Figures and figure supplements

Rescue of cognitive function following fractionated brain irradiation in a novel preclinical glioma model

Xi Feng et al
Figure 1. GL261-DTR model allows manageable tumor growth. (A) Survival curve of mice injected with GL261-DTR cells and the response to DT or combined DT and fWBI treatments. DT treatment was given at 1 ug daily between Day 7 and 12, the combined DT and fWBI treatment group received extra DT treatment at 1 ug every other day between Day 31 and 45. **p_tumor + DT = 0.0076, **p_tumor + DT + fWBI = 0.0033, Mantel-Cox Log-rank test compared to the tumor only group. (B) BLI of the tumor only and the tumor +DT groups. DT treatment resulted in delayed tumor growth. (C) BLI of mice received combined DT and fWBI treatment. Irradiation further delayed tumor growth, and extra DT treatment was able to delay growth of tumors when they recur. (tumor only no treatment control, DT and DT +fWBI treatments were randomly allocated among tumor bearing mice, N = 5 – 7 each treatment group, median survival is labeled next to the curve of each group, sample size was selected to minimize the number of animals used for this pilot experiment.

DOI: https://doi.org/10.7554/eLife.38865.002
Figure 1—figure supplement 1. Response of GL261-DTR cells to 48 hr’ DT treatment in vitro. Treatment with 10 ng/ml DT killed all GL261-DTR cells in 48 hr.
DOI: https://doi.org/10.7554/eLife.38865.003
Figure 2. CSF-1R inhibitor treatment improves survival and prevents fWBI-induced memory deficits in glioma bearing mice. (A) No memory deficits were seen in GL261-DTR bearing mice at 16 days after tumor implantation. ns = not significant, ****p<0.0001, paired t-test. N = 5, error bars show mean ±SEM. (B) Improved survival of GL261-DTR bearing mice with concurrent DT, fWBI and/or CSF-1R inhibitor treatment. Timeline of treatments and behavior tests are labeled on the X-axis. Novel Object Recognition (NOR) tests were performed between day 28 and 31, or day 46 and 49, for the tumor +DT group or all other groups, respectively. \( *p_{\text{tumor + DT + fWBI}} = 0.0178 \), \( *p_{\text{tumor + DT + PLX}} = 0.0167 \), \( **p_{\text{tumor + DT + PLX + fWBI}} = 0.0029 \), Mantel-Cox Log-rank test compared to the tumor +DT group, survival curves show combined results from two independent experiments, N = 11–15. (C) The results of NOR tests, recognition memory was impaired by fWBI, and CSF-1R inhibitor treatment during fWBI was able to prevent this impairment. The tumor +DT + fWBI group showed memory deficits, this was prevented by CSF-1R inhibitor treatment, combined results from two independent experiments, N = 8–12, ns = not significant, \( **p_{\text{tumor + DT}} = 0.0011 \), \( ***p_{\text{tumor + DT + PLX}} = 0.0006 \), \( ***p_{\text{tumor + DT + PLX + fWBI}} = 0.0001 \), paired t-test. All treatments were randomly allocated among tumor bearing animals. F = familiar object, N = novel object.

DOI: https://doi.org/10.7554/eLife.38865.005
Figure 2—figure supplement 1. (A) DT and fWBI single treatments significantly prolonged survival of tumor bearing mice (**p=0.0021, ****p<0.0001). Combined DT and fWBI treatment further prolonged survival compared to no treatment (****p<0.0001) and each single treatments (****p<0.0001, *p=0.0178, compared to DT and fWBI treatments, respectively). (B) CSF-1R inhibitor (PLX5622) treatment did not significantly improve survival. No deficits in motor function or anxiety in GL261-DTR bearing mice. N

\text{tumor only} = 5, N

\text{tumor+DT} = 11, N

\text{tumor+PLX} = 5, N

\text{tumor+fWBI} = 5, N

\text{tumor+PLX+fWBI}=5, N

\text{tumor+DT+fWBI}=15, p values were calculated by comparing two curves using the Mantel-Cox test. (C) There was no difference among the groups in velocity of mice tested by the Open Field test (day 1 of NOR test). (D) There was no significant difference among the groups in time spent in the center of arena tested by the Open Field test (day 1 of NOR test). N = 5–12 each group. Results shown in Figures A and B are combined from three experiments. Results shown in Figure C and D are combined from two independent experiments.

DOI: https://doi.org/10.7554/eLife.38865.006
Figure 3. CSF-1R inhibitor treatment prevents fWBI-induced microglia activation in glioma bearing mice. (A) Plots of the CD11b mean fluorescent intensity of microglia in the contralateral hemisphere of tumor bearing mice. There was significant decrease of microglial CD11b MFI in CSF-1R inhibitor treated mice. There was significant CSF-1R treatment effect F(1,14) = 38.77, p<0.0001 with no significant fWBI effect or interaction. (B) Quantifications of Iba1 + signal covered area. Two-way ANOVA revealed significant PLX5622 treatment effect, F(1,13) = 39.14, p<0.0001, no significant radiation effect F(1,13) = 0.01122, p=0.9173 and significant interaction between PLX5622 treatment and fWBI, F(1,13) = 4.793, p=0.0474. There was a trend of increase in the tumor + DT + fWBI group (p=0.3044), and significant reduction in the tumor + DT + PLX group (**p=0.0017) and the tumor + DT + PLX + fWBI group (**p=0.0017), compared to the tumor + DT group. (C) Quantifications of CD68 + signal covered area. Two-way ANOVA revealed significant CSF-1R inhibitor treatment effect, F(1,13) = 53.07, p<0.0001, no significant radiation effect F(1,13) = 3.559, p=0.0817, and significant interaction F(1,13) = 7.529, p=0.0167. (D) Representative images of Iba1 and CD68 staining, showing Iba1 staining in red, CD68 staining in white and DAPI in blue. Scale bar = 50 um. (A-C, Two-way ANOVA with Dunnett’s multiple comparisons test vs the tumor + DT group, ns = not significant, *p<0.05, **p<0.01. Each dot represents value from one mouse (A) or the mean values of Iba1 and CD68 (B and C) staining quantification of 3 snapshots in the hippocampus, N = 4–5. Error bars show mean ±SEM.

DOI: https://doi.org/10.7554/eLife.38865.009
Figure 3—figure supplement 1. Tumor growth after treatment and timeline of flow cytometry analyses. BLI imaging during the week of fWBI treatment and timeline of treatments (related to Figures 3 and 4). All mice were euthanized between day 22 and 24.

DOI: https://doi.org/10.7554/eLife.38865.010
Figure 4. Treatment with a CSF-1R inhibitor alters myeloid cell composition in the contralateral hemisphere. (A) Plot of microglia (CD11b + CD45 lo) numbers in treated tumor bearing mice. There was significant CSF-1R inhibitor treatment effect (F(1,8) = 31.45, p=0.0005), but no significant radiation effect or interaction between these two treatments. Two-way ANOVA with Dunnett post-hoc comparisons against the tumor +DT group, ns = not significant, *p<0.05. (B) Plot of inflammatory monocyte (CD11b + CD45hiLy6Chi) numbers in treated tumor bearing mice. There was significant radiation effect (F(1,8) = 10.68, p=0.0114) and CSF-1R inhibitor treatment effect (F(1,8) = 23.49, p=0.0013), as well as significant interaction (F(1,8) = 5.52, p=0.0467) between these two treatments, two-way ANOVA with Dunnett post hoc comparisons against the tumor +DT group, ns = not significant, *p<0.05. Error bars show mean ±SEM values. Each dot represents cell count from a mouse, N = 4–5. DOI: https://doi.org/10.7554/eLife.38865.013
Figure 4—figure supplement 1. Changes in myeloid cells in the ipsilateral hemisphere and growth of tumors with single treatments. (A) Plot of microglia numbers in tumor bearing mice. (B) Plot of inflammatory monocyte numbers. (C) Plot of BLI signals after single treatments. (D) Plot of correlation between BLI signal (X axis) and monocyte numbers in the ipsilateral brain (Y axis). N = 3–5 each group.

DOI: https://doi.org/10.7554/eLife.38865.014