Luciferase Expression Elicits an Anti-Tumor Immune Response in GL261 Murine Glioma Models

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Introduction

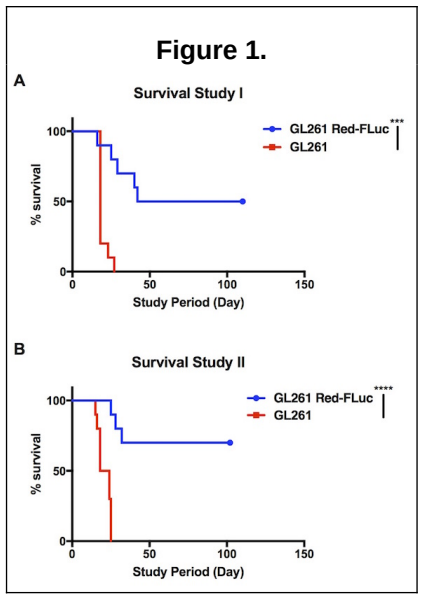
There has been renewed interest in immune therapy for glioblastoma due to the successes of these treatments in systemic tumors. Preclinical models that accurately recapitulate the immune suppressive properties of human gliomas are essential to evaluate such therapies. GL261 murine glioma cells are widely used as an in vivo model of glioma. The luciferase expressing cell line, GL261 Red-FLuc, has been widely used in the literature to enable non-invasive tumor monitoring in preclinical studies. It is unclear if these cells are equivalent, particularly when applied to investigating tumor immunology.

Methods

C57BL/6 mice (n=20 in each group) underwent stereotaxic, intracranial implantation with GL261 or GL261 Red-FLuc cells at 5x104cells/5uL and were assessed for survival. Flow cytometric analysis was performed on sacrificed mouse brain lysate which gated for T-cell infiltration and macrophages. Immunohistochemistry examination of immune cell populations in formalin-fixed paraffin embedded GL261 or GL261 Red-FLuc implanted brains from sacrificed mice was performed. Each parental cell line was assessed in vitro by CFSE proliferation assay, TGF-Beta2 ELISA, and proteome profiler immunoassay for broad cytokine analysis.

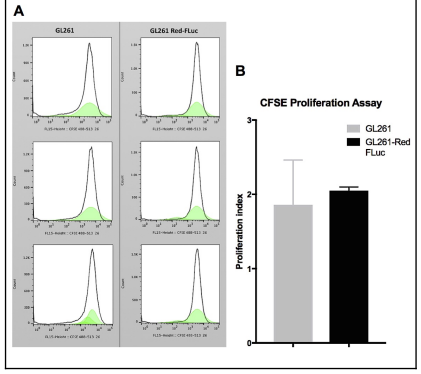
Results

Figure 1.



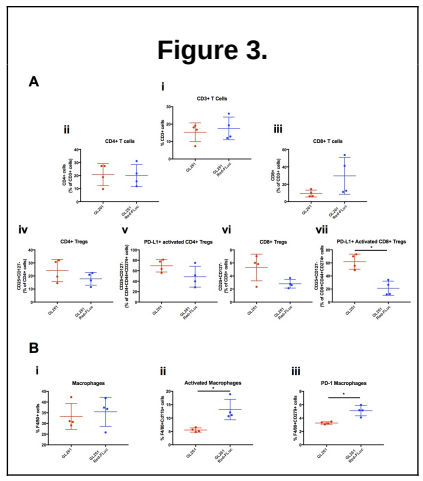
Median survival for GL261 implanted mice was 20.9± 1.3 days with all animals progressing to a moribund state, while median survival was not reached for animals implanted with GL261 Red-Fluc cells $P<0.001$ by Kaplan -Meier analysis.

Figure 2.



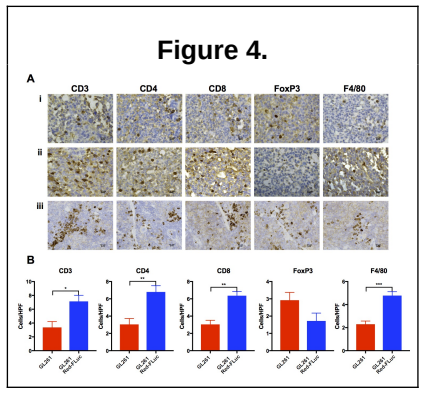
Proliferation assays revealed no difference between these cell lines.

Figure 3.



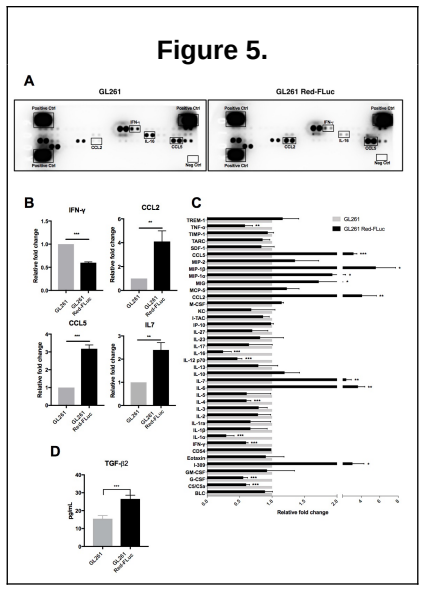
Flow cytometric analysis reveal greater T-cell infilration and a higher frequency of activated macrophages in GL261 Red-FLuc. In addition, there was markedly greater frequency of Treg cells in GL261 brains with upregulation of immune supressive marker, PD-L1, also correlating.

Figure 4.



IHC of sacrificed mouse brain reveal greater T-cell infiltration defined by markers CD3, CD4, and CD8 and macrophage defined by F4/80 in GL261 Red-Fluc-implanted brain. An increase of immunosuppressive T-regulatory cells defined by FoxP3+ T-cell subset was seen in GL261.

Figure 5.



Proteomic results demonstrated a significant difference in 16 of the 40 cytokines profiled. These include profiinflammatory cytokines such as IL-6 and IL-7 and chemoattractants CCL5 and CCL2 which were significantly upregulated in GL261 Red-FLuc. Broad spectrum cytokine analysis experiments were performed repeatedly in 3 independent experiments with duplicates for cytokines each time. In addition, TGFB2 ELISA assay demonstrate a consistent 2-fold increase in GL261 Red-FLuc cells in 4 independent experiments assayed in triplicate each time for intraassay precision.

Conclusions

The significantly different survival outcomes between mice injected with GL261 or GL261 Red-FLuc clearly establish reasons to question the pre-clinical use of this cell line. Further investigation revealed GL261 Red-Fluc cells create a pro-inflammatory microenvironment when implanted intracranially in C57BL/6 which is different from the tumor microenvironemnt created by non-transfected GL261 cells. GL261 cells appear to more acurately recapitulate the immunosuppressive properties characteristic of GBM as shown by the increase in Treg demonstrated by flow cytomtry and IHC. These findings suggest that investigators who seek to evaluate immune therapeutics in a C57BL/6 background may consider utilizing unaltered GL261 cells over GL261 Red-Fluc cells.

References

1.Nduom, E. K., Weller, M.,&Heimberger, A. B. (2015). Immunosuppressive mechanisms in glioblastoma. Neuro-Oncology, 17(Suppl 7), vii9–vii14. 2.Clark, A. J., Safaee, M., Oh, T., Ivan, M. E., Parimi, V., Hashizume, R., Parsa, A. T. (2014). Stable luciferase expression does not alter immunologic or in vivo growth properties of GL261 murine glioma cells. Journal of translational medicine, 12(1), 345.3.Wei, J., Nduom, E. K., Kong, L. Y., Hashimoto, Y., Xu, S., Gabrusiewicz, K., ...&Ivan, C. (2015). MiR-138 exerts anti-glioma efficacy by targeting immune checkpoints. Neuro-oncology, 18(5), 639-648.