tumors (2, 8, 16, 18) and for established cell lines from human tumors (1, 19, 20).

If these xenografted tumors can be considered typical of tumors encountered in the clinic, then this discrepancy between intrinsic radioresponsiveness and clinical radioresponsiveness confirms the data that were previously obtained from animal tumors and cell lines that suggest that differences in intrinsic cellular radiosensitivity are not a major factor in determining differences in therapeutic response of human tumors. There may be exceptions to this general statement, as the wide “shoulder” on the melanoma cell survival curve suggests, and further studies are required with a variety of tumors to determine the frequency of such exceptions. However, in general our results indicate that extrinsic factors such as tumor vascularity and the presence of differing populations of hypoxic cells are likely to be of much greater importance in determining clinical response to radiotherapy. These cannot be determined by the in vitro techniques described in this paper; in vivo cell survival studies are necessary for their further investigation, and these are currently in progress.

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