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Amelioration of Glioblastoma multiforme by 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. GBM usually arises from the glial cells known as astrocytes and it is commonly located in the supratentorial region (cortical lobes),usually affecting the frontal lobe. A unique feature of this tumor is its rapid local growth and spread making the prognosis very poor with a 5-year survival rate less than 5 %.Treatment of GBM remains challenging. Multiple therapeutic interventions are used for GBM including 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 an oncolytic viral therapy using an autonomous rat parvovirus H-1 (H-1PV). Our hypothesis combines the beneficial effects of simulated microgravity and oncolytic viral therapy to lyse tumor cells through induction of apoptosis, decreased cell proliferation and or induction of an immune response. This proposal provides the foundations to construct novel breakthroughs in the treatment of GBM.

Keywords: Brain tumor; clinostat; Epidermal Growth Factor Receptor (EGFR),incidence; mutations; parvovirus H1; primary malignant brain tumors; EGFR mutations; Parvovirus H1; Chromosomal loss, Genetically modified viruses; Suicide genes; Neurosurgery; Radiotherapy; Clinical Neurology.

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Abbreviations :

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

1. Introduction

Glioblastoma multiforme is the most common aggressive malignant primary brain tumor affecting approximately 3.19 per 100,000 persons in the United States with an incidence 1.6 times higher in males compared to females. This is the highest incidence among malignant brain tumors. 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.GBM is more common in males compared to females(3.97 vs. 2.53).(1)

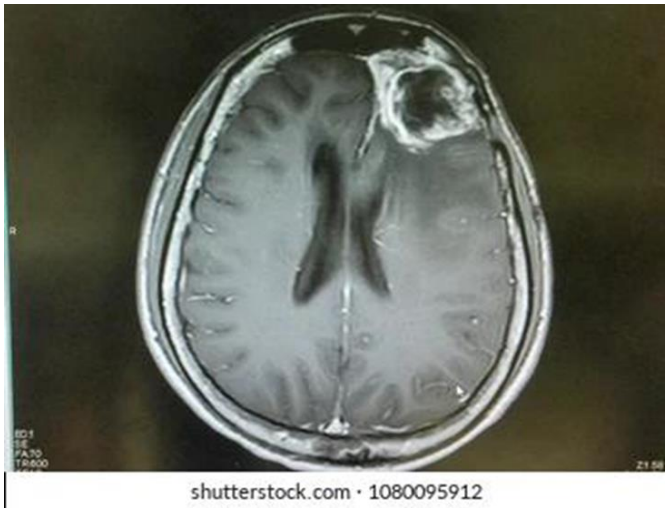

Figure 1. : MRI showing Glioblastoma in the left frontal lobe of the brain.. Reproduced with permission from**.** https://www.shutterstock.com/image-photo/mri-brain-show-left-frontal-gliblastoma-1080095912.

GBM usually arises from the glial cells known as astrocytes and it is commonly located in the supratentorial region (cortical lobes),usually affecting the frontal lobe. It is multiformed grossly. Microscopically gross transverse sections how areas of hemorrhage and necrosis, pleomorphic nuclei and cells, pseudopalisading necrosis, and microvascular proliferation .(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 less than 5 %.(1)

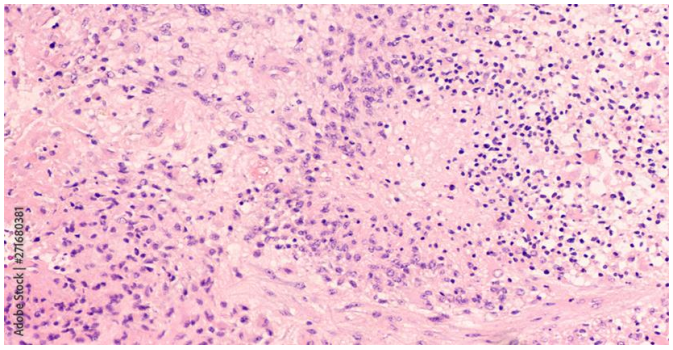

Figure 2. Microscopic image showing histology of a glioblastoma multiforme (GBM). Necrosis and vascular proliferation are diagnostic features of this high grade malignant tumor. Reproduced with permission from https://stock.adobe.com/search?load_type=search&native_visual_search=&similar_content_id=&is_recent_search=&search_type=usertyped&k=glioblastoma+multiforme&asset_id=271680381.

Multiple risk factors have been linked to GBM such as exposure to radiation, a weak immune system, and increased age. The median age of diagnosis of 64 years. It is uncommon in children accounting only approximately 3% of all brain and CNS tumors reported among individuals aged 0 to 19 years. Other risk factors include high socioeconomic status. Decreased susceptibility to allergy and the use of anti-inflammatory medications have been linked to increased risk for GBM. (1)

Several genetic and molecular mechanisms have been identified to play a role in the development of GBM. Primary *de novo* GBM without evidence of a less malignant precursor is associated with Epidermal Growth Factor Receptor (EGFR)over-expression, pleitrophin mutation, and loss of chromosome 10. Secondary GBM arising from a low grade astrocytoma or anaplastic astrocytoma is associated with IDH1 mutations, TP53

mutations causing alpha synclean protein over expression; the same protein involved in Parkinson's disease , and chromosome 19q loss (3),(4),(5),(6)

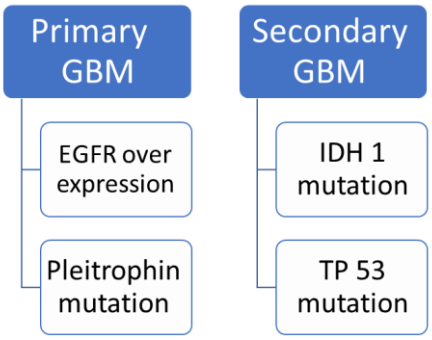

Figure 3. :Schematic diagram to represent the genetic and molecular mechanisms of primary and secondary glioblastoma multiforme (GBM). GBM: Glioblastoma multiforme; EGFR: Epidermal Growth Factor Receptor (EGFR).

Treatment of GBM remains challenging .Multipletherapeutic interventions are used for GBM including surgical resection of the tumor, 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. 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) which is commonly the case (7).Survival benefits have been reported with postoperative radiation therapy to doses of 5,000–6,000 (8).Common chemotherapeutic agents used for GBM include temozolomide which can be given concomitant with radiotherapy and after it. Bevacizumab can be used along with temozolomide (TMZ) to improve progression free survival (9). Despite all these therapeutic interventions, GBM has a very poor prognosis with a 5-year survival rate less than 5 %, hence novel interventions are required for treatment of this highly malignant tumor.

2. Methods- the Hypothesis

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.

Malignant glioma cells (Takeda, 2009) 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 (GMSCF), and parvovirus H1 at 37 º C with a humidified 5% CO2 atmosphere will be placed in a simulated microgravity using a clinostat-based three-dimensional (3D) culture system. The necrosis of malignant glioma cells week by verified by inverted contrast phase and quantum microscopy, flow cytometry mitochondrial membrane potentials. After two weeks of this intervention, all malignant glioma cells will be dead.

3. Results and discussion

Evidence from in vitro, animal and clinical studies were collected and evaluated. The data described in the next slides suggest that our hypothesis could provide a new therapy for glioblastoma multiforme. We aim to conduct an experiment to confirm this hypothesis and, if confirmed, to conduct clinical trials:

A) Simulated microgravity effect on tumor cells :

1. -Simulated microgravity effects on Glioblastoma multiforme:-

Multiple studies have been conducted by Kentucky State University to examine the effect of microgravity during spaceflights on different tumor cells including Glioblastoma multiforme and the results showed that microgravity causes decreased cell proliferation,

decreased secretory activity, and induction of apoptosis. Simulated microgravity can be produced on earth by floating or using a device called 3D Clinostat to generate gravitational waves in 3 dimensions resulting in an environment with an average of 10^{-3} G (10),(11)

A study was conducted in Japan to examine the effects of simulated microgravity using a 3D clinostat on cell proliferation of Glioblastoma multiforme, mitochondrial activity and sensitivity of tumor cells to cisplatin; a chemotherapy drug. 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 group; cells under 100 G force and CL group; 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 rhodamine123 was used to measure the mitochondrial membrane potential. A Define first FACS caliber flow cytometer was used to measure cell cycle distribution. The results of this experiment showed that after 3 days, simulated microgravity induces Glioblastoma cells growth inhibition by worsening mitochondrial activity and increasing the sensitivity of tumor cells to cisplatin (12)

2 Simulated Microgravity effects on Thyroid Cancer cells :

Different ESA ground-based facilities, including the RPM and the 2D and 3D clinostat S-g, have been used to expose thyroid cancer cells. These exposures have resulted in a variety of changes in the exposed TCC, including early changes to the cytoskeleton, ECM, focal adhesion molecules, proliferation, the rate of apoptosis, migration, and growth ([13, 14, 15, 16, 17, 18) The development of MCS was the main discovery. After varying exposure times, the examined TCC and cells from other cancers (such as breast carcinoma and prostate carcinoma) developed in r- and s-g in the form of 3D MCS (19, 20, 21, 22, 23, 24, 25, 26).

B) Oncolytic Viral Therapy:

HSV and Melanoma .-1

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 (eg, intravenously) to induce direct lysis of tumor cells and/or systemic induction of an immune response against the tumor cells. Due to increased research on virus biology and tumor immunology, there is increased interest in oncolytic viruses. A clinical trial of advanced melanoma was treated with herpes simplex virus type 1 (HSV-1) encoding granulocyte-macrophage colony-stimulating factor (GM-CSF). This virus, called Ltalimogenelaherparepvec, is expected to be approved by the FDA in the near future. (28

Parvo Virus and Glioblastoma multiforme .-2

Parvo H1 virus (12) has been used in experiments on glioblastoma multiforme due to its kinetics being able to cross the blood brain barrier.(29

Multiple factors are involved in clearing the viral particles in Glioblastoma multifore cells such as TRAF3, INF-related factor 3, INF7 and RIG-1. These factors activate JAK-STAT pathway which activate PKR pathway leading to termination of protein synthesis and cell death .(16) The other mechanism by which oncolytic viruses cause tumor cell death is induction of a systemic immune response , multiple cytokines such as type 1 INF , TNF alpha, INF gamma and IL-12 which play a role in stimulation of antigen presenting cells(APCs) such as dendiritic cells then activation of T-helper cells CD 4 and C-cytotoxic cells CD 8 occurs leading to tumor cell lysis.(30

Glioblastoma cells may 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.(31)

3. -.Reovirus and Pancreatic cancer :

A reovirus being tested in a Phase II clinical study for pancreatic cancer is called Oncolytics Biotech Inc., Calgary, Alberta, Canada[32]. The research is still inconclusive. However, it has been demonstrated that administering reovirus intraperitoneally in the management of peritoneal metastases in hamsters with pancreatic ductal adenocarcinoma carcinomatosis was efficient and secure[33.

Overexpression of CD46, a viral entrance receptor also present in many cancer cells, is required for measles virus development. An altered measles virus previously demonstrated oncolytic activity in pancreatic tumor xenografts in mice, leading to tumor shrinkage and improved survival[35]. In a different study, the virus was changed to specifically target prostate stem cell antigen, a protein found in pancreatic cancer.

Overexpression of CD46, a viral entrance receptor also present in many cancer cells, is required for measles virus development. 34) In a prior work, mice with pancreatic tumor xenografts treated with a modified measles virus shown oncolytic activity, which resulted in tumor shrinkage and improved survival[35]. In a different investigation, the virus was changed to target the purine nucleoside phosphorylase drug and the protein prostate stem cell antigen, which is expressed in pancreatic cancer. The researchers came to the conclusion that immunocompromised mice treated with viral treatment showed anticancer activity[36]

C) Limitations of our hypothesis :

Unfavorable results would be decreased efficacy due to the inhibitory effects of microgravity on the human immune system which is one of the mechanisms oncolytic viruses utilize to abort the growth of tumor cells. We suggest adding Immune stimulants such as GM-CSF to escape this inhibitory effect. (

Conclusion :

The treatment of glioblastoma remains a challenge. We are pleased to publish our hypothesis that could offer a novel therapy for this highly malignant CNS tumor. We aim to conduct an experiment to confirm this hypothesis and, if confirmed, to conduct clinical trials.

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