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Late Effects of Radiation Prime the Brain Microenvironment for Accelerated Tumor Growth

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Abstract

Purpose: Glioblastoma (GBM) remains incurable, despite state-of-the-art treatment involving surgical resection, chemotherapy, and radiation. GBM invariably recurs as a highly invasive and aggressive phenotype, with the majority of recurrences within the radiation therapy treatment field. Although a large body of literature reporting on primary GBM exists, comprehensive studies of how prior irradiation alters recurrent tumor growth are lacking. An animal model that replicates the delayed effects of radiation therapy on the brain microenvironment, and its impact on the development of recurrent GBM, would be a significant advance.

Methods and Materials: Cohorts of mice received a single fraction of 0, 20, 30, or 40 Gy Gamma Knife irradiation. Naïve, nonirradiated mouse GBM tumor cells were implanted into the ipsilateral hemisphere 6 weeks postirradiation. Tumor growth was measured by magnetic resonance imaging, and animal survival was assessed by monitoring weight loss. Magnetic resonance imaging results were supported by hematoxylin and eosin histology.

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Conflict of interest: none.

Results: Tumorous lesions generated from orthotopic implantation of nonirradiated mouse GBM tumor cells into irradiated mouse brain grew far more aggressively and invasively than implantation of these same cells into nonirradiated brain. Lesions in irradiated brain tissue were significantly larger, more necrotic, and more vascular than those in control animals with increased invasiveness of tumor cells in the periphery, consistent with the histologic features commonly observed in recurrent high-grade tumors in patients.

Conclusions: Irradiation of normal brain primes the targeted cellular microenvironment for aggressive tumor growth when naïve (not previously irradiated) cancer cells are subsequently introduced. The resultant growth pattern is similar to the highly aggressive pattern of tumor regrowth observed clinically after therapeutic radiation therapy. The mouse model offers an avenue for determining the cellular and molecular basis for the aggressiveness of recurrent GBM.

Summary

For many years, research on recurrent glioblastoma has largely focused on therapy-induced *immediate* radiobiologic changes to the tumor and surrounding tissues. In this study, we demonstrate that substantially *delayed* effects of irradiation on the brain microenvironment lead to a remarkable pattern of aggressive tumor regrowth. This affords new insights into the highly aggressive tumor regrowth pattern observed after radiation therapy and provides an avenue for determining the cellular basis for the aggressiveness of recurrent glioblastoma.

Introduction

Glioblastoma (GBM) is a highly aggressive and incurable malignant neoplasm of the brain. Together with surgical resection and chemotherapy, radiation therapy (RT) is central to standard of care treatment for newly diagnosed GBM (1, 2). Nonetheless, GBM tumors invariably recur, the vast majority within the first year (3) and within close proximity (2 cm) to the RT treatment field (4).

Much of the current focus of research into GBM recurrence has been on the role of glioma stem cells (5–7). Although substantial work has emphasized the immediate radiobiologic damage to the tumor and surrounding tissues (8–10), delayed, late, radiation-induced alterations to the brain microenvironment (BME) appear to play an important role in the pattern of tumor regrowth (11). Notably, brain-associated microglia and macrophages may contribute significantly to the resistance and recurrence of gliomas (12). Delayed BME RT effects include chronic inflammation, damage to vasculature, hypoxia, stromal effects, and suppressed immune response (11).

Therefore, we sought to determine the impact of late radiation-induced changes in the BME on tumor growth. To accomplish this, we developed a model in which naïve tumor cells (ie, cells having undergone no radiation-induced genetic or molecular changes) are implanted into brain parenchyma irradiated 6 weeks prior, and tumor growth was measured by magnetic resonance imaging (MRI). Animal morbidity, survival, and histologic evaluations were also assessed.

Methods and Materials

Cohorts ($n = 5$ each) of female BALB/c mice (Harlan Laboratories, Indianapolis, IN) received single-fraction Gamma Knife irradiation doses of 0, 20, 30, or 40 Gy (50% isodose). Nonirradiated glioblastoma tumor cells (DBT) were implanted in the ipsilateral hemisphere 6 weeks postirradiation (13). Anatomic MRI and histology (hematoxylin and eosin [H&E] staining) showed no evidence of frank radiation necrosis or structural changes at these irradiation doses (14, 15). (We note that laboratory mice are roughly half as radiation sensitive [re LD₅₀] as humans. Thus, a 20 Gy mouse dose corresponds approximately to a 10 Gy human exposure) (16, 17).

Contrast-enhanced T₁-weighted MRI scans were acquired every 3 days, starting 10 days postimplantation, until mice were either sacrificed (due to 20% weight loss) or died of disease progression. Formalin-fixed brain tissue sections were stained with H&E. Tumor-lesion volumes were derived from MRI; comparison of tumor volumes across groups used repeated measures one-way analysis of variance. Mortality was assessed using Kaplan-Meier analysis of survival followed by a 2-tail log-rank test. Further details can be found in Supplementary Material (available online at <https://doi.org/10.1016/j.jrobp.2018.08.033>).

Results

DBT cells implanted in previously irradiated brain tissue showed markedly enhanced tumor growth patterns, similar to recurrent GBM patterns observed clinically (Fig. 1). Furthermore, tumor contrast enhancement patterns were characteristic of highly necrotic and vascular tissue, whereas in the control group (implantation into nonirradiated brain), tumor contrast enhancement patterns were characteristic of well-circumscribed homogeneous tumor tissue. Hypointense regions in the contrast-enhancing tumor core for the irradiated groups reveal tumor necrosis and hemorrhage (see the histologic results in the following).

Figure 2 (left) shows anatomic MRI tumor/lesion volumes on postimplantation day 17. The group irradiated 6 weeks before implantation demonstrated much larger lesion volumes ($P < .001$) and a significant decrease ($P < .01$) in median survival compared with the nonirradiated controls (Fig. 2, right).

Figure 3 (left) shows standard H&E staining for 4 representative mice, respectively receiving 0, 20, 30 or 40 Gy radiosurgically, 6 weeks before DBT cell implantation. Employing low optical resolving power (top row), tumors implanted in previously irradiated brain tissue demonstrated an aggressive growth pattern, with large, mixed areas of hemorrhage and tumor necrosis. Under higher optical resolving power (bottom row), tumors growing in previously irradiated brain tissue showed prominent histologic changes, including tumor cell loss, necrosis, and hemorrhage—changes not seen in the control group. In contrast to the well-circumscribed tumor edges observed in the control group, tumors implanted in the previously irradiated brain also developed increased tumor satellites, indicating increased tumor invasiveness. The H&E stains in Figure 3 (right) demonstrate that vasculopathic changes (*) in DBT tumor growing in irradiated brain (bottom) are similar to those seen for human GBM (top).

Discussion

Delayed radiation effects within the BME may contribute to a higher risk of local GBM recurrence through increased radioresistance and induction of aggressive, invasive tumor growth, which is often observed in recurrent GBM (9, 10). A better preclinical modeling approach is needed to determine the underlying mechanisms of this phenomenon, to improve outcomes for patients with recurrent GBM. The model proposed herein focuses specifically on the role of delayed, late-evolving effects of radiation on the BME. Irradiation of normal brain 6 weeks before naïve tumor cell implantation induces aggressive, invasive tumor growth, similar to that clinically observed in patients with recurrent GBM, suggesting a BME-induced, tumor-extrinsic effect. Similar results were seen in a second orthotopic murine GBM model, GL261, when implanted into immunocompetent C57BL/6 mice. Specifically, on day 18 postimplantation, the average GL261 lesion size was larger in irradiated brain versus nonirradiated brain, and irradiated brains were more prone to support the growth of tumors with necrosis and hemorrhage, as assessed histologically.

Therapeutic resistance in locally aggressive recurrent gliomas may be mediated by radiation-altered BME. Ongoing work is aimed at gaining a deeper understanding of the exact mechanism(s) mediating this postirradiation BME effect on tumor growth. Two potential tumor-extrinsic factors at play include (1) Radiation-induced endothelial cell dysfunction leading to hypoperfusion and hypoxia due to impaired vasculature and decreased vascular density; and (2) delayed effects of radiation on the BME may induce a change in the inflammatory milieu, as seen in wound healing, leading to a potentially skewed immune-suppressive phenotype with impaired immune cell homing and recruitment.

Conclusions

The model described herein simulates the late, delayed effects of radiation on the BME, leading to aggressive, invasive tumor regrowth. It provides an important platform for (1) assessing different radiation doses, timing, and fractionation schedule; (2) evaluating the cellular and molecular basis for glioma tumor resistance; and (3) developing and testing therapeutic strategies targeted at modulating the BME that can be translated to the clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Representative postcontrast, T1-weighted images of mice with tumor lesions at postimplantation day 17. The magnetic resonance images for each radiation dose are 3 contiguous images from the same animal.

Fig. 2. (left) Tumor-lesion volumes at postimplantation day 17 for mice that received 0 Gy (nonirradiated, $n = 5$) or 20 to 40 Gy (irradiated, $n = 15$) irradiation ($P < .01$). (right) Kaplan-Meier survival curves for the same two groups of mice. Survival in mice irradiated six weeks prior to tumor implantation is significantly shorter than that of the non-irradiated group ($P < .01$), as measured by a two-tail log-rank (Mantel-Cox) test.

Fig. 3.

Representative hematoxylin and eosin-stained brain sections. (left) The mice whose histology is shown here were sacrificed on day 17 (0 Gy), day 21 (20 Gy), day 21 (30 Gy), and day 17 (40 Gy). Scale bar: 2.3 mm (top) and 100 μm (bottom). (right) Hematoxylin and eosin sections of human glioblastoma (top) and glioblastoma tumor in previously irradiated brain (bottom) show similar vasculopathic changes (*).