Increased Metastatic Dissemination in Human Melanoma Xenografts after Subcurative Radiation Treatment: Radiation-induced Increase in Fraction of Hypoxic Cells and Hypoxia-induced Up-Regulation of Urokinase-Type Plasminogen Activator Receptor

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Abstract

Cancer patients showing local failure after radiation treatment have increased probability for developing metastatic disease. The mechanisms behind this observation have not been identified. In the present work, metastatic spread after inadequate radiation therapy was studied by using R-18 human melanoma xenografts as models of cancer in humans. Pi-monidazole was used as a hypoxia marker, and hypoxia and urokinase-type plasminogen activator receptor (uPAR) expression were detected by immunohistochemistry. R-18 tumors regrowing after subcurative irradiation showed a higher frequency of lymph node metastasis than unirradiated tumors. The expression of uPAR was up-regulated in hypoxic tumor regions, and the fractions of hypoxic and uPAR-positive cells were two-fold higher in regrowing irradiated tumors than in untreated tumors. Treatment with anti-uPAR antibody blocked metastasis almost completely in irradiated as well as unirradiated tumors. The metastatic frequency was higher in tumors regrowing after irradiation than in unirradiated tumors because the irradiation induced tumor hypoxia, and tumor hypoxia induced up-regulation of uPAR.

Introduction

Radiation therapy is an important modality for the treatment of primary tumors and regional cancer disease. Patients achieving local tumor control after radiation therapy show better survival rates than patients with recurrent local disease, and this has been attributed primarily to the observation that local treatment failure increases the probability of developing metastatic disease in distant organ sites (1). Studies of experimental tumors have confirmed that the incidence of metastases can increase after radiation treatment of the primary tumor (2). The first major work examining the effect of local tumor irradiation on metastatic frequency in an experimental cancer model was reported in 1949 by Kaplan and Murphy (3). They used a transplantable mouse mammary carcinoma and showed that mice given subcurative radiation treatment developed pulmonary metastases more frequently than unirradiated control mice and attributed the effect to the longer survival time of the irradiated mice. Since then, a large number of experimental studies have been performed, and the majority of those showed that the incidence of metastases increased after irradiation of the primary tumor (2). Interestingly, increased metastasis occurred frequently after radiation doses that were insufficient to control the primary tumor and rarely after curative radiation treatments. Convincing data explaining why many experimental tumors show increased metastasis after subcurative radiation treatment have not been provided thus far. However, several possible mechanisms have been proposed, including radiation-induced DNA changes increasing the metastatic propensity of the tumor cells (4), radiation-induced abscopal effects leading to enhanced capability of metastatic organ sites to support secondary tumor growth (5), radiation-induced vascular damage facilitating tumor cell intravasation (6), and radiation-induced tumor cell death leading to the formation of metastasis-promoting agents in necrotic tumor regions (7).

Recent studies have demonstrated that tumor hypoxia can promote metastasis by up-regulating the expression of genes involved in the metastatic process (8). Because tumors recurring after radiation therapy generally show higher fractions of hypoxic cells than untreated tumors (9), we hypothesized that subcurative radiation treatment could promote metastasis by increasing the fraction of hypoxic cells in the primary tumor. The purpose of the present study was to test this hypothesis, and to do so, experiments were performed with xenografted tumors of the R-18 human melanoma cell line. R-18 tumors develop spontaneous lymph node metastases in BALB/c-nu/nu mice, and it has been shown that hypoxia promotes metastasis in R-18 tumors by up-regulating the expression of uPAR (10). This transmembrane receptor focuses the formation of plasmin to the cell surface, and plasmin facilitates tumor invasion and metastasis by degrading matrix proteins directly and by activating several metalloproteinases (11). The experiments reported here gave results consistent with our hypothesis, showing that tumors regrowing after radiation treatment can have increased metastatic propensity because of radiation-induced hypoxia and hypoxia-induced up-regulation of gene products promoting metastasis.

Materials and Methods

Mice and Tumors. Adult female BALB/c-nu/nu mice, 8–10 weeks of age, maintained as described elsewhere (10), were used as host animals for xenografted tumors. Unless otherwise stated, tumors were initiated from R-18 monolayer cell cultures (12). Approximately 3.5 × 10⁵ cells suspended in 10 μl of Ca²⁺- and Mg²⁺-free HBSS were inoculated intradermally into the left mouse flank (10). Tumor volume (V) was calculated as V = π/6 × ab², where a is the longer and b is the shorter of two orthogonal diameters (12). The animal experiments were approved by the Institutional Committee on Research Animal Care and were performed according to the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Marketing, and Education (New York Academy of Sciences, New York, NY).

Radiation Treatment. A Siemens Stabilipan X-ray unit, operated at 220 kV, 19–20 mA, and with 0.5-mm copper filtration, was used for irradiation. The mice were anesthetized with ketamine (33 mg/kg) and azaperone (25 mg/kg), and the tumors were irradiated at a dose rate of 5.1 Gy/min, using a radiation field of 15 × 15 mm (13).

Metastasis Assay. The primary tumors were resected at predetermined times after they were initiated, and the hosts were examined for the presence of external lymph node metastases, i.e., enlarged lymph nodes, in the interscapular, submandibular, axillary, or inguinal region twice a week. The mice

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were euthanized 3 months after the primary tumor was resected, when moribund, or when scored to be positive for external metastases and were then examined for the presence of lymph node metastases in the abdomen and mediastinum. Metastases in the abdomen and/or mediastinum were always found in moribund mice. Histological examinations confirmed that enlarged lymph nodes, external as well as internal, always contained metastatic deposits. Mice were scored to be metastasis negative if enlarged lymph nodes could not be detected by autopsy 3 months after the primary tumor was resected, because previous long-term experiments have shown that mice appearing healthy and without enlarged external lymph nodes at 3 months after primary tumor resection also are free from lymph node metastases 3 months later (10, 12).

Treatment with Neutralizing Antibody. Anti-urokinase-type plasminogen activator receptor (uPAR) treatment, anti-angiogenic motility factor receptor (AMFR) treatment, or anti-hepatocyte growth factor receptor (HGFR; also known as Met) treatment was given by using an anti-human uPAR mouse monoclonal antibody (IgG1), an anti-human AMFR rat monoclonal antibody (IgM), or an anti-human HGFR mouse monoclonal antibody (IgG2A; R&D Systems, Abingdon, United Kingdom). The antibodies show no cross-reactivity with recombinant murine uPAR, recombinant murine AMFR, or recombinant murine HGFR. The treatments consisted of 15 doses of 25 μg of antibody given in 24-h intervals. In each experiment, control mice were treated at 24-h intervals with 15 doses of 25 μg of an irrelevant anti-human monoclonal antibody of the same isotype (IgG1, IgM, or IgG2A) as the receptor blocking antibody. Antibody solutions were diluted in PBS and administered in volumes of 0.25 ml by i.p. injection.

Immunohistochemical Detection of Hypoxia and uPAR Expression. Pimonidazole [1-[(2-hydroxy-3-piperidinyl)propyl]-2-nitroimidazole], administered as described elsewhere (13), was used as a marker of tumor hypoxia. Tumors were fixed in phosphate-buffered 4% paraformaldehyde, and immunohistochemistry was performed by using a peroxidase-based indirect staining method (10). Anti-pimonidazole rabbit polyclonal antibody (a gift from Professor J. A. Raleigh, Department of Radiation Oncology, University of North Carolina School of Medicine, Chapel Hill, North Carolina) or anti-human uPAR mouse monoclonal antibody (American Diagnostica, Greenwich, CT) was used as primary antibody. Diaminobenzidine was used as chromogen, and hematoxylin was used for counterstaining. Quantitative studies of hypoxia or the expression of uPAR were based on four cross-sections of each tumor. Area fractions showing positive pimonidazole or uPAR staining were determined by image analysis (13).

Statistical Analysis. Experimental data are presented as arithmetic mean ± SD unless otherwise stated. Statistical comparisons of data sets were performed by using the Student’s t test (single comparisons) or by one-way ANOVA (multiple comparisons) when the data sets complied with the conditions of normality and equal variance. Under other conditions, comparisons were performed by nonparametric analysis using the Mann-Whitney rank-sum test (single comparisons) or the Kruskal-Wallis one-way ANOVA on ranks (multiple comparisons). The Bonferroni’s method (parametric tests) or the Dunnett’s method (nonparametric tests) was used to identify data sets that differed from the control data in multiple comparisons. Probability values of \( P < 0.05 \), determined from two-sided tests, were considered significant. The statistical analysis was performed by using SigmaStat statistical software (Jandel Scientific GmbH, Erkrath, Germany).

Results

R-18 tumors were exposed to single doses of 10 or 15 Gy to investigate whether the incidence of lymph node metastases changed after subcurative radiation treatment. The irradiation was performed at day 25 after the tumors were initiated, i.e., when they had attained a volume of \( \sim 100 \text{ mm}^3 \). These radiation treatments did not result in local tumor control but caused significant dose-dependent tumor growth delays (Fig. 1A). The metastasis experiments involved seven groups of mice, i.e., three groups with unirradiated control tumors and four groups with irradiated tumors. Unirradiated tumors were resected at day 25 (corresponding to the time of irradiation), day 40, or day 55. Irradiated tumors were removed at day 40 or day 55, i.e., at day 15 or day 30 after they were exposed to 10 or 15 Gy. The times at which the primary tumors were irradiated or resected are indicated by arrows in Fig. 1A.

The percentage of mice that developed lymph node metastases was used as a parameter for metastatic frequency. The metastatic frequency was influenced significantly by the radiation treatments (Fig. 1B). Only 5% of the mice had developed metastases at the time of irradiation, i.e., at day 25 after tumor initiation. Unirradiated tumors showed a higher metastatic frequency than irradiated tumors at day 40 (10 Gy, \( P = 0.00027 \); 15 Gy, \( P = 0.000051 \)). The metastatic frequency increased from day 25 to day 40 in unirradiated tumors (\( P < 0.00001 \)) but not in irradiated tumors. At day 40, unirradiated tumors had grown to volumes of \( \sim 500 \text{ mm}^3 \), whereas the volumes of irradiated tumors were not different from those at irradiation. In contrast, irradiated tumors showed a higher metastatic frequency than unirradiated tumors at day 55 (10 Gy, \( P = 0.00098 \); 15 Gy, \( P = 0.00026 \)). The metastatic frequency increased between day 40 and day 55 in irradiated tumors (10 and 15 Gy, \( P < 0.00001 \)) but not in unirradiated tumors. Irradiated tumors showed significant regrowth in this period and had attained volumes of \( \sim 500 \text{ mm}^3 \) (10 Gy) or \( \sim 200 \text{ mm}^3 \) (15 Gy) at day 55. The increase in metastatic frequency from day 40 to day 55 in irradiated tumors was larger than that from...
day 25 to day 40 in unirradiated tumors (10 Gy, $P = 0.0023$; 15 Gy, $P = 0.00039$). Taken together, the data in Fig. 1B demonstrate that the majority of the metastatic cells were disseminated when the primary tumors had volumes within the range of 100–500 mm$^3$, and that tumors regrowing after radiation treatment metastasized more frequently than unirradiated tumors.

Primary tumors from these metastasis experiments were subjected to studies of tumor hypoxia and uPAR expression. The tumors showed highly heterogeneous staining for pimonidazole as well as for uPAR. Foci of hypoxic cells and foci of uPAR-positive cells were seen throughout the tumor parenchyma. The uPAR-positive foci were generally 1.3–1.5-fold larger than the hypoxic foci. The remaining tissue showed no detectable pimonidazole staining and very weak uPAR staining, i.e., the boundary line between stained and unstained cells was sharp for both pimonidazole and uPAR. Examinations of adjacent sections demonstrated a high degree of colocalization of uPAR and pimonidazole staining (Fig. 2, A and B). Quantitative colocalization studies were performed in two tumors from each of the seven groups of mice. These studies showed that the uPAR-positive foci covered 97.2 ± 2.0% ($n = 14$) of the area that stained positive for pimonidazole, and pimonidazole staining was seen in 70.2 ± 4.5% ($n = 14$) of the area occupied by uPAR-positive foci.

Associations between metastatic frequency and tumor hypoxia or uPAR expression were searched for by measuring the fraction of hypoxic cells and the fraction of uPAR-positive cells in all primary tumors from one of the four metastasis experiments presented in Fig. 1B. The area fraction showing positive pimonidazole staining and the area fraction showing positive uPAR staining were used as parameters. Irradiated tumors differed from unirradiated tumors in hypoxic fraction (Fig. 2C) and in uPAR-positive fraction (Fig. 2D). Both fractions were low at the time of irradiation, i.e., at day 25 after tumor initiation. Unirradiated tumors had a higher hypoxic fraction and a higher uPAR-positive fraction than irradiated tumors at day 40 (10 and 15 Gy, $P < 0.000010$ for both fractions). Both fractions increased from day 25 to day 40 in unirradiated tumors ($P < 0.000010$ for both fractions) but not in irradiated tumors. On the other hand, irradiated tumors showed a higher hypoxic fraction (10 Gy, $P = 0.00060$; 15 Gy, $P < 0.000010$) and a higher uPAR-positive fraction (10 and 15 Gy, $P < 0.000010$) than unirradiated tumors at day 55. Both fractions increased between day 40 and day 55 in irradiated tumors (10 and 15 Gy, $P < 0.000010$ for both fractions) but not in unirradiated tumors. The increases from day 40 to day 55 in irradiated tumors were larger than the increases from day 25 to day 40 in unirradiated tumors [hypoxic fraction (10 Gy, $P = 0.00015$; 15 Gy, $P = 0.000074$); uPAR-positive fraction (10 Gy, $P = 0.000092$; 15 Gy, $P = 0.000048$)]. Thus, the radiation-induced changes in metastatic frequency (Fig. 1B) were strongly correlated to the radiation-induced changes in hypoxic fraction (Fig. 2C) and uPAR-positive fraction (Fig. 2D), i.e., tumors regrowing after radiation treatment not only had a higher metastatic frequency than unirradiated tumors but also showed a higher hypoxic fraction and a higher uPAR-positive fraction.

The specific role of uPAR in the development of metastases was investigated by treating host mice with neutralizing antibody against uPAR. The experiments involved six groups of mice, i.e., three groups treated with anti-uPAR antibody and three control groups treated with an irrelevant antibody. Unirradiated tumors were given 15 daily antibody treatments from day 25 until tumor resection at day 40, whereas tumors irradiated with 10 or 15 Gy at day 25 were given 15 daily antibody treatments from day 40 until tumor resection at day 55. The anti-uPAR treatment had no significant effect on the growth of the primary tumors, i.e., the volumes of the primary tumors at resection were not different in anti-uPAR-treated and control mice (0 Gy, $461 ± 185$ mm$^3$ versus $485 ± 178$ mm$^3$; 10 Gy, $514 ± 192$ mm$^3$ versus $476 ± 181$ mm$^3$; 15 Gy, $180 ± 53$ mm$^3$ versus $189 ± 57$ mm$^3$). In contrast, the metastatic frequency was influenced significantly by the anti-uPAR treatment (Fig. 3A). The control groups showed metastatic frequencies consistent with the data in Fig. 1B. The anti-uPAR treatment resulted in reduced metastatic frequencies in both unirradiated ($P < 0.000010$) and irradiated (10 and 15 Gy, $P < 0.000010$) tumors. In fact, the anti-uPAR treatment blocked metastasis almost completely in all three treatment groups.

Moreover, the role of uPAR in the metastatic process was compared with that of AMFR and HGFR, two other cell surface proteins known to promote lymph node metastasis in a wide variety of tumor types. Six groups of mice with unirradiated tumors were included in the experiments, i.e., one group treated with anti-uPAR antibody, one group treated with anti-AMFR antibody, one group treated with anti-HGFR antibody, and three control groups treated with irrelevant antibody. Three different control antibodies were used, thus ensuring that the receptor blocking antibody and the corresponding control antibody were of the same isotype. The mice were treated with 15 daily doses of antibody from day 25 until tumor resection at day 40. The metastatic frequency was reduced after the anti-uPAR treatment ($P < 0.000010$), consistent with the data in Fig. 3A, whereas neither the anti-AMFR treatment nor the anti-HGFR treatment influenced the metastatic frequency significantly (Fig. 3B). The three control groups showed metastatic frequencies that were not different.

The mechanisms underlying the increases in hypoxic fraction, uPAR-positive fraction, and metastatic dissemination in tumors regrowing after irradiation were studied further by performing experiments attempting to determine whether the increases were a result of permanent radiation-induced genetic changes in the melanoma cells. The experiments involved four groups of mice with untreated tumors. The primary tumors were initiated from monolayer cultures (one group) or 55-day-old intradermal tumors (three groups, i.e., untreated nonmetastatic tumors, highly metastatic tumors irradiated with 10 Gy at day 25, or highly metastatic tumors irradiated with 15 Gy at day 25) and were resected at day 40 after initiation. The volumes of the tumors at resection (~500 mm$^3$) did not differ among the groups, implying that the growth rate of the second generation primary tumors, i.e., the primary tumors derived from disaggregated tumors, was similar to...
that of the first generation primary tumors, *i.e.*, the primary tumors derived from cultured cells. Moreover, the hypoxic fractions of the primary tumors initiated from tumors irradiated with 10 Gy (12.2 ± 2.5%; *n* = 20) or 15 Gy (10.3 ± 2.7%; *n* = 18) were not different from those of the primary tumors initiated from unirradiated tumors (11.5 ± 2.9%; *n* = 19) or monolayer cell cultures (11.1 ± 2.6%; *n* = 19). Finally, the metastatic frequency did not differ among the groups (Fig. 4), *i.e.*, the primary tumors initiated from irradiated tumors metastasized at the same frequency as the primary tumors initiated from unirradiated tumors or monolayer cultures.

**Discussion**

Effects of radiation treatment of the primary tumor on the incidence of distant metastases have been studied in several transplantable murine tumors (2). The outcome of the experiments was influenced significantly by two factors, the radiation dose and the survival time of the host mice. In general, subcurative doses resulted in increased metastasis, whereas curative doses had no significant effect on the metastatic frequency. In many studies, the mice were euthanized and examined for metastases when moribund, and because irradiated mice had longer survival times than unirradiated control mice, the increased incidence of metastases after irradiation may have been primarily a secondary effect of the prolonged survival (2). In the present work, R-18 tumors were treated with subcurative doses of 10 or 15 Gy, and irradiated tumors showed a higher metastatic frequency between day 40 and day 55 than did unirradiated tumors between day 25 and day 40. During these 15-day intervals, mean tumor volume increased from ~100 mm³ to ~500 mm³ (control and 10 Gy) and from ~50 mm³ to ~200 mm³ (15 Gy). The dissemination of metastatic cells per unit time and tumor volume was, therefore, higher in tumors regrowing after irradiation than in untreated tumors. Consequently, our study demonstrated unequivocally that tumors subjected to inadequate radiation treatment can have increased metastatic propensity.

Mechanisms underlying observations of increased metastatic growth after inadequate radiation therapy have not been studied extensively (2). However, several possible mechanisms have been suggested, including DNA changes, abscopal effects, microvascular damage, and tumor necrosis induced by the radiation therapy (4–7). The increased metastatic frequency in R-18 tumors regrowing after irradiation probably did not involve any of these possibilities. Thus, it was shown that primary tumors initiated from irradiated tumors metastasized with the same frequency as primary tumors initiated from untreated tumors and primary tumors initiated from monolayer cultures. We have also shown that the metastatic frequency of tumors transplanted to the left flank is not influenced by local irradiation of the right flank. Moreover, R-18 tumors disseminate via the lymphatics, and neither irradiated nor untreated R-18 tumors show necrotic regions at volumes of 500 mm³.

The increased metastasis in R-18 tumors regrowing after irradiation was rather associated with tumor hypoxia induced by the irradiation, because the fraction of hypoxic cells was ~2-fold higher in irradiated than in untreated tumors. The increase in hypoxic fraction was probably not a secondary effect of radiation-induced genetic changes in the melanoma cells, because primary tumors initiated from irradiated tumors metastasized at the same frequency as the primary tumors initiated from unirradiated tumors or monolayer cultures.

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tumors and primary tumors initiated from untreated tumors or monolayer cultures showed similar hypoxic fractions. More likely, regrowing irradiated tumors showed higher hypoxic fractions than unirradiated tumors because of radiation-induced damage in the tumor stroma, leading to impaired angiogenesis and reduced blood supply, a phenomenon known as the tumor bed effect (9). Rodent tumors regrowing after subcurative radiation treatment generally have higher hypoxic fractions than unirradiated tumors owing to the tumor bed effect (9), and it has been shown that recurrent human tumors have lower oxygen tensions and are more aggressive than previously unirradiated tumors (14).

We propose that R-18 tumors regrowing after irradiation showed a higher metastatic frequency than unirradiated tumors because the irradiation induced hypoxia in the primary tumor and hypoxia induced up-regulation of uPAR. This interpretation of our data is in agreement with several recent observations connecting metastasis to hypoxia: (a) tumor hypoxia can induce expression of genes promoting metastasis by activating DNA transcription factors such as hypoxia inducible factor-1α (8); (b) KHT-C murine fibrosarcomas subjected to experimentally imposed hypoxic stress in vivo or in vitro show increased frequency of pulmonary metastasis (15); (c) high hypoxic fractions promote pulmonary and lymph node metastasis in untreated human melanoma xenografts (8, 10); and (d) human soft tissue sarcomas and squamous cell cervix carcinomas having low oxygen tensions are usually highly aggressive and show elevated metastatic dissemination (8).

Three observations reported here strongly suggest that the increased metastatic frequency in R-18 tumors regrowing after irradiation was mediated primarily by hypoxia-induced up-regulation of uPAR: (a) histological examinations showed that uPAR-positive foci colocalized with pimonidazole-positive foci, implying that uPAR was up-regulated in hypoxic regions of the tumors; (b) the tissue area fractions with positive uPAR or pimonidazole staining were higher in irradiated than in unirradiated tumors; and (c) treatment with neutralizing antibody against uPAR blocked metastatic dissemination almost completely in both irradiated and unirradiated tumors.

Other studies of the R-18 melanoma supporting this suggestion have been reported elsewhere (10). Western and Northern blot analyses of R-18 cells exposed to hypoxia in vitro showed that the levels of uPAR protein and mRNA increased gradually with time under hypoxia and were enhanced by factors of 8–10 after 16–24 h, whereas the expression of other main members of the plasminogen activation system was not influenced significantly by hypoxia. Studies of R-18 tumors in vivo showed that the incidence of lymph node metastases, the hypoxic fraction of the primary tumor, and the uPAR-positive fraction of the primary tumor increased with similar kinetics during primary tumor growth, and that metastatic tumors had ~1.5-fold higher hypoxic fractions and ~1.4-fold higher uPAR-positive fractions than nonmetastatic tumors of the same size.

We cannot exclude the possibility that metastasis-promoting gene products other than uPAR also were up-regulated in hypoxic regions of R-18 tumors and hence contributed to the increase in metastatic frequency after irradiation. AMFR and HGFR, similar to uPAR, are cell surface proteins known to play important roles in tumor cell migration, invasion, and metastasis, and studies of tumor cells in vitro have shown that hypoxia can promote cell motility and invasion in collagen gels by up-regulating AMFR (16) or HGFR (17). However, it is unlikely that AMFR or HGFR contributed significantly to the metastatic spread in R-18 tumors, because immunohistochemical preparations of R-18 tumors just show weak and homogeneous staining for AMFR and HGFR, i.e., neither is up-regulated in hypoxic tumor regions. Moreover, treatment with blocking antibody against AMFR or HGFR did not inhibit lymph node metastasis in R-18 tumors. These observations do of course not exclude the possibility that hypoxia may promote metastasis in other tumor models by up-regulating AMFR (16) or HGFR (17).

Spontaneous metastasis is positively correlated to the expression of proangiogenic factors in some human melanoma xenografts (18), and several proangiogenic factors are up-regulated by hypoxia (8). However, hypoxia-induced up-regulation of proangiogenic factors probably did not contribute to the increased metastatic propensity in R-18 tumors regrowing after irradiation, because immunohistochemical studies of R-18 tumors have revealed that staining indicating up-regulation of vascular endothelial growth factor, interleukin 8, angio- genin, or platelet-derived endothelial cell growth factor does not colocalize with pimonidazole staining, and that mean or hot spot microvascular density does not correlate with the fraction of hypoxic cells.

Our study may have significant implications for the radiation therapy of tumors as well as for cancer treatment in general. The observation that tumors regrowing after inadequate radiation treatment can have elevated metastatic propensity implies that postirradiation recurrences should be subjected to curative treatment as early as possible after their diagnosis to prevent (further) metastatic spread. The observation that radiation-induced hypoxia can increase the metastatic propensity of tumors may be relevant for treatment modalities other than radiation therapy also, because tumors can show increased hypoxia after several types of treatment including photodynamic therapy, hyperthermia, and some forms of chemo- and immunotherapy (19). Moreover, the tumor endothelium has been recognized as an important target for the treatment of cancer, and novel treatment strategies based on the use of angiogenesis inhibitors, i.e., agents inhibiting tumor neovascularization, or vascular targeting agents, i.e., agents destroying established tumor microvasculature, have shown great promise in recent preclinical studies (20). Some of the strategies may lead to a significant increase in the fraction of viable hypoxic cells in the tumor tissue. Therefore, studies investigating the possibility that antiangiogenic or vascular targeting treatments may cause increased metastatic dissemination because of treatment-induced hypoxia are required.

In summary, the present work suggests that tumors regrowing after inadequate radiation therapy can have elevated metastatic propensity, primarily because the radiation therapy can induce an increase in the fraction of hypoxic cells and hypoxia can up-regulate the expression of genes promoting metastatic dissemination.

References

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