Amelioration of Glioblastoma multiforme by the combination of Simulation microgravity and Oncolytic Viral therapy.

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)

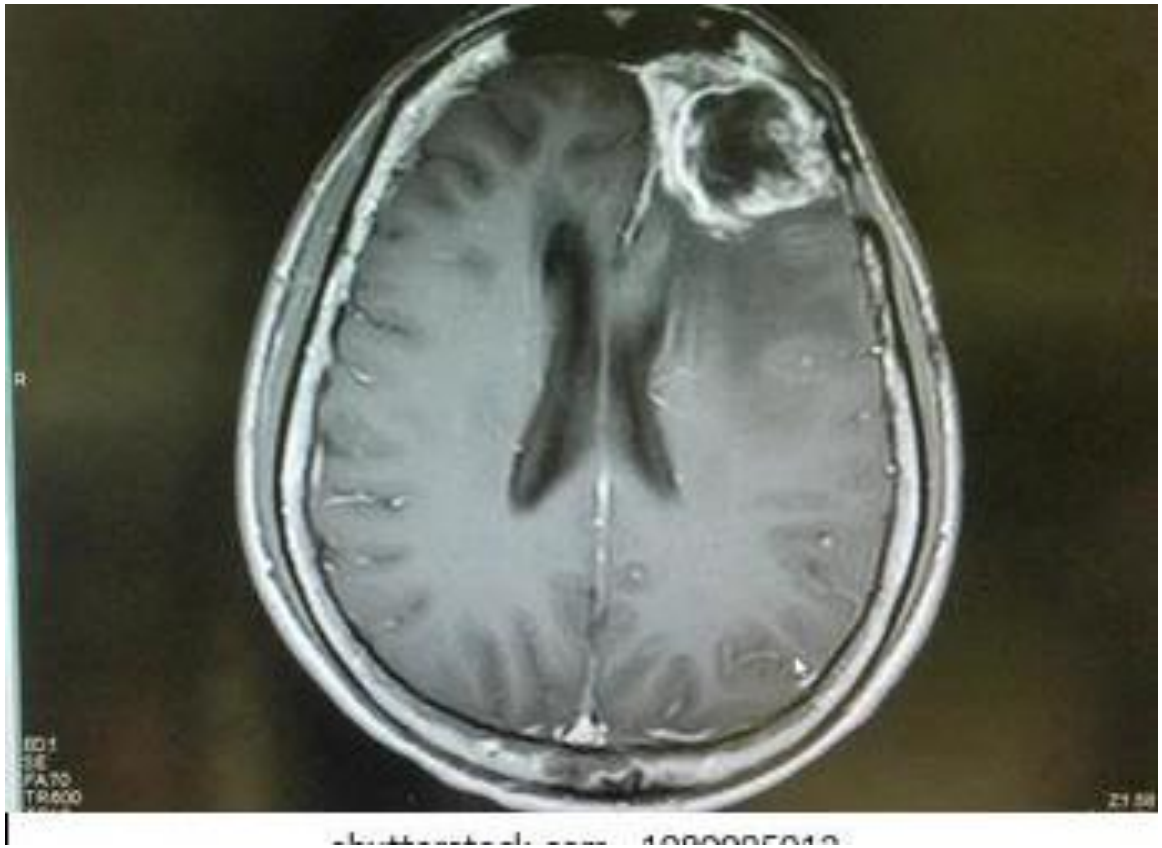

Fig 1 :

MRI showing Glioblastoma in the left frontal lobe of the brain..
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Introduction

- 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)

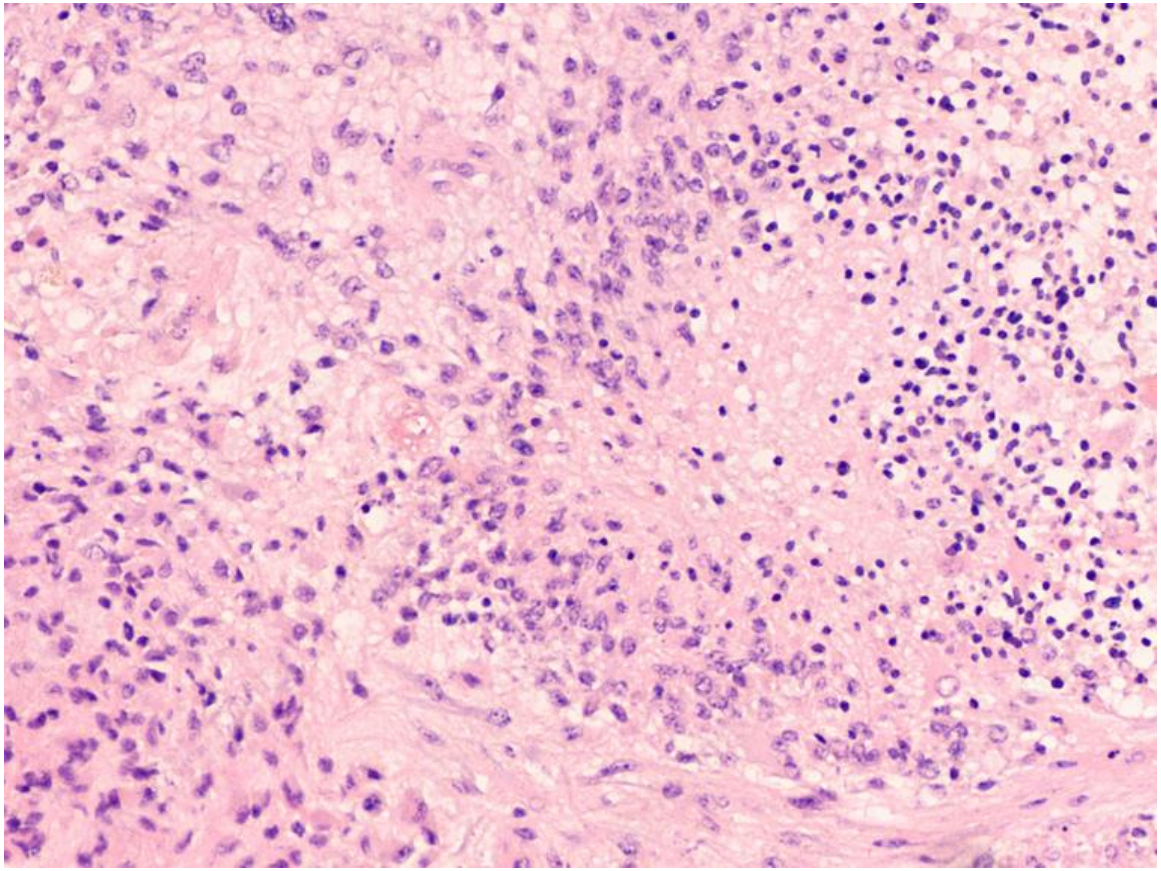

Fig 2:

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

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The etiology of Glioblastoma multiforme

- 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 withIDH1 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)

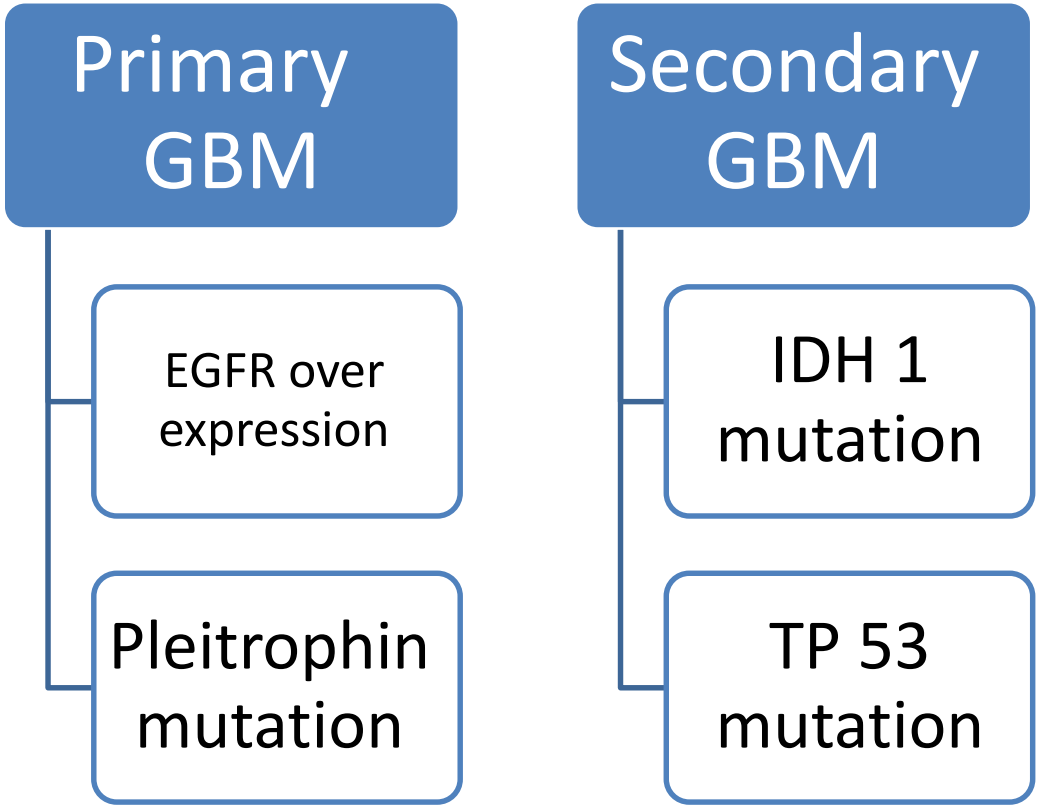

Fig 3 :

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)

Methods- 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 cytometrymitochondrial membrane potentials. After two weeks of this intervention, all malignant glioma cells will be dead.

Results- Consequences of the hypothesis

- 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

Discussion

- **A) Simulated microgravity effect on tumor cells :**

1- Simulated microgravity effects on Glioblastoma multiforme:

- Multiple studies have been conducted by Gliolab at Kentucky state University to examine the effect of microgravity during spaceflights on different tumor cells including GBM and the results showed that microgravity caused decreased cell proliferation, decreased secretory activity, and induction of apoptosis(10). Simulated microgravity can be produced on earth by floating or using a device to generate gravitational waves in 3 dimensions resulting in an environment with an average of 10^{-3} G called 3D clinostat(11)

Discussion

- A study was conducted in Japan to examine the effects of simulated microgravity using a 3D clinostat on cell proliferation of glioblastima 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 cell growth inhibition by worsening mitochondrial activity and increases the sensitivity of tumor cells to cisplatin,12)

Fig 4:

This figure shows a 3D clinostat device used to generate gravitational waves in 3 dimensions resulting in an environment with an average of 10^{-3} G

Discussion

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).

Discussion

- **B)Oncolytic Viral Therapy:**

- **1- Melanoma and HSV-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.
- A clinical trial on advanced melanoma showed that tumor was lysed by a genetically modified herpes simplex virus type 1 (HSV-1) encoding granulocyte-macrophage colony-stimulating factor (GM-CSF). This virus is called Ltalimogenelaherparepvec and is expected to be approved by the FDA in the near future. (28

Discussion

- **2- Glioblastoma multiforme and Parvovirus H1:**

- Parvo virus H1 (12)which 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 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 Parvovirus H1 cause glioblastoma 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

Discussion

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 multiforme 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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