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Proceeding Paper

Amelioration of Glioblastoma Multiforme via the Combination of Simulated Microgravity and Oncolytic Viral Therapy †

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Abstract: Glioblastoma multiforme (GBM) is the most common aggressive malignant primary brain tumor, afflicting approximately 3.19 per 100,000 persons in the United States with an incidence 1.6 times higher in males compared to females. Arising from the glial cells known as astrocytes, GBM is commonly located in the supratentorial region (cortical lobes) affecting the frontal lobes. A unique feature of this tumor is its rapid local growth and spread making the prognosis very poor with a 5-year survival rate of 6.9%. The treatment of GBM remains challenging. Multiple therapeutic interventions are used for GBM, including the surgical resection of the tumor, radiotherapy and chemotherapy. Other experimental methods for the treatment of GBM include immune therapy, gene therapy, simulated microgravity therapy and oncolytic viral therapy. We propose a combination therapy of simulated microgravity using a clinostat-based three-dimensional culture system with oncolytic viral therapy using an autonomous rat parvovirus H1. Our hypothesis combines the beneficial effects of simulated microgravity and oncolytic viral therapy to lyse tumor cells through the induction of apoptosis, decreased cell proliferation and/or the induction of an immune response. This proposal provides the foundations to construct novel breakthroughs in the treatment of GBM.

Keywords: brain tumor; clinostat; chromosomal loss; epidermal growth factor receptor (EGFR); genetically modified viruses; mutations; neurosurgery; parvovirus H1 (H-1PV); primary malignant brain tumors; radiotherapy; suicide genes

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1. Introduction

Glioblastoma multiforme is the most common aggressive malignant primary brain tumor, affecting approximately 3.26 per 100,000 persons in the United States with an incidence 1.6 times higher in males compared to females. GBM has the highest incidence of malignant brain tumors and a median survival rate of 8 months. The median age of diagnosis of 65 years. It is uncommon in children accounting for 2.7% of all brain and CNS tumors reported among individuals aged 0 to 19 years. The incidence is highest in the northeast and lowest in the south-central region of the United States. Whites have the highest incidence rates for GBM and it is more common in males compared to females (4.08 vs. 2.55) [1].

GBM arises from glial cells known as astrocytes and it is commonly located in the supratentorial region (cortical lobes), usually affecting the frontal lobe (Figure 1). GBM has many forms on gross examination. Transverse sections under the microscope show areas of hemorrhage and necrosis, pleomorphic nuclei and cells, pseudopalisading necrosis, and microvascular proliferation (Figure 2) [2]. A unique feature of this tumor is its rapid local growth and spread, making the prognosis very poor with a 5-year survival rate of 6.9% [1].

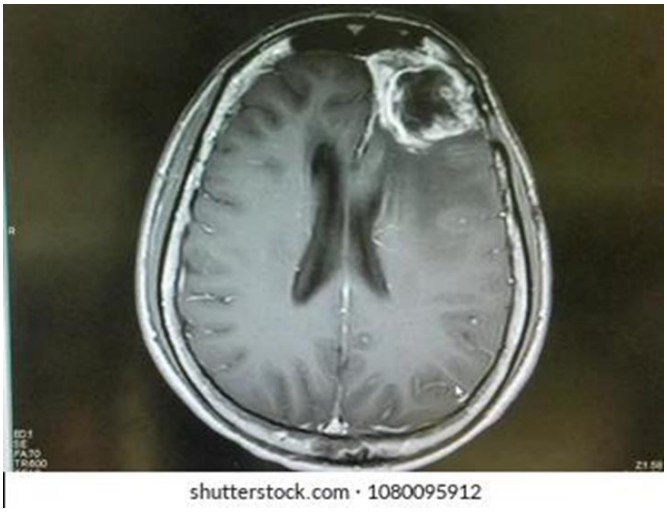

Figure 1. Coronal section of magnetic resonance imaging showing glioblastoma mutiforme in the left frontal lobe of the brain. The right side of the image represents the left side of the brain. https://www.shutterstock.com/image-photo/mri-brain-show-left-frontal-gliblastoma-1080095912, accessed on accessed on 9 April 2022.

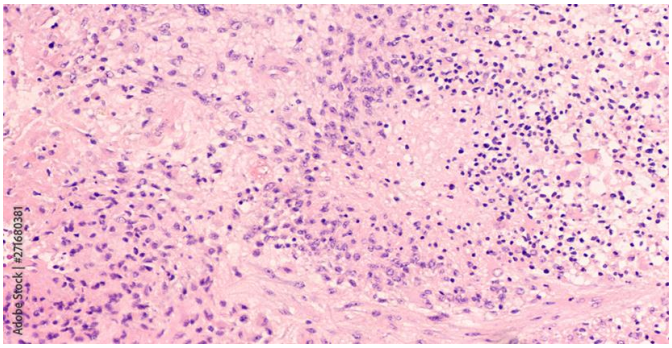

Figure 2. Microscopic image showing histology of a glioblastoma multiforme. Necrosis and vascular proliferation are diagnostic features of this high-grade malignant tumor. https://stock.adobe.com/search?load_type=search&native_visual_search=&similar_content_id=&is_recent_search=&search_type=usertyped&k=glioblastoma+multiforme&asset_id=271680381, accessed on 9 April 2022.

Multiple risk factors linked to GBM include exposure to radiation, a weak immune system, and increased age. Other risk factors are high socioeconomic status, decreased allergy susceptibility, and the use of anti-inflammatory medications [1].

Several genetic and molecular mechanisms have been identified to play a role in the development of GBM (Figure 3). Primary de novo GBM without evidence of a less malignant precursor is associated with epidermal growth factor receptor (EGFR) overexpression, pleitrophin mutation and a loss of chromosome 10. Secondary GBM arising from a low-grade astrocytoma or anaplastic astrocytoma is associated with IDH1 mutations and TP53 mutations causing excessive expression of alpha synuclean protein, the same protein involved in Parkinson's disease and chromosome 19q loss [3–6].

The treatment of GBM remains challenging, despite multiple therapeutic interventions including surgical resection of the tumor [6], post operative radiotherapy, and chemotherapy. The extent of resection is determined after assessing the preoperative prognosis of the case and the location of the tumor [6]. Since GBM is a highly aggressive tumor, total surgical resection would lead to permanent neurological deficits, especially if the tumor is located in critical areas (cortical areas) [7]. Increased survival results from postoperative radiation therapy to doses of 5000–6000 [8]. Common chemotherapeutic agents used for GBM include temozolomide (TMZ) which can be given concomitant with radiotherapy and after it. Bevacizumab can be used along with TMZ to improve progression of free

survival [9]. Despite all these therapeutic interventions, GBM has a very poor prognosis with a 5-year survival rate of 6.9%; hence, novel interventions are required for the treatment of this highly malignant tumor.

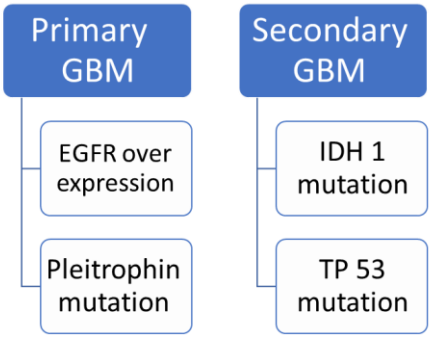

Figure 3. Schematic diagram to represent the genetic and molecular mechanisms of primary and secondary glioblastoma multiforme (GBM). GBM: glioblastoma multiforme; EGFR: epidermal growth factor receptor.

2. Materials and Methods

We hypothesize that the growth of malignant glioma cells will be aborted by the application of a combination of simulated microgravity and oncolytic viral therapy [10]. Malignant glioma cells in Dulbecco's modified medium supplemented by penicillin (100 units/mL), streptomycin (100 micrograms/mL), 10% fetal bovine serum, white blood cells, granulocyte-monocyte colony-stimulating factor and parvovirus H1 at 37 °C with a humidified 5% CO_2 atmosphere will be placed in simulated microgravity using a clinostat-based three-dimensional (3D) culture system. The necrosis of malignant glioma cells will be verified by inverted contrast phase and quantum microscopy, as well as flow cytometry mitochondrial membrane potentials. We hypothesize that all malignant glioma cells will die after two weeks of this protocol [10].

3. Results and Discussion

Evidence from in vitro, animal and clinical studies suggest that our hypothesis could provide a new therapy for glioblastoma multiforme. We aim to confirm our hypothesis through clinical trials.

3.1. Simulated Microgravity Effects on Tumor Cells

3.1.1. Simulated Microgravity Effects on Glioblastoma Multiforme

Microgravity during space flights on different tumor cells including glioblastoma multiforme showed that microgravity causes decreased cell proliferation, decreased secretory activity, and the induction of apoptosis. Simulated microgravity can be produced on earth by floating or using a clinostat-based 3D culture system, resulting in an environment with an average of 10^{-3} G [10,11].

The effects of cisplatin on cell proliferation and mitochondrial activity of glioblastoma multiforme were assessed with simulated microgravity using a 3D clinostat. Various cell lines were used in this study, including the D54MG (human glioma; wild p53), U251MG (human glioma; mutant p53), and T98G (human glioma; mutant p53) cell lines. The samples were divided into 2 groups, (C) cells under 100 G force and (CL) cells incubated in 1 G force generated by a 3D clinostat. An inverted phase contrast microscope was used to examine the morphological changes in tumor cells. The fluorescent dye rhodamine 123 was used to measure the mitochondrial membrane potential. After 3 days, simulated microgravity induced glioblastoma cells growth inhibition by affecting mitochondrial activity and increasing the sensitivity of tumor cells to cisplatin [12].

3.1.2. Simulated Microgravity Effects on Thyroid Cancer Cells

Multiple studies have been conducted to expose thyroid cancer cells to simulated microgravity using the 3D clinostat. These exposures have resulted in a variety of changes including early changes to the cytoskeleton and focal adhesion molecules, decreased proliferation, increased rate of apoptosis, and inhibition of cellular migration [13–18].

3.2. Oncolytic Viral Therapy

A new promising treatment for cancer is oncolytic viral therapy, in which a virus or a genetically modified virus is injected into tumor cells directly or via a systemic route (intravenously) to induce the direct lysis of tumor cells and/or a systemic immune response to attack the tumor cells [19]. For example, an attenuated thymidine kinase-negative mutant of herpes simplex virus-1 (dlsptk) produced prolonged survival in mice with U87 gliomas [20].

Multiple factors are involved in attacking the viral particles in glioblastoma multiforme cells such as TRAF3, INF-related factor 3, INF7 and RIG-1. These factors activate a JAK-STAT pathway to a activate PKR pathway leading to termination of protein synthesis and cell death. Parvovirus also attacks the tumor cells via induction of a systemic immune response with multiple cytokines such as type 1INF, TNF alpha, INF gamma and IL-12. The systemic immune response stimulates antigen presenting cells (APCs) such as dendiritic cells to activate T-helper cells CD 4 and C-cytotoxic cells CD 8 leading to tumor cell lysis [21]. Glioblastoma cells exhibit counteracting evading mechanisms via surface receptors which inactivate the effector immune cells and secrete inhibitory cytokines such as IL-10, TGF-B and IDO to recruit immune suppressive cells. However, the viruses can be genetically modified to escape this suppressive microenvironment [22].

3.2.1. Parvovirus and Glioblastoma Multiforme

Parvo H1 virus has been used in experiments on glioblastoma multiforme due to its kinetics to cross the blood brain barrier [19,23].

3.2.2. Reovirus, Measles Virus, and Pancreatic Cancer

The promising response of an intraperitoneal reovirus for peritoneal metastases of pancreatic ductal adenocarcinoma in hamsters led to an ongoing Phase II clinical trial [24]. A modified measles virus produced tumor shrinkage and greater survival in mice with pancreatic tumor xenografts [25]. A measles virus was also genetically modified to target the purine nucleoside phosphorylase drug and the protein prostate stem cell antigen, which is expressed in pancreatic cancer, resulting in oncolysis in immunocompromised mice treated with viral treatment [26].

4. Conclusions

The treatment of glioblastoma remains a challenge. Simulated microgravity and oncolytic viruses offer promise for the therapy of glioblastoma multiforme.

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Abbreviations

3D	Three-dimensional
EGFR	Epidermal growth factor receptor
GBM	Glioblastoma multiforme
INF	Interferon
RIG-1	Retinoic acid-inducible gene 1
TMZ	temozolimide
TNF	Tumor necrosis factor
TRAF3	TNF receptor-associated factor 3
TRAIL	TNF-related apoptosis-inducing ligand

References

1. Ostrom, Q.T.; Price, M.; Neff, C.; Cioffi, G.; Waite, K.A.; Kruchko, C.; Barnholtz-Sloan, J.S. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2015–2019. *Neuro. Oncol.* **2022**, *24* (Suppl. S5), v1–v95. [CrossRef] [PubMed]
2. D'Alessio, A.; Proietti, G.; Sica, G.; Scicchitano, B.M. Pathological and Molecular Features of Glioblastoma and Its Peritumoral Tissue. *Cancers* **2019**, *11*, 469. [CrossRef] [PubMed]
3. Ryskalin, L.; Biagioni, F.; Morucci, G.; Busceti, C.L.; Frati, A.; Puglisi-Allegra, S.; Ferrucci, M.; Fornai, F. Spreading of Alpha Synuclein from Glioblastoma Cells towards Astrocytes Correlates with Stem-like Properties. *Cancers* **2022**, *14*, 1417. [CrossRef] [PubMed]
4. Elshourbagy, T.; Brašić, J.R.; Syed, A.B. Guidelines for the Diagnosis and Treatment of Parkinson's Disease. *Biol. Life Sci. Forum* **2021**, *9*, 9. [CrossRef]
5. Elshourbagy, T.; Syed, A.B.; Amer, M.A.M.; Brasic, J.R. Precision medicine to identify optimal diagnostic and therapeutic interventions for Parkinson's Disease. *Med. Sci. Discov.* **2021**, *8*, 514–519. [CrossRef]
6. Yong, R.L.; Lonser, R.R. Surgery for Glioblastoma Multiforme: Striking a Balance. *World Neurosurg.* **2011**, *76*, 528–530. [CrossRef]
7. Barani, I.J.; Larson, D.A. Radiation Therapy of Glioblastoma. In *Cancer Treatment and Research*; Springer International Publishing: Cham, Switzerland, 2015; pp. 49–73.
8. Fernandes, C.; Costa, A.; Osório, L.; Lago, R.C.; Linhares, P.; Carvalho, B.; Caeiro, C. *Glioblastoma*; Chapter 11 Current Standards of Care in Glioblastoma Therapy; Creative Commons: Mountain View, CA, USA, 2017; pp. 197–241. [CrossRef]
9. Carter, T.C.; Medina-Flores, R.; Lawler, B.E. Glioblastoma treatment with temozolomide and bevacizumab and overall survival in a rural tertiary healthcare practice. *BioMed Res. Int.* **2018**, *2018*, 1–10. [CrossRef]
10. Topal, U.; Zamur, C. Microgravity, Stem Cells, and Cancer: A New Hope for Cancer Treatment. *Stem Cells Int.* **2021**, *2021*, 1–9. [CrossRef]
11. Takeda, M.; Magaki, T.; Okazaki, T.; Kawahara, Y.; Manabe, T.; Yuge, L.; Kurisu, K. Effects of simulated microgravity on proliferation and chemosensitivity in malignant glioma cells. *Neurosci. Lett.* **2009**, *463*, 54–59. [CrossRef]
12. Svejgaard, B.; Wehland, M.; Ma, X.; Kopp, S.; Sahana, J.; Warnke, E.; Aleshcheva, G.; Hemmersbach, R.; Hauslage, J.; Grosse, J.; et al. Common Effects on Cancer Cells Exerted by a Random Positioning Machine and a 2D Clinostat. *PLoS ONE* **2015**, *10*, e0135157. [CrossRef]
13. Warnke, E.; Pietsch, J.; Wehland, M.; Bauer, J.; Infanger, M.; Görög, M.; Hemmersbach, R.; Braun, M.; Ma, X.; Sahana, J.; et al. Spheroid formation of human thyroid cancer cells under simulated microgravity: A possible role of CTGF and CAV. *Cell Commun. Signal.* **2014**, *12*, 1–11. [CrossRef]
14. Kopp, S.; Krüger, M.; Bauer, J.; Wehland, M.; Corydon, T.J.; Sahana, J.; Nassef, M.Z.; Melnik, D.; Bauer, T.J.; Schulz, H.; et al. Microgravity affects thyroid cancer cells during the TEXUS-53 mission stronger than hypergravity. *Int. J. Mol. Sci.* **2018**, *19*, 4001. [CrossRef] [PubMed]
15. Grimm, D.; Bauer, J.; Kossmehl, P.; Shakibaei, M.; Schönberger, J.; Pickenhahn, H.; Schulze-Tanzil, G.; Vetter, R.; Eilles, C.; Paul, M.; et al. Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. *FASEB J.* **2002**, *16*, 604–606. [CrossRef] [PubMed]
16. Grimm, D.; Wehland, M.; Pietsch, J.; Aleshcheva, G.; Wise, P.; van Loon, J.; Ulbrich, C.; Magnusson, N.E.; Infanger, M.; Bauer, J. Growing tissues in real and simulated microgravity: New methods for tissue engineering. *Tissue Eng. Part B Rev.* **2014**, *20*, 555–556. [CrossRef] [PubMed]
17. Krüger, M.; Melnik, D.; Kopp, S.; Buken, C.; Sahana, J.; Bauer, J.; Wehland, M.; Hemmersbach, R.; Corydon, T.J.; Infanger, M.; et al. Fighting thyroid cancer with microgravity research. *Int. J. Mol. Sci.* **2019**, *20*, 2553. [CrossRef]
18. Grimm, D.; Kossmehl, P.; Shakibaei, M.; Schulze-Tanzil, G.; Pickenhahn, H.; Bauer, J.; Paul, M.; Cogoli, A. Effects of simulated microgravity on thyroid carcinoma cells. *J. Gravit. Physiol.* **2002**, *9*, P253–P256. [PubMed]
19. Kaufman, H.L.; Kohlhapp, F.J.; Zloza, A. Oncolytic viruses: A new class of immunotherapy drugs. *Nat. Rev. Drug Discov.* **2015**, *14*, 642–662. [CrossRef]

20. Martuza, R.L.; Malick, A.; Markert, J.M.; Ruffner, K.L.; Coen, D.M. Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* **1991**, *252*, 854–856. [CrossRef]
21. Clemens, M.J. Targets and mechanisms for the regulation of translation in malignant transformation. *Oncogene* **2004**, *23*, 3180–3188. [CrossRef]
22. McEntee, G.; Kyula, J.N.; Mansfield, D.; Smith, H.; Wilkinson, M.; Gregory, G.; Roulstone, V.; Coffey, M.; Harrington, K.J. Enhanced cytotoxicity of reovirus and radiotherapy in melanoma cells is mediated through increased viral replication and mitochondrial apoptotic signalling. *Oncotarget* **2016**, *7*, 48517–48532. [CrossRef]
23. Geletneky, K.; Hajda, J.; Angelova, A.L.; Leuchs, B.; Capper, D.; Bartsch, A.J.; Neumann, J.-O.; Schöning, T.; Hüsing, J.; Beelte, B.; et al. Oncolytic H-1 parvovirus shows safety and signs of immunogenic activity in a first Phase I/IIa glioblastoma trial. *Mol. Ther.* **2017**, *25*, 2620–2634. [CrossRef] [PubMed]
24. Hirano, S.; Etoh, T.; Okunaga, R.; Shibata, K.; Ohta, M.; Nishizono, A.; Kitano, S. Reovirus inhibits the peritoneal dissemination of pancreatic cancer cells in an immunocompetent animal model. *Oncol. Rep.* **2009**, *21*, 1381–1384. [CrossRef] [PubMed]
25. Galanis, E. Therapeutic potential of oncolytic measles virus: Promises and challenges. *Clin. Pharmacol. Ther.* **2010**, *88*, 620–625. [CrossRef]
26. Baertsch, M.A.; Leber, M.F.; Bossow, S.; Singh, M.; Engeland, C.E.; Albert, J.; Grossardt, C.; Jäger, D.; von Kalle, C.; Ungerechts, G. MicroRNA-mediated multi-tissue detargeting of oncolytic measles virus. *Cancer Gene Ther.* **2014**, *21*, 373–380. [CrossRef] [PubMed]