Glioblastoma Stem Cells Demonstrate Abnormal Mitochondria and a Dependence on Glucose for Cell Proliferation and Survival

Sumedh Subodh Shah BS; Gregor Rodriguez; Zachary Charles Gersey; Winston Walters; Andrew L. Middleton MD; Amade Bregy MD PhD; Ricardo Jorge Komotar MD, FAANS; Jeffrey S. Prince PhD; Regina Graham

University of Miami Brain Tumor Initiative, Department of Neurosurgery, Miller School of Medicine, Miami, FL, USA. & UM

CNS 2015 ANNUAL MEETING NEW ORLEANS LOUISIANA SEPTEMBER 26-30, 2015

Introduction

Glioblastoma multiforme (GBM) is the most common and lethal form of primary brain cancer. A subset of glioblastoma cells, glioblastoma stem cells (GSCs), is treatment resistant and implicated in disease recurrence. There is debate over metabolic pathways—glycolysis versus oxidative phosphorylation—employed by GSCs (1,2). The purpose of our study was to:

- Perform ultrastructure analysis of GSCs with electron microcopy (EM)
- Evaluate potential therapeutic targets to eliminate GSCs.

Methods

GSCs generated from patient-derived tumors, propagated in neurosphere media, and examined for relevant stem-cell markers and ability to generate tumors in nude mice. Ultrastructural analysis completed using Jeol 1400 TEM. Effect of glycolytic inhibitors, 2-deoxyglucose (2-DG), 3-bromopyruvate, and dichloroacetate, on cell viability was determined by MTS assay and Sphere-forming assay.

Results

GSC Indentification

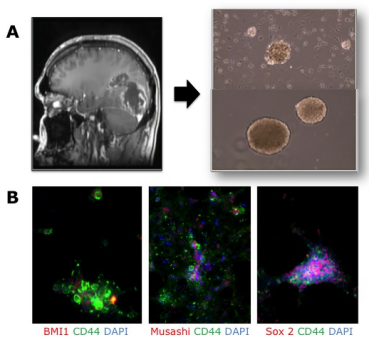
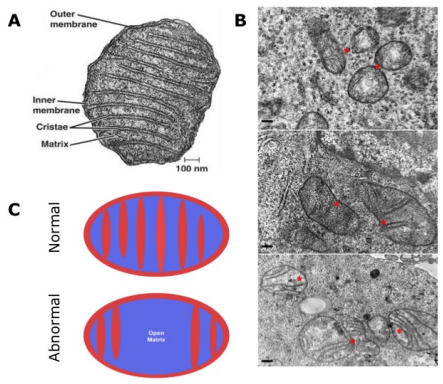


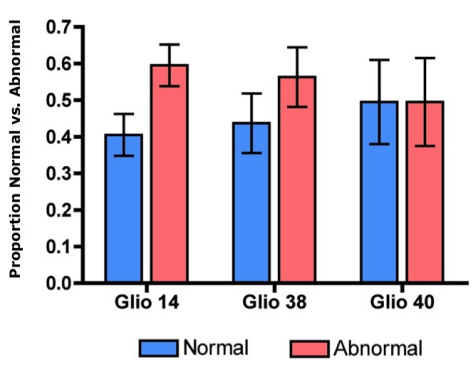
Fig. 1A. MRI of Patient with GBM whose cells were propagated as cell line. B. Stem Cell Makers BMI1, CD44, DAPI, Musashi, and Sox 2.

Ultrastructural Analysis of GSCs



Mitochondrial Analysis. A. Normal Mitochondrion. B. TEM ultrastructural (*). Scale Bar = 0.2m. C. Classification guide used for numerical analysis.

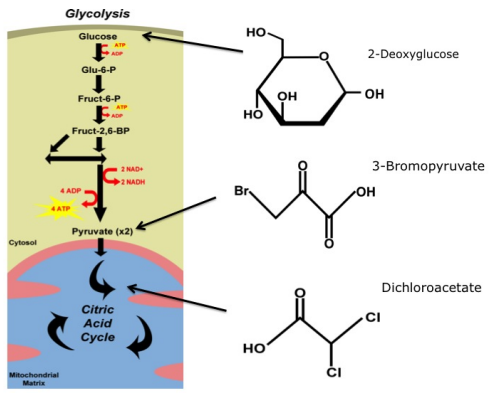
Mitochondrial Distribution in GSCs



Frequency of normal vs. abnormal mitochondria among imaged GBM stem cell lines (mean±SD). All GSCs exhibited >50% abnormal mitochondria.

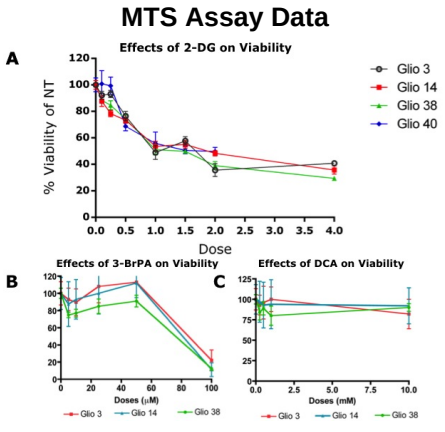
EM analysis of multiple patient-derived GSCs confirmed >50% of mitochondria exhibit cristae loss or inner-fold polarization, indicating less available surface area for oxidative phosphorylation suggesting a metabolic dependence on glycolysis.

Metabolic Inhibitors



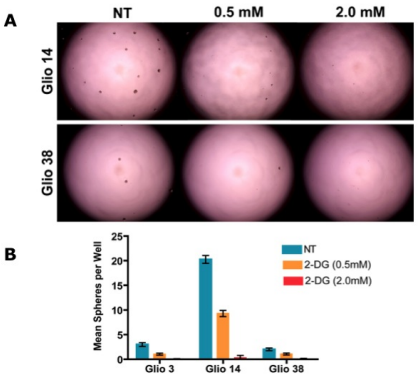
Representation of the glycolytic pathway with the relevant reactions highlighted. Molecular structures of the glycolytic inhibitors used against GSCs. 2-DG and 3-BrPA act as analogs for glucose and pyruvic acid, respectively.

MTS Assay Data



2-DG causes dose dependent loss in cell viability at clinically relevant concentrations. 3-BrPA reduces GSC viability at high concentrations (100M). DCA does not induce remarkable proliferative loss, yet provides insight into the glycolytic nature of GSCs.

Sphere-Forming Assay



Sphere-forming Assay with 2-DG shows dose-dependent loss of stem-cell ability to form neurospheres.

Conclusions

Targeting GSCs is vital in preventing tumor regeneration. EM provides a useful tool in developing experimental therapies. Our EM results indicate that oxidative phosphorylation is severely compromised in GSCs. Glycolytic inhibition proved effective in targeting GSCs and may represent an adjuvant therapy for a disease with minimal survival.

Acknowledgements

This project was supported by the University of Miami Brain Tumor Initiative, the UM Electron Microscopy Laboratory, and the Mystic Force Foundation.

References

1. Yuan S et al. Effective elimination of cancer stem cells by a novel drug combination strategy. Stem Cells 31(1): 23-34.
2. Vlashi E et al. Metabolic state of glioma stem cells and nontumorigenic cells. Proc Natl Acad Sci 108(38): 16062 – 16067.