Influence of Cellular Radiation Sensitivity on Local Tumor Control of Human Melanoma Xenografts Given Fractionated Radiation Treatment

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ABSTRACT

The radiocurability of human melanoma xenografts was studied by treating tumors with multiple fractions of 2.0 Gy and using local tumor control at 180 days as end point. Three melanoma lines (E. F., G. E., M. F.) that are only weakly immunogenic in athymic nude mice (BALB/c-\(\text{n}u/\text{n}u/\text{BOM}\)) were selected for the study. The tumor radiocurability was found to differ considerably among the lines; the radiation doses required to achieve local control of 50% of the tumors irradiated (\(\text{TCD}_{50}\); mean \(\pm\) SE) were 85.0 \(\pm\) 4.7 Gy (E. F.), 60.3 \(\pm\) 5.4 Gy (G. E.), and 99.3 \(\pm\) 5.7 Gy (M. F.). The radiation sensitivity in \textit{vitro} of cells isolated directly from tumors also differed significantly among the lines. The \(\text{TCD}_{50}\) showed positive correlations with the surviving fraction after 2.0 Gy in \textit{vitro}, the surviving fraction after two doses of 2.0 Gy (4-h interval) in \textit{vitro}, and the surviving fraction after 4.0 Gy at a low dose rate (1.25 cGy/min) in \textit{vitro}. Thus, the differences in tumor radiocurability among the lines were mainly a consequence of cellular differences in the capacity to repair radiation damage. Comparisons of measured \(\text{TCD}_{50}\)s with theoretical \(\text{TCD}_{\text{dose}}\)s, calculated from cell-surviving fractions measured in \textit{vitro} after radiation treatment in \textit{vitro} or in \textit{vivo}, suggested that other tumor parameters, \(\text{e.g.}\), rate of repopulation between radiation fractions, also had a significant impact on the \(\text{TCD}_{50}\). However, this study strongly supports the assumptions that the surviving fraction at 2.0 Gy in \textit{vitro} is a useful parameter for prediction of clinical tumor radiocurability.

INTRODUCTION

Predictive assays of tumor radiocurability are required for development of individualized and, hence, improved strategies for radiation treatment of cancer (1–3). Studies of the radiation biology of continuous cell lines (4, 5) and primary cell cultures (6–8) derived from human tumors have suggested that \(\text{SF}_2\) in \textit{vitro} may predict the clinical radiocurability of tumors. This hypothesis is supported by results from two similar, but independent studies of the radioresponsiveness of experimental tumors, one by Røfstad and Brustad (9) involving 5 human melanoma xenograft lines and the other by Bristow and Hill (10) making use of 8 murine tumor lines. The tumors were given fractionated radiation treatment with 2.0-Gy fractions in \textit{vitro}, and tumor radioresponsiveness was found to be correlated with \(\text{SF}_2\) in \textit{vitro} for cells isolated from tumors of the same lines. Specific growth delay and tumor cell survival were used as end points for tumor radioresponsiveness in both studies.

Similar studies of experimental tumors using local control as the end point after treatment in \textit{vivo} have not been reported so far. Experiments intending to determine \(\text{TCD}_{50}\)s for 2.0-Gy fractions involve considerable strain on the host animals and are therefore difficult to perform in a scientifically and ethically proper way (11). Moreover, immune reactivity by the host is often a serious problem in studies of human tumor xenografts (12) and immunogenic murine tumors (11); artificially low \(\text{TCD}_{50}\)s can be measured, particularly in studies involving protracted fractionated treatments. However, \(\text{TCD}_{50}\)s for 2.0-Gy fractions for experimental tumors with well-characterized cellular radiation sensitivity would be of great value in assessment of the power of \(\text{SF}_2\) in \textit{vitro} to predict tumor radiocurability. Even if tumor radioresponsiveness is positively correlated with \(\text{SF}_2\) in \textit{vitro}, tumor radiocurability does not necessarily need to show a similar correlation, for example, if \(\text{SF}_2\) in \textit{vitro}, tumor radioresponsiveness does not necessarily need to show a similar correlation, for example, if \(a\) the repair capacity of tumor cells is different in the beginning and toward the end of a curative fractionated treatment or \(b\) the number of stem cells differs considerably among tumors.

Local control following single-dose irradiation of tumors from 5 human melanoma xenograft lines has been studied in our laboratory (13). \(\text{TCD}_{50}\), host immune reactivity, and the influence of host immune reactivity on the \(\text{TCD}_{50}\) were measured. Three of the lines were found to be just weakly immunogenic in athymic nude mice (BALB/c-\(\text{n}u/\text{n}u/\text{BOM}\)). The \(\text{TCD}_{50}\)s of these lines were not significantly influenced by host immune reactivity, and the lines were judged to be adequate models for tumor radiocurability studies (13). \(\text{SF}_2\) in \textit{vitro} has been found to differ considerably among these lines (9, 14). They were therefore selected for tumor radioresponsiveness studies using 2.0-Gy fractions, and the \(\text{TCD}_{50}\) are reported here. The main purpose of the work was to search for a possible relationship between \(\text{TCD}_{50}\) and \(\text{SF}_2\) in \textit{vitro}, but the impact of other radiobiological parameters on the \(\text{TCD}_{50}\) is also discussed.

MATERIALS AND METHODS

Mice and Tumors. Female BALB/c-\(\text{n}u/\text{n}u/\text{BOM}\) mice, 8 to 10 wk old, were used. They were bred at the animal department of our institute and were kept under specific-pathogen-free conditions. The melanoma xenograft lines (E. F., G. E., M. F.) were originally derived from lymph node metastases of patients admitted to The Norwegian Radium Hospital. Tumor tissue was transplanted directly into athymic mice without previous adaptation to \textit{in vitro} culture conditions. Histologically the parent metastases were similar. They were composed of solid trabeculae and nests of relatively large cells with hyperchromatic vesicular nuclei surrounded by partly abundant eosinophilic cytoplasm. Areas with more spindle-shaped cells were also seen. The cytoplasm contained little or no melanin. Numerous mitotic figures were found.

The melanoma lines were grown serially in athymic mice by implanting tumor fragments, approximately 2 x 2 x 2 mm, s.c. into the flanks of recipient mice. Passages 35 to 60 of the lines were used in the present work. The lines were stable during the period the experiments were carried out, as ascertained by measurements of DNA histograms, volumetric growth rates, and cellular radiation sensitivities. Light and electron microscopic examinations showed that the histological appearance of the xenografts was similar to that of the metastases in the donor patients.

The immune reactivity of the athymic mice against the melanoma lines has been shown to be exceptionally weak (13). The \(\text{TCD}_{50}\) is low for all lines (<40 cells) and not significantly different in untreated and

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3 The abbreviations used are: \(\text{SF}_2\), surviving fraction at 2.0 Gy; \(\text{TCD}_{50}\), radiation dose required to achieve local control of 50% of the tumors irradiated; \(\text{TCD}_{\text{dose}}\), number of tumor cells required to achieve tumors in 50% of the inoculation sites; \(\text{SF}_4\), surviving fraction at 4.0 Gy; \(\text{SF}_{4+2}\), surviving fraction after two doses of 2.0 Gy (4-h interval).
whole-body irradiated mice (5.0 Gy). Moreover, the TD50 is not enhanced significantly in mice immunized by multiple injections of radiation-inactivated tumor cells or by growing a tumor s.c. in the flank and then removing it surgically.

Irradiation. A Siemens Stabilipan X-ray unit, operated at 220 kV, 19 to 20 mA, and with 0.5-mm Cu filtration, was used for irradiation. Tumors in nonanesthetized, air-breathing mice were irradiated at a dose rate of 5.1 Gy/min. A 15- x 15-mm hole through a 2-cm-thick lead block served as a beam-defining aperture. During exposure, the mice were kept in specially made, thin-walled polymethylmethacrylate tubes with a hole in the cranial end through which they could breathe freely. A piston in the tail end positioned the mice firmly in the tubes. A hole was cut in each tube at the position of the mouse flank, through which the tumors protruded. To ensure uniform doses throughout the tumors, the tumors were exposed to radiation by two opposing treatment fields through each of which 50% of the dose in each fraction was delivered. Care was taken to avoid irradiating any normal tissue except the skin surrounding the tumors. The tumor volumes at the time of the first radiation fraction were approximately 400 mm³.

TCD50 Assay. The tumors were irradiated with 4 fractions of 2.0 Gy over 12 h each day, i.e., the interfraction time was 4 h. The total dose rate was varied from 6 to 15 days; i.e., the total radiation dose ranged from 48 to 120 Gy. The tumors were examined twice weekly after irradiation and scored as locally controlled if regrowth was not observed within 180 days after the last radiation fraction. Mice with recurrent tumors were killed when the tumor diameters reached 10 to 12 mm. Cured mice were killed at Day 180 and subjected to necropsy and histological examinations for residual tumor tissue. Mitotic figures, morphologically intact tumor cells, or any other signs of viable melanoma tissue were never seen in the histological sections.

The tumor control data were analyzed by linear regression analysis using the double negative logs model (15). Thus, TCD50 ± SE and D0 ± SE were determined from the relationships

\[-\ln(-\ln P) = \frac{1}{D_0} D - \ln(Nn)\]  
(A)

\[TCD_{50} = D_0 [\ln(Nn) - \ln(2)]\]  
(B)

in which P is the fraction of tumors controlled at dose D, n is the extrapolation number of the cell survival curve, and N is the number of clonogenic cells per tumor. These formulae are based on the assumption that only Poisson statistics influence the slope of the tumor control curve. If any heterogeneity is present, D0 will be overestimated and N underestimated by this method of analysis.

RESULTS

Tumor control curves are presented in Fig. 1; the fraction of controlled tumors (double negative logs scale) is plotted versus total radiation dose. The radiocurability and the cellular radiation sensitivity in vitro differed considerably among the melanoma lines; the TCD50s and the D50s are listed in Table 1. Corresponding TCD50s and D50s for single-dose irradiation (13) are included in Table 1 for comparison. The number of clonogenic cells per tumor, calculated from Equation B, was found to be equal within experimental error for fractionated and single-dose irradiation for all lines; i.e., the tumor control curves for fractionated irradiation (Fig. 1) were consistent with those for single-dose irradiation (13).

The SF2 in vitro and two other radiobiological parameters related to SF2 in vitro, i.e., the surviving fraction after 2 doses of 2.0 Gy (SF2+2) with a 4-h interval in vitro and the SF4 in vitro (low dose rate) are plotted versus TCD50. The rank order of the melanoma lines, i.e., M. F. > E. F. > G. E., was also found to be the same for TCD50 and the cell surviving fraction in vitro, irrespective of the in vitro parameter considered. TCD50 showed a significant correlation with SF2 in vitro (P < 0.01) and a marginally significant correlation with SF4 in vitro (low dose rate) (P ~ 0.05), whereas the relationship to SF2+2 in vitro was not significant (P > 0.10), as revealed by parametric statistical tests.

DISCUSSION

The radiocurability of 3 human melanoma xenograft lines was measured by treating tumors with multiple fractions of 2.0 Gy and using local tumor control at 180 days as end point. The TCD50 was found to be correlated with SF2 in vitro. Local tumor control determined experimentally in laboratory animals is directly analogous to curative intent in clinical radiation therapy. Long-term survival of cancer patients is significantly improved when local control of the primary tumor and regional disease is achieved (16). Thus, radiobiological studies of experimental tumors using TCD50 assays have greater relevance to clinical radiation therapy than do studies using any of the other common assays.

Comparisons with Theoretical TCD50s. The 3 melanoma xenograft lines have been characterized in detail with respect to cellular radiation sensitivity; cell surviving fractions have been

![Table 1 Parameters of tumor control curves](image)

<table>
<thead>
<tr>
<th>Melanoma</th>
<th>Fractionated irradiation</th>
<th>Single-dose irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCD50 (Gy)</td>
<td>D0 (Gy)</td>
</tr>
<tr>
<td>E. F.</td>
<td>85.0 ± 4.7*</td>
<td>5.42 ± 0.66</td>
</tr>
<tr>
<td>G. E.</td>
<td>60.3 ± 5.4</td>
<td>4.25 ± 0.61</td>
</tr>
<tr>
<td>M. F.</td>
<td>99.3 ± 5.7</td>
<td>6.58 ± 0.75</td>
</tr>
</tbody>
</table>

*Mean ± SE.

![Table 2 Cell-surviving fractions in vitro](image)

<table>
<thead>
<tr>
<th>Melanoma</th>
<th>SF2</th>
<th>SF2+2</th>
<th>SF4 (low dose rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. F.</td>
<td>0.36 ± 0.07*</td>
<td>0.15 ± 0.05</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>G. E.</td>
<td>0.18 ± 0.06</td>
<td>0.039 ± 0.009</td>
<td>0.080 ± 0.014</td>
</tr>
<tr>
<td>M. F.</td>
<td>0.46 ± 0.08</td>
<td>0.17 ± 0.04</td>
<td>0.28 ± 0.04</td>
</tr>
</tbody>
</table>

*Mean ± SE.
measured in vitro after irradiation of tumor cell suspensions in vitro and solid tumors in vivo (9, 13, 14). Comparisons of measured TCD95s with theoretical TCD95s calculated from tumor cell survival curves are useful in attempts to find the radiobiological parameters which are decisive for the measured TCD95s (11, 17).

Theoretical TCD95s were therefore calculated from tumor cell survival curves using the formula described by McNally and Sheldon (18).

\[
\text{TCD95} = D_0 \left( \ln n - \ln \frac{2}{N} \right)
\]

This equation is based on the assumptions that the tumor cell survival \( S \) curve at high radiation doses has the form

\[
S = n \cdot \exp(-D/D_0)
\]

and the tumors can recur from one cell. The number of clonogenic cells per tumor \( N \) for tumors with a volume of approximately 400 mm³ has been measured experimentally to be 4.5 \( \times 10^6 \) (E. F.), 1.0 \( \times 10^6 \) (G. E.), and 2.5 \( \times 10^6 \) (M. F.) (13).

Table 3 shows theoretical TCD95s and \( D_0 \)s for fractionated irradiation calculated from SF2 in vitro, i.e., from survival curves of the form

\[
S = (SF2)^n, \quad \text{where } N \text{ is the number of 2.0-Gy fractions and } 2N \text{ is the radiation dose. The TCD95s and } D_0 \text{s in Table 3 are considerably lower than the measured TCD95s and } D_0 \text{s, suggesting that tumor radiocurability was not governed solely by SF2 in vitro.}

Table 4 shows theoretical TCD95s and \( D_0 \)s derived from in vitro cell survival curves measured for tumors irradiated with multiple fractions of 2.0 Gy in vivo (Fig. 2 in Ref. 9). Two different survival curves were analyzed for each melanoma line, one pertaining to a conventional and the other to a superfractionation regimen. The conventional regimen consisted of 5 fractions of 2.0 Gy each week, one fraction each weekday. The total treatment time was varied from 1 to 3 wk; i.e., total doses of 10, 20, and 30 Gy were used. In the superfractionation regimen, 3 fractions of 2.0 Gy with 4-h intervals were given each day. The total treatment time was varied from 2 to 5 days; i.e., the total doses used were 12, 18, 24, and 30 Gy.

The parameters of the tumor control curves for fractionated irradiation (Table 1) can for all lines be seen to be within the same range as those derived from the two tumor cell survival curves (Table 4). The slightly higher \( D_0 \)s in Table 1 than for superfractionation in Table 4 can probably be attributed to tumor heterogeneity. The data in Tables 1 and 4 thus suggest that the radiobiological parameters which were decisive for tumor radioresponsiveness also were governing tumor radiocurability.

Prediction of Tumor Radiocurability. Previous work with the same 3 and 2 other melanoma xenograft lines has shown that tumor radioresponsiveness is correlated with SF2 in vitro (9). Radiation treatment was given in 2.0-Gy fractions according to the conventional and the superfractionation regimens described above. No correlations were found when tumor radioresponsiveness was analyzed against growth and microenvironmental parameters, e.g., volume-doubling time, fraction of cells in G1-G0, S, and G2 + M, growth fraction, cell loss factor, potential doubling time, volume fraction of necrosis, fraction of radiobiologically hypoxic cells, and capillary density (9). The radioresponsiveness of the tumors could not be explained solely from SF2 in vitro; the tumors were too resistant in vivo. This was mainly due to repopulation between radiation fractions, although hypoxia (19) and potentially lethal damage (PLD) repair (20) also might have contributed somewhat to the resistance in vivo. The repopulation did not differ significantly among the melanoma lines and therefore did not influence their rank order in radioresponsiveness, only the absolute level.

This work has shown that also the radiocurability of tumors treated with multiple 2.0-Gy fractions is correlated with SF2 in vitro. However, SF2 in vitro is not the only radiobiological parameter that exerted an influence on TCD50; tumor radiocurability was probably governed by the same parameters as those governing tumor radioresponsiveness (Tables 1, 3, and 4), i.e., the rate of repopulation during the treatment period in addition to SF2 in vitro. This suggests that the repair and proliferative capacity of the surviving clonogenic tumor cells did not change significantly throughout the course of a complete fractionated treatment; measurement of tumor radioresponsiveness was based on 5 to 15 fractions, whereas the TCD95s corresponded to 30 to 50 fractions.

The 3 melanoma lines studied here differed considerably in tumor radiocurability, mainly as a consequence of cellular differences in the capacity to repair radiation damage. The present...
results strongly support the suggestions of Fértil and Malaise (5) and Deacon et al. (4) that the clinical radiocurability of tumors may be positively correlated with the initial slope of their in vitro cell survival curves. Clinical investigations of the predictive value of SF2 in vitro have been initiated for squamous cell carcinoma of the head and neck (6) and of the uterine cervix (8), and similar studies involving other histological types of cancer should be encouraged. However, such studies should also include explorations of the predictive value of cell proliferation parameters, for example, by using flow cytometric techniques similar to those described by Begg et al. (21).

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