Integrated Systems and Technologies: Mathematical Oncology

Acute and Fractionated Irradiation Differentially Modulate Glioma Stem Cell Division Kinetics

Xuefeng Gao, J. Tyson McDonald, Lynn Hlatky, and Heiko Enderling

Abstract

Glioblastoma multiforme (GBM) is one of the most aggressive human malignancies with a poor patient prognosis. Ionizing radiation either alone or adjuvant after surgery is part of standard treatment for GBM but remains primarily noncurative. The mechanisms underlying tumor radioresistance are manifold and, in part, accredited to a special subpopulation of tumorigenic cells. The so-called glioma stem cells (GSC) are bestowed with the exclusive ability to self-renew and repopulate the tumor and have been reported to be less sensitive to radiation-induced damage through preferential activation of DNA damage checkpoint responses and increased capacity for DNA damage repair. During each fraction of radiation, non-stem cancer cells (CC) die and GSCs become enriched and potentially increase in number, which may lead to accelerated repopulation. We propose a cellular Potts model that simulates the kinetics of GSCs and CCs in glioblastoma growth and radiation response. We parameterize and validate this model with experimental data of the U87-MG human glioblastoma cell line. Simulations are conducted to estimate GSC symmetric and asymmetric division rates and explore potential mechanisms for increased GSC fractions after irradiation. Simulations reveal that in addition to their higher radiosensitivity, a shift from asymmetric to symmetric division or a fast cycle of GSCs following fractionated radiation treatment is required to yield results that match experimental observations. We hypothesize a constitutive activation of stem cell division kinetics signaling pathways during fractionated treatment, which contributes to the frequently observed accelerated repopulation after therapeutic irradiation. Cancer Res; 73(5); 1–10. ©2012 AACR.

Major Findings

The calibrated cellular Potts model reproduces experimentally observed in vitro and in vivo ratios of glioma stem cells (GSC) in the U87-MG cell line when the frequency of GSC symmetric division events is 35%. The model verifies that acute and fractionated irradiation yield enrichment in GSCs due to their reduced radiosensitivity. GSC radioresistance alone, however, while reproducing the 4-fold enrichment in GSCs after acute irradiation, is insufficient to yield the 6-fold enrichment after fractionated irradiation with equal total dose. An additional prolonged increase in GSC symmetric division events or a significantly shortened GSC cell cycle after repeated exposure is required to reproduce experimentally observed GSC ratios after fractionated irradiation.

Introduction

Glioblastoma multiformes (GBM) are among the most lethal primary human malignancies, possessing rapid growth, high invasiveness, and treatment resistance. Standard treatment for GBM comprises surgical resection of the gross tumor mass followed by radiotherapy of 60 Gy in 30 to 33 fractions of 1.8 to 2 Gy (9). The prognosis for patients with GBM remains poor because of refractory response to radiation and other treatments (10, 11). Therapeutic failure is due, in part, to tumor cell heterogeneity derived from both genetic and epigenetic sources (12). GBMs frequently recur after treatment with irradiation (IR; ref. 13), indicating survival of tumor-initiating cells (14, 15). Studies from our group and other laboratories have shown that the number of cancer colonies correlates with the frequency of cancer stem cells (16). GBMs frequently recur after treatment with irradiation (IR; ref. 13), indicating survival of tumor-initiating cells. It has been shown that only a subpopulation of cells—the so-called glioma stem cells (GSC)—are able to initiate brain tumors in mouse models (14, 15). Studies from our group and other laboratories have shown that the number of cancer colonies correlates with the frequency of cancer stem cells (16, 17). The population of GSCs has been shown to be highly resistant to IR due to more efficient DNA damage response mechanisms and environmental survival cues (11, 18–20). By detecting the expression of CD133 (Prominin-1), a pivotal marker of putative GSCs, the percentage of GSCs as well as their population size was found to be increased after radiation exposure (11, 20, 21). Importantly, irradiation did not induce CD133 expression in CD133 (Prominin-1), a pivotal marker of putative GSCs, the percentage of GSCs as well as their population size was found to be increased after radiation exposure (11, 20, 21). Importantly, irradiation did not induce CD133 expression in CD133− cells, confirming that increase in CD133− cells is due to proliferation of the original CD133− subpopulation (11). Surviving GSCs are able to initiate secondary tumors with enhanced aggressiveness and
Quick Guide to Equations and Assumptions

We use a cellular Potts model (CPM; refs. 1, 2) to simulate tumor development and response to irradiation. Cell behavior is determined by intrinsic parameters and interactions with adjacent cells, dependent on population-level changes in effective energy $E$ (i.e., Hamiltonian function), which determines cell structure, motility, adhesion, and response to extrinsic signals:

$$E = \frac{1}{2} \sum_{\text{neighbors} i, j} J(\tau(\sigma(i)), \tau(\sigma(j))) \left( I - \delta(\sigma(i), \sigma(j)) \right) + \frac{1}{2} \lambda_{\text{surface}}(\sigma) \left( \sigma(i) - \sigma\left(\sigma(i) \sigma(j) \right) - V\left(\sigma(i) \sigma(j) \right) \right)^2$$

where $\vec{i} = (x_i, y_i)$ and $\vec{j} = (x_j, y_j)$ denote neighboring lattice sites, $\sigma(\vec{i})$ denotes the cell at lattice site $(\vec{i})$, and $J(\tau(\sigma(i)), \tau(\sigma(j)))$ denotes the contact energy per unit area between cells at neighboring lattice sites. $V(\sigma)$ and $s(\sigma)$ are the volume (total number of pixels in the cell) and surface area that are constrained close to the target volume $V\text{target}$ and surface area $s\text{target}$, respectively; $\lambda_{\text{volume}}(\sigma)$ and $\lambda_{\text{surface}}(\sigma)$ denote the inverse volume compressibility and inverse membrane compressibility of the cell; and $\delta$ is the Kronecker delta with $\delta(\sigma(i), \sigma(j)) = (0, \text{if } \sigma(\vec{i}) \sigma(\vec{j}) \text{ and } 1, \text{if } \sigma(\vec{i}) \sigma(\vec{j}) \text{ )}.$

The cell lattice evolves through attempts by cells to extend their boundaries into neighboring cells’ lattice sites, displacing the neighboring cells that currently occupy those sites. For each index-copy attempt, we randomly select a cell boundary pixel (source) and attempt to displace a randomly chosen neighboring cell pixel (target). The effective energy change, $\Delta E$, is calculated by assuming the source cell displaces the target cell at that pixel. If $\Delta E$ is negative (i.e., the change is energetically favorable), the index-copy attempt is accepted. If $\Delta E$ is positive, the index-copy attempt is accepted with probability $P$ (i.e., Boltzmann acceptance function):

$$P = e^{-\Delta E/T_m}$$

where $T_m$ determines the amplitude of cell membrane fluctuations (equivalent to effective cell motility). These cell rearrangement dynamics utilize relaxation Monte-Carlo–Boltzmann–Metropolis dynamics (3, 4). On an $N$ sites lattice, $N$ displacement attempts are made in each Monte-Carlo step (MCS). The translation of experimental time into MCS depends on the average ratio of $\Delta E/T_m$ (2). Simulations are conducted in the open-source CompuCell3D simulation environment (2) on a 4,000 × 4,000 square lattice with periodic boundary conditions and $T_m = 50$ [c.f., Eq. (B)].

Assumptions

Glioblastomas are heterogeneous with subpopulations of glioma stem cells (GSC) and non–stem cancer cells (CC). The kinetics and interactions of both populations dictate dynamics of the population as a whole.

Basic cell kinetics

Cells are considered individual entities with a cell cycle (with length $T_c$) and a limited proliferation capacity $\rho = [0, \rho_{\text{max}}]$. For GSCs, $\rho_{\text{max}} = \infty$. At each cell division, GSCs undergo symmetric division with probability $p_s$ to produce 2 GSCs with identical features, or with probability $1 - p_s$ asymmetric division to produce a GSC and a CC with limited proliferation capacity $\rho_{\text{max}}$. The proliferation capacity $\rho$ is decremented at each CC division and inherited by both daughter CCs. CCs die when a proliferation attempt yields $\rho < 0$ (Fig. 1). To model proliferation, cell target volume $V\text{target}(\sigma)$ is increased with growth rate $k$ until the actual cell volume $V(\sigma)$ is doubled. If the local environment is not favorable for cell growth and cells cannot reach their target volume [i.e., the effective energy change, $\Delta E$, for a cell to increase in size is so large that the Boltzmann acceptance probability $P$ (c.f., Eq. B) becomes infinitesimal], cells are considered growth arrested, or quiescent.

Radiation response model

After exposure to irradiation, cells become arrested in the cell cycle and attempt to repair radiation-induced DNA damage (5). The probability of successful repair and thus cell survival after application of dose $d$ is modeled using the established linear–quadratic (LQ) model:

$$S = e^{-d/(\alpha + \beta d)}$$
decreased latencies relative to untreated tumors (11, 22). The survival and increase of the GSC population during radiation therapy may be a leading cause of accelerated and more aggressive GBM recurrence after radiation therapy.

In the present study, we found that resistance of GSCs to IR alone is insufficient to explain the experimentally observed increased GSC ratios after fractionated radiation treatment. We show that an additional radiation-induced shift in GSC division in favor of symmetric division or a faster cycling time reproduces the reported in vitro and in vivo enrichment of GSCs (21). On the basis of our previous studies (8, 16, 23, 24), we develop a cellular Potts model (CPM) that simulates cancer stem cell–driven GBM growth and radiation response. In particular, this model estimates symmetric and asymmetric GSC division rates before and after irradiation and simulates the growth dynamics of the irradiated GBM population with

\[
d\alpha \text{d} describes cell killing due to a single event and \beta d^2 \text{ describes cell killing after combination of two independent, potentially repairable events, with } \alpha \text{ and } \beta \text{ being cell-specific radiosensitivity parameters (6). We introduce } \xi \text{ and } \lambda \text{ as radiation protection factors for quiescent cells and GSCs, respectively. The basic LQ model has been extended to take into account the effects of interfraction tumor repopulation (6). Alternatively, the LQ model can be applied repeatedly as independent events at discrete time intervals if interfraction population dynamics are simulated by a tumor growth model (7, 8). We assume the cells that are fated to die undergo cell death at the next division attempt, which is achieved by setting the proliferation capacity of a hit cell to } \rho = 0.\]

![Figure 1. Schematic of cell division fate model. A, GSCs (red circle) divide with rate } k, either symmetrically with probability } p_s \text{ or asymmetrically with probability } 1 - p_s \text{, where one daughter cell is a non–stem CC (green hexagon) with proliferation potential } r_{\text{max}}. \text{ B, non–stem CCs (green hexagon) grow with rate } k \text{ and produce } 2 \text{ CCs with decremented proliferation potential } r/ C_0 \text{ if } r > 0, \text{ or die if proliferation potential is exhausted (} r = 0). \text{ C, flowchart of simulation process.}
and without radiation-induced constitutive changes in GSC division kinetics.

Materials and Methods

Cell culture

The U87-MG cell line was obtained from American Type Culture Collection where cell line authentication and species identification was conducted. Cells were grown in Minimum Essential Medium (MEM; Invitrogen Life Technologies) supplemented with 10% FBS (Lonza) and maintained at 37°C with 5% CO₂ in humidified air. Time-lapse video microscopy was used to monitor cell behavior. Images were taken every 15 minutes, by using a digital camera (Photometric Coolsnap HQ2 CCD) coupled to an inverted microscope. We used CellTrack (25), an open-source software, for cell tracking and motility analysis to estimate cell proliferation and migration rates.

For clonogenic survival assays, single-cell suspensions were irradiated with the Gammacell 40 irradiator (MDS Nordion, Inc.) with doses ranging from 0 to 16 Gy at 0.48 Gy per minute. Irradiated cells were plated in 10-cm dishes at low density. Irradiated cells were plated in 10-cm dishes at low density. Cells were fixed with 70% ethanol and stained with 0.2% crystal violet 14 days after irradiation. Cell colonies of 50 cells or more were scored.

Simulation process

Cellular kinetics are simulated through the following steps (Fig. 1):

1) Increase the target volume of cell σ with fixed rate k.
2) If the volume of cell σ is doubled \(|v(\sigma) \geq 2v_0|\) cell division is attempted.
   a. If cell σ is a GSC, it divides symmetrically with probability \(p_s\) to produce 2 GSCs, or asymmetrically with probability \(1 - p_s\) to produce a GSC and a cancer cell (CC) with limited proliferation potential \(\rho_{\text{max}}\) (see next section).
   b. If cell σ is a CC, both daughter CCs inherit a decremented proliferation capacity \(\rho - 1\).
3) If the proliferation capacity of cell σ is exhausted (\(\rho = 0\)), the death of cell σ is invoked by setting \(V_{\text{target}}(\sigma) = 0\).
4) Cell σ is labeled as currently quiescent when no increase in cell volume \(v(\sigma)\) has occurred for consecutive simulated \(t = 6\) hours (180 MCS).
5) If a cell σ is irradiated, the cell gets arrested in its cell cycle for a period of time \(T_r\).
6) If a cell σ is unrepaired when re-entering the cycle [calculated by Eq. (C)], cell death is evoked by setting proliferation capacity \(\rho = 0\).

Parameterization

We set the initial size of a CC in the CPM to \(v_0 = 4 \times 4\) pixels. We estimated the average diameter of U87-MG cell, \(r\), \(\textit{in vitro}\) at approximately 10 μm \((n = 20)\). Using these measurements, 1 pixel equals \(2.5 \times 2.5\) μm². The average migration speed of U87-MG cells, \(w\), \(\textit{in vitro}\) is 23.4 μm/h \((n = 24;\) Fig. 2A), which is in good agreement with the literature (26, 27). The average replacement of cells in CPM is approximately 0.31 pixels MCS⁻¹ (Fig. 2B). Equating experimental and simulated cell migration speeds implies that 30 MCS approximate 1 hour. By tracking 2 consecutive mitotic events of individual U87-MG cells, we obtained the cell-cycle length to be \(T_c = 25\) hours \((n = 22)\). Therefore, the growth rate of \(\textit{in silico}\) cells is \(k = (V_{\text{target}} - v_0)/T_c = 0.0213\) pixel MCS⁻¹, where \(V_{\text{target}} = 2v_0\) represents the doubling volume of CCs. The proliferation potential of CCs is set to \(\rho_{\text{max}} = 10\), which has previously been shown to enable fast tumor growth (16).

Radiation-induced cell-cycle arrest of U87-MG cells is observable through a decreased mitotic index immediately after irradiation that returns to control levels after 16 hours (5). We assign a cell-cycle arrest time \(0 \leq T_a \leq 16\) hours for each cell from a uniform distribution. Using clonogenic assays for long-term survival of U87-MG cells after single doses of radiation ranging from 0 to 16 Gy, we derived for sensitivity parameters in the LQ model [Eq. (C)] the unconstrained best-fit values of \(\alpha = 0.3859\) and \(\beta = 0.01148\) (Fig. 3). Radioresistance of quiescent cells is a major determinant for treatment success (28), and the spatial distribution of proliferating cells and their killing by radiation plays a crucial role in the redistribution of quiescent cells into the cell cycle (8). For radiosensitive proliferating cells, we assume \(\xi = 1\), and a reduced sensitivity of \(\xi = 0.5\) is assumed for non-cycling quiescent cells (29, 30).

To match the reported increase in GSC fraction after different doses of irradiation (21), survival probabilities above 100% are required, which implies that different \(\sigma\) and \(\beta\) values for GSCs alone are insufficient to explain the reported data. Nevertheless, GSC radiation response parameters need to be reliably identified in the future. From the dose–response survival curves (Fig. 3), we estimate the GSC radiation protection factor \(\lambda_{\text{GSC}} = 0.1376\) by applying 4.5-fold lower apoptosis rate than CCs \((\lambda_{\text{CC}} = 1)\) at 3 Gy radiation as reported (11). Model parameters with their values are listed in Table 1.

Results

Tumor population growth

To compare \(\textit{in silico}\) tumor population formation with observed \(\textit{in vitro}\) morphologies, we place a GSC in the center of the computational lattice and monitor tumor growth for 15 days. The seeded cell conducts a random walk and gives rise to two daughter cells that subsequently repeat the aforementioned dynamics. An initially sparse collection of cells forms an \(\textit{in silico}\) tumor population (Fig. 2D) comparable with \(\textit{in vitro}\) U87-MG growth (Fig. 2C).

Estimation of GSCs symmetric division rate

The fraction of U87 CD133+ cells \(\textit{in vitro}\) and \(\textit{in vivo}\) has recently been estimated to be 1.8% to 3.0% (31), which is comparable with 2.5% ± 2.12% GSCs reported in primary GBM specimens. The frequency of GSCs depends pivotally on the frequency of symmetric GSC division events \((p_s\) in our model) to expand the stem cell pool. To estimate the symmetric division rate of GSCs, we simulate tumor growth with...
varying $p_s$ values. By comparing the frequency of GSCs in \textit{in silico} tumors of $10^5$ cells with the reported fraction of CD133$^+$ cells, we estimated the symmetric division rate to be 35\% to 45\% ($p_s = 0.35$–0.45) for the U87-MG cell line (Supplementary Fig. S1). Taking the lower boundary, that is, $p_s = 0.35$, we simulate the growth of 5 tumors from one GSC to a population of about $10^5$ cells with average of 1.81\% GSCs ($n = 5$; Fig. 4).

**Enrichment of GSCs**

We simulate continuing tumor growth either without (control) or with exposure to IR and compare GSC fractions and tumor progression rates. Using available data in the literature, we simulate single-dose irradiation with 6 Gy. At 48 hours after irradiation, the simulated percentage of GSCs is 7.4\%, approximately 4.1-fold enrichment relative to the untreated control (1.81\%, c.f. Fig. 4A), reproducing the increased ratios of GSCs reported in the literature (3- to 5-fold; refs. 11, 21). The observed enrichment is due to (i) a larger fraction of CCs being killed by IR and (ii) GSCs re-entering the proliferation cycle.

Surprisingly, GSC enrichment to 10.33\% after fractionated radiation of $3/2$ Gy as reported experimentally (21) was not observed in the CPM, even if increased damage repair in GSCs (11) yields no GSC cell-cycle arrest and immediate
advancement in the cell cycle (Fig. 4A). This disparity indicates that the increase in the proportion of GSCs was not only caused by selection of a radioresistant subpopulation but also an increase in GSC population. Therefore, we hypothesize that repeated exposure to radiation might alter the division kinetics of GSCs. The underlying mechanisms might be comparable with activated signaling pathways such as Sonic Hedgehog (SHh), Notch, Wnt, and EGFr receptor (EGFR) that enable the expansion of the somatic stem cell pool through increased symmetric division during normal tissue development and wound healing (32). Radiation has furthermore been shown to activate the AKT/cyclin D1/Cdk4 pathway in human glioblastoma cells (33), which yields a significant decrease in cell growth rate of 15 to 16 hours in human embryonic stem cells (34). As single-dose IR of 2 Gy did not cause a significant shorter cell-cycle time compared to somatic cells due to an abbreviated G1 phase (34), the radiation response in human glioblastoma cells (33) suggests that there might be an underline mechanism that modulates both mechanisms modulated by half of the estimated difference when altered individually (Fig. 4A).

Accelerated tumor repopulation

Simulated tumors of $10^7$ cells treated with fractionated $3 \times 2$ Gy IR reach pretreatment size 6 days after treatment start (3 days after final dose; Fig. 4B), and 3 weeks after treatment start the average mass of irradiated tumors exceeds that of untreated control tumors. While control tumors took 14 days to double their cell number (to $2 \times 10^7$ cells), tumors treated with fractionated IR doubled in 10 days. The overall tumor population growth rates (not to be confused with cell growth rate $k$) after treatment with fractionated IR for all hypotheses (i–iii) are unanimously greater than 0.05 (Table 2), whereas untreated control tumors grow at a rate of 0.0389, which is in excellent agreement with previous untreated glioblastoma growth rate estimates (35, 36). Tumor composition 30 days after treatment began, however, is remarkably different for tumors after fractionated irradiation following the different hypotheses discussed above. While tumors with a shortened $T_c = 12$ hours [hypothesis (ii)] have the fewest stem cells, their total cell number is the highest due to frequent production of CCs. Tumors formed by GSCs with largest $p_s = 0.75$ [hypothesis (i)] contain the largest number of GSCs, but the least overall total number of cells due to the longer cell cycle compared hypotheses (ii) and (iii; Fig. 4B and C).

Table 1. Model parameters and values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>Cell diameter</td>
<td>10 μm</td>
<td>In vitro U87-MG cell line</td>
</tr>
<tr>
<td>$w$</td>
<td>Cell migration speed</td>
<td>23.4 μm/h</td>
<td>In vitro U87-MG cell line</td>
</tr>
<tr>
<td>$T_c$</td>
<td>Cell-cycle time</td>
<td>25 hours</td>
<td>In vitro U87-MG cell line</td>
</tr>
<tr>
<td>$k$</td>
<td>Cell growth rate</td>
<td>0.0213 pixel MCS⁻¹</td>
<td>In vitro U87-MG cell line</td>
</tr>
<tr>
<td>$r_{max}$</td>
<td>Non–stem cell proliferation capacity</td>
<td>10</td>
<td>Assumed from ref. 16</td>
</tr>
<tr>
<td>$p_s$</td>
<td>GSC symmetric division rate</td>
<td>0.35</td>
<td>Estimated using data from ref. 21</td>
</tr>
<tr>
<td>$T_a$</td>
<td>Cell-cycle arrest time post-IR</td>
<td>Random in 0–16 hours</td>
<td>In vitro U87-MG cell line (5)</td>
</tr>
<tr>
<td>$a$</td>
<td>Radiosensitivity of single-hit killing</td>
<td>0.3859</td>
<td>In vitro U87-MG cell line</td>
</tr>
<tr>
<td>$β$</td>
<td>Radiosensitivity of double-hit killing</td>
<td>0.01148</td>
<td>In vitro U87-MG cell line</td>
</tr>
<tr>
<td>$λ_{GSC}$</td>
<td>Radioprotection of GSCs</td>
<td>0.1376</td>
<td>Estimated from in vitro U87-MG cell line and ref. 11</td>
</tr>
<tr>
<td>$ζ$</td>
<td>Radioprotection of noncycling cells</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

We have presented a CPM of GSC and CC kinetics in glioblastoma growth and response to IR. The study aimed to identify GSC division modes that generate observed frequencies of stem cells in GBM, as well as the role of GSCs in GBM response to IR and subsequent accelerated repopulation. The key cell kinetics parameters in the model were calibrated using experimental data obtained in our laboratory as well as from the literature, yielding simulations of population growth that are in good agreement with experimental results. We obtained
the best agreement with reported fractions of GSCs if symmetric division occurs in 35% to 45% of mitotic GSC events. While these division probabilities reproduce experimentally observed enrichment ratios after single-dose irradiation, our simulations reveal that such frequencies are insufficient to reproduce the 6-fold enrichment observed after exposure to identical dose delivered in smaller fractions.

We hypothesized that fractionated radiation increases activation of GSC signaling pathways such as AKT/cyclin D1/Cdk4, SHH, Notch, Wnt, and EGFR, which have been shown to orchestrate stem cell survival, self-renewal, proliferation, and differentiation during normal tissue development and repair (ref. 32; Fig. 5). While these pathways are tightly regulated in normal tissue, it is conceivable that their regulation is aberrant in tumors (Fig. 5). Recently, Lathia and colleagues (37) showed that GSC division mode is regulated by growth factors including EGF and fibroblast growth factor (FGF). EGF-mediated signaling pathways have been shown to contribute to increased migration, survival, proliferation, and self-renewal capacity (38) and to maintain the stemness of GSCs with enhanced malignant phenotypes (39). SHH-Gli signaling is required for self-renewal and tumorigenic potential of GSCs, and its inhibition is able to prevent GSCs proliferation (40). Notch signaling is highly involved in regulating self-renewal and expansion of many different types of normal and cancer stem cells (41). Inhibition of Notch signaling in GSCs (e.g., by γ-secretase inhibitor) attenuates the formation of neurosphere-like colonies, increases neuronal differentiation, reduces CD133+ cell fraction in vitro, and decreases tumorigenicity in vivo (42), whereas its overexpression has been linked to GSC radioreistance (43) much like Wnt signaling (44–46). Particularly, Notch2 was markedly upregulated in glioma cells treated with fractionated radiation compared with single-dose radiation (21), lending further support to our hypothesis. Increased activation of Notch-1 following fractionated radiation was also found in breast cancer stem cells conceiving a possible mechanism for the accelerated repopulation of breast tumors following radiotherapy (47).

Model simulations show that either an increased symmetric division rate or a faster cell cycle of GSCs after repeated exposure to radiation is sufficient to reproduce the elevated enrichment observed experimentally. Frequencies of symmetric GSC division even larger than the herein estimated 75% have been reported recently if exposed to the right milieu of growth factors (37), and aforementioned literature suggests that fractionated irradiation is capable of creating such environment. Observations or references to the alternative hypothesis of a postirradiation cell cycle in glioma cells as short as 12 hours, however, are unbeknownst to the authors. It is, nevertheless, conceivable that the increased activation of stem cell division kinetics signaling pathways as discussed above partially modulates both cell-cycle progression and symmetric division of GSCs. The simulated example with $p_s = 55\%$ and $T_c = 18.5$ hours (both mechanisms modulated by half of the estimated difference when altered individually) reproduces the experimental data similarly well with biologically relevant parameter values. Increase in GSC symmetric division rate appears to be the required mechanism for increasing the GSC pool, which is in line with previous calculations that regulation of self-renewal is essential for efficient repopulation in the healthy hematopoietic system.

### Table 2. Comparison of tumor composition and growth rates after simulated IR schedules (means ± SD, $n = 5$)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of GSCs</th>
<th>% of GSCs</th>
<th>Post-IR population growth rate</th>
<th>No. of total tumor cells 30 d after IR begins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2,137 ± 311</td>
<td>1.81% ± 0.21%</td>
<td>0.0389</td>
<td>347,973 ± 19,788</td>
</tr>
<tr>
<td>6 Gy</td>
<td>2,411 ± 318</td>
<td>7.4% ± 0.83%</td>
<td>0.0445</td>
<td>313,866 ± 15,043</td>
</tr>
<tr>
<td>$3 \times 2$ Gy, $p_s = 0.75$ hypothesis (i)</td>
<td>5,291 ± 707</td>
<td>10.44% ± 0.85%</td>
<td>0.0527</td>
<td>367,254 ± 12,540</td>
</tr>
<tr>
<td>$3 \times 2$ Gy, $T_c = 12$ h hypothesis (ii)</td>
<td>6,591 ± 801</td>
<td>10.84% ± 0.82%</td>
<td>0.0535</td>
<td>403,629 ± 22,444</td>
</tr>
<tr>
<td>$3 \times 2$ Gy, $p_s = 0.55$, $T_c = 18.5$ h hypothesis (iii)</td>
<td>5,908 ± 851</td>
<td>10.99% ± 1.1%</td>
<td>0.0539</td>
<td>395,517 ± 14,605</td>
</tr>
</tbody>
</table>

### Figure 5. A, normal stem cell (SC) response to tissue injury adapted from the work of Beachy and colleagues (32). B, proposed GSC response to fractionated irradiation exposure.

![Figure 5](image-url)
(48). Future experiments will need to identify which of the discussed hypotheses is prevalent after fractionated irradiation of glioblastoma cells.

Taken together, the presented study reveals that subthalamic perturbation of a primary GBM selects for and increases the more aggressive subpopulation of GSCs, yielding accelerated repopulation, fast recurrence and worsens prognosis, as previously hypothesized (11, 22). If GSCs survive irradiation, recurring tumors show increased population growth rates. Consequently, pretreatment tumor sizes will be acquired relatively shortly after irradiation with inadequate dose and inevitably such treated tumors will outgrow untreated control. While the diffuse nature of GBM is the major cause for tumor recurrence, fractionated radiation-induced increase in GSC self-renewal capacity and/or accelerated cell-cycle progression are conceivable as general mechanisms contributing to tumor resistance and accelerated repopulation after apparently adequate radiation treatment.

For computational convenience, we focused in our study on early microscopic tumors. Tumors smaller than diffusion-limited size (~1 mm in diameter, 10^6 cells) are fully oxygenated (49), and therefore hypoxia can be ignored in the presented model. It is of note, however, that cancer stem cell driven solid tumor growth is self-similar and population composition at smaller sizes is representative for later tumors (16, 50). For the applicability of the treatment model, we might argue that such microscopic tumors exist in the target area of radiation. GBMs are highly infiltrative, and while surgery removes the bulk of the tumor, an appreciable number of CCs and microscopic foci have diffused beyond surgical margin throughout the brain (51).

We used in vitro data to parameterize radiosensitivity parameters of the linear–quadratic model (Eq. 3). The obtained α and β values are an order of magnitude larger than estimated by Rockne and colleagues (52) to match clinical response of primary GBMs. By scaling the radiosensitivity of quiescent cells (ζ) as well as GSCs (λ), however, the linear sensitivity parameter α of the overall population matches their estimated parameters from clinical response data (52).

The only parameter in the presented model that we are unable to estimate (and to our knowledge has not been reported in the literature) is the proliferation capacity \( p_{\text{max}} \) of CCs. We assumed \( p_{\text{max}} = 10 \) based on previous simulation results that revealed aggressive tumor progression for this parameter value (16). This parameter, however, plays a pivotal role in modulating the frequency of cancer stem cells in solid tumors (50). Therefore, the estimated GSC symmetric division rate of 35% to 45% to yield 1.8% to 3.0% of GSCs in the total tumor population is only an estimate that is closely linked to the chosen CC proliferation capacity. Further experimental work into identifying the proliferation capacity of CCs is required to confidently derive GSC division modes pre- and posttherapeutic intervention. Furthermore, our studies were conducted on the U87-MG cell line that was reported in the literature (21). This cell line has limited capability to recapitulate the in vivo biology of human glioblastoma, and further validation on primary GSCs is required before profound conclusions for treatment planning can be drawn.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the NIH. No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: X. Gao, J.T. McDonald, L. Hlatky, H. Enderling

Development of methodology: X. Gao, H. Enderling

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.T. McDonald, L. Hlatky

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Gao, J.T. McDonald, L. Hlatky, H. Enderling

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