Local Tumor Control following Single Dose Irradiation of Human Melanoma Xenografts: Relationship to Cellular Radiosensitivity and Influence of an Immune Response by the Athymic Mouse

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ABSTRACT

The potential usefulness of untreated congenitally athymic adult mice as hosts for human tumors in radiocurability studies was investigated using five human melanoma xenograft lines (E.E., E.F., G.E., M.F., V.N.). 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 (TCD<sub>50</sub> values) ranged from 29.6 ± 2.1 (SE) to 67.9 ± 3.5 Gy. Since the clinical relevance of experimentally determined TCD<sub>50</sub> values depends on to what extent they are modified by a host immune response, a possible immune reactivity against the melanomas was investigated by comparing the radiocurability data with cell survival data measured in vitro after irradiation in vivo and by performing quantitative tumor transplantability studies. The radiocurability and the cell survival data were found to agree well for the E.E., G.E., and M.F. melanomas. Moreover, the number of tumor cells required to achieve tumors in 50% of the inoculation sites (TD<sub>50</sub> values) in untreated and in whole-body irradiated mice were similar, suggesting that the TCD<sub>50</sub> values measured for these lines were not significantly influenced by a host immune response. On the other hand, the E.E. and V.N. melanomas showed significantly lower TCD<sub>50</sub> values in vivo than predicted theoretically from the in vitro cell survival data and a significantly lower number of tumor cells required to achieve tumors in 50% of the inoculation sites in whole-body irradiated than in untreated mice, suggesting that the radiocurability of these two lines was enhanced due to an immune response by the host. Athymic mice may thus express a significant immune reactivity against some human tumor xenograft lines but not against others. Consequently, TCD<sub>50</sub> values measured for human tumors in athymic mice cannot be considered to be clinically relevant unless it has been verified that the tumors are not exposed to an immune reaction in the untreated host or the host has been rendered immunologically blank by immunosuppressive treatment.

INTRODUCTION

Since Povlsen and Rygaard (1, 2) in the early 1970s showed that human tumor tissue heterotransplanted into congenitally athymic mice was accepted and gave rise to tumors with histology similar to that of the parent tumor in the donor patient, human tumor xenografts have been used extensively in studies of effects of various treatment strategies (3, 4). Thus, it has been shown that the response to radio- and chemotherapy of human tumor xenografts generally correlates well with clinical responsiveness (5, 6). The majority of the therapeutic studies have been performed using tumor growth delay in vivo or cell survival measured in vitro as end points (3–6). Local tumor control has not been used regularly as an end point, mainly because there has been some concern that immune reactions by the athymic mouse might be active against human tumor xenografts and hence could enhance the frequency of tumor control artificially (5, 7).

Indeed, there is considerable evidence that the growth and treatment response of human tumors xenografted into athymic mice may be influenced seriously by a residual host immune reactivity (5). Thus, some human tumors fail to grow in adult athymic mice (7). The take rate of others is enhanced in newborn mice and in adult athymic mice given sublethal whole-body irradiation or treatment with antilymphocyte serum (8, 9). Local control has been observed in some tumors following low dose chemotherapy, i.e., doses that caused minor growth delays in nontreated mice (7). Moreover, it has been shown that athymic mice exhibit an unusually high level of natural killer cells (10) and an elevated macrophage activity (11) compared with conventional mice. Recent studies have also shown that certain lymphoid cells in athymic mice express surface antigens characteristic of mature T-lymphocytes (12).

An experimental model for studying local control of human tumors following radiation treatment would be particularly useful since eradication of the primary and regional disease is the principal aim of curative clinical radiotherapy. Experimental data showing whether and to what extent TCD<sub>50</sub> values of human tumor xenografts may be artificially reduced due to a host immune response are sparse (5). The main purpose of the present work was therefore to investigate the potential usefulness of human tumor xenografts growing in untreated congenitally athymic adult mice as a clinically relevant model for experimental studies of tumor radiocurability. Five human melanoma xenograft lines, which have been the subject of extensive growth and radiobiological studies at our institute (5, 13), were used for this purpose. TCD<sub>50</sub> values following single dose irradiation were determined from tumor control results at 180 days posttreatment. The tumor control data were analyzed in relation to cell survival data measured in vitro following single dose irradiation in vivo, since problems caused by a possible immune response by the mice are considered to be least severe in studies where the tumors are removed from the hosts within 1 day posttreatment and then cultivated in an immunologically neutral environment (7). Moreover, a possible host immune response against the melanomas was also studied by determining TD<sub>50</sub> values for the tumor cells in whole-body irradiated and in untreated mice.

MATERIALS AND METHODS

Mice and Tumors. Female BALB/c-nu/nu/BOM mice, 8–10 weeks 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.E., E.F., G.E., M.F., V.N.) 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 in vitro culture conditions. Histologically the parent metastases were similar. They

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were composed of solid trabecules 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 35–60 of the melanomas were used in the present work. The melanomas were kinetically stable during the period the experiments were carried out, as ascertained by flow cytometric measurements of DNA histograms and measurements of volumetric growth rates. Light and electron microscopic examinations showed that the histological appearance of the xenografts was similar to that of the metastases in the donor patients.

Irradiation. A Siemens Stabilipan X-ray unit, operated at 220 kV, 19–20 mA, and with 0.5 mm Cu filtration, was used for irradiation.

Tumors in nonanesthetized air-breathing mice and tumors in asphyxiated mice were irradiated at a dose rate of 5.1 Gy/min. The mice were asphyxiated by cervical dislocation 15 min before irradiation to obtain hypoxic tumors. A 15 x 15-mm hole through a 2-cm-thick lead block served as beam-defining aperture. During exposure the mice were kept in specially made, thin-walled polymethylmethacrylate tubes with holes 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 tumor volumes, the tumors were exposed to irradiation by two opposing treatment fields through each of which 50% of the dose was delivered.

Care was taken to avoid irradiating any normal tissue except the skin surrounding the tumors. The tumor volumes at the time of irradiation were approximately 400 mm³.

Colonv Assay. The fraction of surviving cells in the tumors after irradiation was measured in vitro using a soft agar colony assay similar to that developed by Courtenay and Mills (14). The tumors were dissected free from the mice immediately after irradiation or 24 h after irradiation to allow for PLD-repair. Single cell suspensions were prepared from the tumors using a standardized mechanical procedure; the tumors were put into plastic bags with 20 ml culture medium [Ham’s F-12 medium with 20% fetal calf serum, penicillin (250 mg/liter), and streptomycin (50 mg/liter)] and disaggregated for 30 s with a stomacher (Lab-Blender 80; Seward Laboratory, London, United Kingdom). The resulting suspensions were filtered through 30-μm nylon mesh.

The cells were counted using a hemocytometer. Melanoma cells (100 Gy), up to 100,000 cells/2 ml was added on the top of the agar 5 days after seeding and then changed weekly. A stereomicroscope was used to count colonies. Cells were asphyxiated by cervical dislocation 15 min before irradiation to obtain hypoxic tumors. A 15 x 15-mm hole through a 2-cm-thick lead block served as 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 tumor volumes, the tumors were exposed to irradiation by two opposing treatment fields through each of which 50% of the dose was delivered.

The tumor control data were analyzed by linear regression analysis using the double negative log model (18). Thus, TCD₅₀ ± SE and theoretical D₀ ± SE were determined from the relationships

\[
S = n \cdot \exp(-D/D₀)
\]

and the tumors can recur from one cell (17).

The tumor control data were analyzed by linear regression analysis using the double negative log model (18). Thus, TCD₅₀ ± SE and theoretical D₀ ± SE were determined from the relationships

\[
-\ln(-\ln P) = \frac{1}{D₀} D - \ln(Nn)
\]

where P is the fraction of tumors controlled at dose D. A t test was used to determine whether two D₀ values or two TCD₅₀ values were significantly different.

RESULTS

Radiation survival curves for tumors irradiated in vivo and assayed in vitro are shown in Fig. 1. The D₀ values of the curves differed considerably among the melanoma lines (Table 1). Tumors irradiated in air-breathing and in asphyxiated mice and excised immediately after irradiation showed similar D₀ values. The D₀ values for tumors excised 24 h after irradiation were higher than those for tumors excised immediately after irradiation. Thus, the data suggest that all tumors, irrespective of line, possessed a significant fraction of hypoxic cells that showed PLD-repair.
The results from two independent experiments with each line are presented in Table 4. The two experiments gave similar results. The TD50 values in whole-body irradiated mice were similar for all melanoma lines. The E.F., G.E., and M.F. melanomas showed TD50 values in untreated and whole-body irradiated mice that were not significantly different. In contrast, the TD50 values in untreated mice were higher than those in whole-body irradiated mice for the E.E. and V.N. melanomas, i.e., the same lines that showed higher theoretical than measured TCD50 values.

DISCUSSION

Local control of the primary tumor and regional disease has been shown to have a significant impact on long-term survival of patients with several histological types of cancer (20). A tumor is controlled when not a single clonogenic cell survives or can express its reproductive potential after therapy. Local tumor control determined experimentally in laboratory animals is directly analogous to curative intent in clinical radiotherapy. Thus, TCD50 assays of the radiation response of experimental tumors have greater relevance to clinical radiotherapy than any of the other assays commonly used. A suitable TCD50 assay for experimental studies of the radiocurability of human tumors would therefore be particularly welcome.

The potential usefulness of untreated, adult congenitally athymic mice as hosts for human tumors in radiocurability studies was investigated here using five human melanoma xenograft lines. The radiocurability differed considerably among the lines; the TCD50 values ranged from 29.6 ± 2.1 Gy for the E.E. melanoma to 67.9 ± 3.5 Gy for the E.F. melanoma.

The TD50 values calculated from the slopes of the tumor control curves in Fig. 2 are compared with the measured TD50 values (Fig. 1) in Table 2. The measured TD50 values refer to tumors irradiated in vivo in air-breathing mice, excised 24 h after irradiation, and then assayed in vitro. The calculated TD50 values tended to be higher than the measured ones, probably caused by tumor heterogeneity. However, the difference was not statistically significant for any of the melanoma lines.

A comparison of measured and theoretical TCD50 values is presented in Table 3. The measured TCD50 values were derived from the data in Fig. 2. The theoretical TCD50 values were calculated from the survival curves in Fig. 1 (air-breathing mice, seeding in vitro 24 h after irradiation). The measured and the theoretical TCD50 values agreed well for the E.F., G.E., and M.F. melanomas. On the other hand, the measured TCD50 values for the E.E. and V.N. melanomas were significantly lower than those calculated theoretically.

Tumor transplantability studies in untreated and whole-body irradiated mice were performed to investigate whether a host immune response was active against any of the melanoma lines.

Since immunological mechanisms probably do not have a significant influence on local tumor control by radiotherapy in
the human situation, the relevance of these TCD50 values depends on to what extent they are modified by an immune response by the athymic mouse host. A possible immune reactivity against the melanomas was investigated by comparing the radiocurability data with cell survival data measured in vitro after irradiation in vivo and by performing quantitative transplantability studies. The radiocurability and the cell survival data were found to agree well for the E.F., G.E., and M.F. melanomas (Tables 2 and 3). Moreover, the TDS0 values in untreated and whole-body irradiated mice were similar for these lines (Table 4), suggesting that the TCD50 values were not significantly influenced by an immune reaction against the tumors.

On the other hand, the E.E. and V.N. melanomas showed significantly lower TCD50 values in vivo than predicted theoretically from the in vitro cell survival data. The D0 values calculated from the tumor control curves were not significantly different from those measured in vitro, suggesting that the cellular radioresponsiveness in vivo was similar to that measured in vitro after tumor disaggregation. The number of effective clonogenic cells per tumor was therefore significantly lower in vivo than predicted from the cell clonogenicity in vitro, probably caused by an immune reaction against the tumors in vivo. This suggestion is strongly supported by the observation that the TDS0 values for these two melanoma lines were significantly lower in whole-body irradiated than in untreated mice. Consequently, the radiocurability of the E.E. and V.N. melanomas was probably modified significantly by a host immune response. Other, similar studies have also indicated an immune reaction against human tumors in athymic mice. Thus, Zietman et al. (25) studied a pharyngeal squamous cell carcinoma, a malignant glioma, and a fibrosarcoma and found lower TDS0 values in whole-body irradiated than in untreated control mice. The pharyngeal squamous cell carcinoma was also reported to show higher TCD50 values in immunosuppressed than in control athymic mice (23).

In conclusion, adult athymic mice express a significant immune reactivity against some human tumor xenograft lines but not against others. This immune reactivity may influence tumor radiocurability and hence modify TCD50 values. Consequently, TCD50 values for human tumors growing in athymic mice cannot be considered to be of clinical relevance unless it has been verified that the tumors are not exposed to an immune reaction in the untreated host or the host has been rendered immunologically blank by immunosuppression. Three human melanoma xenograft lines that are not significantly influenced by the residual immune reactivity present in untreated adult athymic mice are reported here. These xenograft lines thus show promise for further studies on mechanisms governing the probability to achieve local control of human tumors by radiotherapy, as well as for testing the therapeutic effect of chemical agents with potential to modify human tumor radiocurability.
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