Is Tumor Cell Radiation Resistance Correlated with Metastatic Ability? 1

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ABSTRACT

Patients who experience local failure following radiation treatment of epithelial malignancies exhibit a substantially higher rate of distant metastasis than those patients who achieve permanent local control. This fact has raised concern that the local failure to control the primary/regional tumor may serve as a marker of a particularly malignant neoplasm, i.e., high metastatic activity and radiation resistance. If this were true, there would be no gains in survival by increasing the efficacy of treating the primary/regional disease because the new local controls would develop distant metastasis. To investigate this concept, the relationship between distant metastasis probability and tumor cell radiation resistance has been studied by examining laboratory and clinical data (in vitro and in vivo assays) from six collaborating centers. TCD50's (radiation dose which inactivates half of the irradiated tumors) and incidence of distant metastasis in mice with local control have been evaluated for 24 murine tumor systems. SF2's (surviving fraction after 2 Gy) were determined in vitro for cell lines from 8 human, 13 mouse, and 15 rat tumors/tumor sublines and the metastatic activity assessed after injection of the cells into syngeneic murine hosts and xenogenic hosts for the human tumors. SF2's of cells from carcinomas of the head/neck, cervix, and endometrium which were controlled locally by radiation surgery from four centers were compared for those which did and those which did not metastasize. The total number of patients studied was 222. The cumulative distributions of SF2's of locally controlled tumors which did and did not metastasize were not different in each of the data sets. Similarly, there was no demonstrable relationship between TCD50's and metastatic frequency in local control mice. Furthermore, the SF2's of murine and human tumor cell lines did track with metastatic activity. Radiation sensitivity of clinical and laboratory tumors did not correlate with metastatic activity in studies from data of six centers.

INTRODUCTION

Clinical and laboratory animal tumor data clearly demonstrate that subjects which fail locally after radiation ± surgery to their primary tumor experience a higher incidence of distant metastasis than those who maintain local control. This has been reported for a wide range of tumor types and anatomic sites (1–14).

An important question for research in radiation oncology is: does local regrowth following modern treatment methods serve as a marker of an inherently very aggressive neoplasm, i.e., one which is highly likely to produce metastatic disease and is also radiation resistant? If this were true, a greater success in the treatment of the primary disease would not yield increased survival rates because the new local controls would fail distantly due to metastatic disease. The implications of this concept of the biology of local failures is that there are two classes of tumors: (a) those which are currently treated locally with success, a variable proportion of which establish metastases (the actual frequency depending on the histological type, grade, and size of tumor); and (b) tumors which fail locally and are associated with distant failure in virtually all instances.

An alternate scenario would be that the probabilities of local control and of distant metastasis are independent. The consequence of such independence would be that the proportion of patients who achieve local control but develop distant metastasis is constant as local control frequency is increased by use of more effective local treatment (e.g., higher radiation doses, use of radiation sensitizers, etc.). Hence, the observed greater frequency of distant metastases in patients with local failure than in those with local control would reflect metastases produced by the regrowing tumor. Accordingly, treatments which realize higher local control rates would be expected to increase disease-free survival rates to the extent that the local failures had contributed to distant metastasis.

Here, we present the results of a search for a relationship between the frequency of development of distant metastasis and the cellular radiation sensitivity based upon laboratory and clinical data from six centers.

MATERIALS AND METHODS

This study analyzed three categories of data: (a) parameters of cellular radiation sensitivity measured in vitro, i.e., the proportion of cells which survive a single dose of 2 Gy or SF2 2 and a descriptor of the slope of the initial portion of the survival curve, α; these have been generated for cell lines from the primary tumors of patients who achieved local control and who are with distant control or distant failure; (b) SF2's of rat tumor cells and the distant metastasis frequency from isografted tumors; and (c) TCD50's of isografted tumors versus distant metastasis frequency in mice with local control. In these analyses, metastasis data are considered only for subjects with local control of the irradiated tumors in order that results not be confounded by the metastases which develop from the persistent/regrowing tumor. The coefficient of variation of the reported SF2 and TCD50 values for independent cell and tumor systems have been in the range of 30–40% and 10–15%, respectively. The coefficients of variation for repeat assays of SF2 on cells from a single cell suspension have been ~10 and ~20% for cell suspensions prepared from different biopsy samples from one tumor. This means that there is a substantial heterogeneity between tumors of a common histological type; accordingly, there is a realistic basis for examining the relationships between radiobiological characteristics and various parameters of biological behavior.

For details of the experimental methods and procedures see these references: for SF2 assays, Paterson Institute for Cancer Research, Manchester (16); Cross Cancer Institute, Edmonton (17); Institut Gustave Roussy, Paris (18); UT MD Anderson Cancer Center, Houston (19); Penn State University

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1 The abbreviations used are: SF2, surviving fraction after 2 Gy; TCD50, radiation dose which (on the average) would be expected to control one-half of the irradiated tumors.

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Fig. 1. Cumulative distributions of SF$_2$ values for tumors which did and did not establish distant metastasis in human patients whose primary tumor was successfully treated (local control). A, data for carcinomas of the uterine cervix from the Christie Hospital, Manchester, United Kingdom. B, data for carcinomas of the uterine cervix and the endometrium from the Cross Cancer Institute. C, data for carcinomas of the head/neck from the Institut Gustave Roussy, Paris. CI, SF$_2$ values; C2, $\alpha$ values. D, data for carcinomas of the head/neck from the UT MD Anderson Cancer Center. DF, distant failure.

Continuous distributions is rejected if the distribution of the absolute difference between the values of the two observed cumulative distributions falls into the critical region corresponding to a significant level, $P = 0.05$ (27).

For the regression lines in Figs. 2 and 3, the least squares method was used to find the best linear unbiased estimators for the intercept and the slope parameter in the regression model. Regression plots were fitted with 95% upper and lower limits with respect to the individual predicted values. A two-tailed test was used to determine if the slope parameter differed from zero.
RADIATION RESISTANCE AND METASTASIS

RESULTS

Distant Metastasis and SF₂'s of Human Tumors Which Were Controlled Locally by Radiation Treatment. SF₂'s for tumors controlled locally by radiation ± surgery and which did or did not develop distant metastasis are presented from four centers, Fig. 1, A–D. These assays were performed in an investigation of the value of SF₂ as a predictor of the probability of local control by radiation ± surgery. This present study of the SF₂ and distant metastasis is based upon separate analyses which have not been components of previously published material. The techniques for cell culture and scoring cell survival varied between the centers. Survival fraction is counted as colony formation at Manchester and Edmonton. The results at the M. D. Anderson Cancer Center and Paris are based upon the CAM (cell adhesive matrix) method, which derives the survival fraction from population growth curves following radiation. For details of the experimental procedures, see below.

At the Christie Hospital, Manchester, West et al. (16) have determined SF₂'s for 88 Stage II and III squamous cell carcinomas of the uterine cervix which were controlled locally by radiation alone. Cell survival was determined using the Courtenay-Mills soft agar method with fresh biopsy tissue; colony formation was the end point for cell survival; radiation was a single dose of 2 Gy. In Fig. 1A, the cumulative distribution of SF₂'s for tumors which were locally controlled are plotted separately for the distant controls (n = 53) and distant failures (n = 13). The SF₂'s were identical for the sensitive one-half of the distant controls and failures. There was, however, a nonsignificant trend for higher SF₂'s for the more resistant tumors which failed distantly. This indicates a slight increased frequency (not significant) for the most resistant tumors to develop metastasis.

Allalunis-Turner et al. (17) of the Cross Cancer Institute, Edmonton, have determined the SF₂ for 27 carcinomas of the uterine cervix (n = 13) and endometrium (n = 14) treated successfully in terms of local control by radiation alone. The assays for cellular radiation sensitivity were performed upon early passage cells from biopsy specimen. ¹³⁷Cs radiations were used to deliver single doses to cells in suspension cultures. The end point was colony formation. The SF₂'s for the carcinoma of the cervix and endometrium cell lines were the same, and for this report, they are considered together. The cumulative distribution of the SF₂'s in this series of local control patients with distant control or distant failure are shown in Fig. 1B. There is a virtual overlay of the two distributions of SF₂'s.

Girinsky et al. (18) at the Institut Gustave Roussy, Paris, determined the SF₂ and α values for 58 squamous cell carcinomas of the head/neck which had local control following treatment by radiation ± surgery. The end point for cell survival was cell population increase using the CAM assay. The cumulative distribution of the head/neck SF₂ and α values for locally controlled tumors with distant control (n = 44) or distant failure (n = 14) is shown in Fig. 1C1 and Fig. 2. Here, there is a slight (not significant) displacement of the SF₂ curve for the distant failures to the right; this was not found for the α curve.

Brock and Peters at the M. D. Anderson Cancer Center, Houston, have determined SF₂ for a series of patients treated by surgery and postoperative radiation for their squamous cell carcinomas of the
head/neck region. The assay was the CAM method which has been utilized extensively by their group (19). Fresh tumor tissue was disaggregated, and the resultant cell suspension was plated onto a CAM plate. Survival fractions were derived from the analysis of the postradiation population increase. The cumulative distribution of the SF₂₅ for locally controlled tumors are given in Fig. 1D for tumors which established distant metastasis (n = 13) and those which remained localized (n = 36). There is no difference in the distributions of the SF₂₅.

**Artificial Metastasis versus SF₂₅ for Tumor Cell Lines Tested in Experimental Animals.** Table 1 lists the SF₂₅ and "artificial metastatic" activity for 16 cell lines derived from the mammary carcinoma 13762NF of the Fischer 344 rat. Portions of this material have been published previously (20, 26, 28, 29). Cells were ¹³⁷Cs-irradiated at 3–5 h after placement in plastic culture vessels; colony formation was scored at 7 days. Metastatic capacity was assessed by counting the number of tumor nodules in the lung at 23 days after i.v. injection of 5 x 10⁴ tumor cells and the number and sizes of metastases at various nonpulmonary anatomic sites. The data given here are from two series of experiments. The rank order of the metastatic activity (1+, 2+, 3+, or 4+) is given for each tumor line independently by D. Welch at Penn State (Table 3). The raw cell survival data were provided to J. Efird, who computed the SF₂₅ at MGH. The scattergram of SF₂₅ and metastatic activity are shown in Fig. 2. They do not indicate a relationship between SF₂₅ and metastatic activity. The rank order of the metastatic activity was the same for the artificial metastasis and for spontaneous metastasis following transplantation to s.c. tissue.

**Distant Metastasis in Local Control Mice and TCD₅₀.** TCD₅₀ and the proportions of mice with local control and distant metastases for 21 tumor systems of diverse histological types are presented in Tables 2 and 3. These results were provided by three laboratories on 21 tumor systems of diverse histological types. The data given here are from two series of experiments. The rank order of the metastatic activity (1+, 2+, 3+, or 4+) is given for each tumor line independently by D. Welch at Penn State (Table 3). The raw cell survival data were provided to J. Efird, who computed the SF₂₅ at MGH. The scattergram of SF₂₅ and metastatic activity are shown in Fig. 2. They do not indicate a relationship between SF₂₅ and metastatic activity. The rank order of the metastatic activity was the same for the artificial metastasis and for spontaneous metastasis following transplantation to s.c. tissue.

**DISCUSSION**

The results presented here from six institutions provide no evidence for a correlation between frequency of metastasis development and cellular radiation resistance (SF₂₅) or TCD₅₀. Welch et al. (20) reported earlier for the rat mammary adenocarcinoma 13762NF that there was no relationship between cellular radiation sensitivity and ability of the tumor cells to establish tumor following i.v. injection of cells. Additional data on this point from their laboratory have been included in this report.

Allam et al. (30) have reported metastatic activities and SF₂₅ for 21 cell lines derived from human (8) and murine (13) tumors. The radiation was administered as single doses to exponential phase cells growing on plastic. Fig. 4 shows the resultant data which do not indicate that the more radioresistant cell lines produced a higher incidence of metastasis.

We know of no other published data directly relevant to this point. Indirectly related is the report by Rofstad (31), who determined the SF₂₅ for cell lines derived from primary malignant melanomas and their metastatic deposits in lung. The cumulative distribution of the
There is no separation between the SF₂s for the primary and metastatic cell lines. Rofstad (31) pointed out that there was a trend for the most sensitive primary tumors to have metastatic lesions comprised of cells which were more radiation resistant than those of the primary melanomas. Such a trend was not evident for primary melanomas of intermediate to high radiation resistance.

**Tumor Stage, SF₂, and Distant Metastasis.** For virtually all solid tumors studied, there is clear evidence of an increasing probability of distant metastasis with increasing size and/or T (T-N-M classification; tumor-node-metastasis) stage of the primary lesion. The Goldie-Coldman hypothesis (32) posits that there is a progression in the degree of malignancy with increase in the number of tumor cells, i.e., with increasing number of cell divisions there are mutations toward higher grades of malignancy and, hence, ability to establish metastasis. There are now data pertinent to the question of a relationship between distant metastasis probability, SF₂, and lesion size. Girinsky et al. (18) reported that the mean SF₂ for squamous cell carcinoma of the head/neck region were 0.40, 0.37, and 0.40 for T stages T₂, T₃, and T₄, respectively. The data for N stages were 0.40, 0.40, and 0.38 for N₀, N₁, and N₂, respectively. They also reported α values; there was no change in α value with T or N stage. Allalunis-Turner et al. (17) found SF₂s of 0.29 and 0.30 for squamous cell carcinoma of the uterine cervix and adenocarcinoma of the uterine corpus; there was no correlation of SF₂ and ploidy. In a separate analysis of the carcinoma of the cervix results, the SF₂s were 0.28, 0.33, and 0.22 for Stages IB, 2B, 3B, and 4, respectively. For the adenocarcinoma of the uterine body, SF₂s were 0.33 and 0.28 for Stages IB and 4A. West et al. (16) found SF₂s to be 0.45, 0.44, and 0.50 for stages I, II and III, respectively. Both West et al. (16) and Girinsky et al. (18) but not Allalunis-Turner et al. (17) and Brock et al. (19) found higher local failure rates for the tumors with high SF₂ and/or lower α values.

The observed increase in metastatic frequency with tumor size is expected to be, at least in part, a function of the increase in number of tumor clonogens. The latter would also cause the radiation dose required to inactivate the tumor to increase. These two consequences would be expected in the absence of changes in the biological characteristics of the constituent cells.

**Radiation Sensitivity and Metastatic Activity in Oncogene-Transfected Cell Lines.** There have been several reports of changes in the inherent cellular radiation sensitivity of nontransformed mammalian cells by transfecting with one or more oncogenes. The term “inherent cellular radiation sensitivity” is used here to designate radiation sensitivity as determined for cells in vitro in exponential growth phase and viability assessed by clonogenic assay.

Fitzgerald et al. (33) reported that transfection by N-ras of the immortalized but not transformed mouse embryo fibroblast cell line NIH 3T3 was characterized by significantly higher D₀ (2.1 versus 1.4 Gy) and plating efficiency (32 versus 4%) when irradiated at 2 Gy min⁻¹ but had no greater resistance for 5 cGy min⁻¹. Kasid et al. (34) observed that a human keratinocyte cell line immortalized by the adenovirus AD12 and SV40 and then transformed by Kirsten murine sarcoma virus was not of increased resistance (D₀) while cells transformed by treatment with chemical carcinogens were more resistant, i.e., D₀s were 4.1 and 4.3 versus 3.5 Gy (dose rate was 85 cGy min⁻¹). Sklar (35) also found that the D₀s for NIH 3T3 transfected with Ch-ras, v-H-ras, N-ras, or v-K-ras were significantly increased over that for control NIH 3T3 cells, namely, 2–2.4 Gy versus 1.4 Gy. McKenna et al. (36) reported that H-ras + EIA or H-ras + myc transformed rat embryo fibroblast (REC) cell lines were more resistant than normal REC cells, namely, D₀ was 1.1 for control cells and varied between 1.4–2.6 Gy for six cell lines. The H ras + EIA cell lines were resistant but did not produce metastasis while the H-ras + myc were of comparable resistance but were highly metastatic. Cheong et al. (37) have reported that the increased radiation resistance of REC cells transformed by H-ras and v-myc is exhibited predominantly during the S phase of the cell replication cycle. There are no published studies on alterations of radiation sensitivity and metastatic activity of tumor cells by transfection with an oncogene(s).

Multiple independent processes participate in the repair of radiation damaged DNA and these are genetically determined (38). Similarly, establishment of a metastasis depends upon the successful completion of a number of complex steps, many of which are under genetic control (39). The metastatic activity varies markedly among tumors of a given histological type. This presumably reflects the number and character of genes suppressed and/or activated (if activated, the extent of amplification), which are relevant to the metastatic process. A basis for examining a correlation between radiation resistance and metastatic activity is that there might be interaction between some of these gene functions which would result in an increasing radiation resistance with increasing malignancy. The available measurements of cellular radiation sensitivity and metastatic aggressiveness indicate that if there is a relationship between the two phenomena, it is weak or not frequent.

The clear implication of these findings is that the use of radiation treatment strategies which achieve higher probabilities of local control will be associated with gains in patient survival. The magnitude of the
gain in disease-free survival would be limited by the frequency of distant metastasis among patients who are controlled locally by the new treatment methods; this should be approximately the same as among patients who achieve local control by current treatment procedures.

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REFERENCES

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