Targeting the GLUT1/TUBB4 Interaction to Disrupt the Warburg Effect in Mesenchymal Subtype Glioblastoma

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Disclosures

- None to report.
Introduction

Glioblastoma (GBM) is an aggressive diffuse glioma with poor prognosis due to lack of sound therapeutic options. As in many cancers, GBMs create a hypoxic microenvironment & switch to glycolytic metabolism from oxidative phosphorylation in what is known as the Warburg Effect. Here, we hypothesize that strategically inhibiting influx of glucose via targeting the GLUT1/TUBB4 axis may reverse the Warburg Effect and slow tumor progression.
Methods

- Datamining studies and immunohistochemical staining were conducted on human GBM (hGBM) specimens to determine GLUT1 status.
- Mass spectrometric analysis was performed on GLUT1 precipitated from mesenchymal subtyped cancer stem cells (CSCs).
- Limiting dilution assays were conducted using the GLUT1 inhibitor fasentin (25 & 50uM) and novel colchicine derivative CR-42-24 (1 & 2 uM) to determine if proliferative abilities can be reduced in mesenchymal CSCs in vitro.
- Reactive oxidative species assays were carried out on control mesenchymal GBM CSCs and GBM CSCs treated with fasentin to assess for a shift toward oxygen-consumptive metabolism with GLUT1 inhibition.
Results

Figure 1. GLUT1 expression in TCGA cohorts. (A,B) We observed upregulation of GLUT1 in GBM relative to lower-grade gliomas ($p < 0.0001$) and in non G-CIMP compared to G-CIMP GBMs. (C) GLUT1 expression is high in mesenchymal subtype. (D) Kaplan-Meier curves show increased GLUT1 corresponds to decreased survival.
Results

Figure 2. GLUT1 and TUBB4 are highly expressed in hGBM surgical biopsies. (A). Using immunohistochemical analysis, we stained GLUT1 and TUBB4 with specific antibodies on consecutive sections. (Brown, diaminobenzidine; light blue, nuclear counter stain with DAPI). (B). Mass spectrometry demonstrated peptide sequence matches that indicate a protein-protein association between GLUT1 isolated from GBM CSCs and TUBB4A & TUBB4B.
Results

Figure 3. Limiting dilution assay demonstrated a dose-dependent attenuation of mesenchymal CSC growth at 2 weeks in response to the GLUT1 inhibitor fasentin (A) and novel tubulin inhibitor CR 42-24 (B).
Figure 4. Reactive oxidative species (ROS) assay demonstrated that the GLUT1-specific inhibitor fasentin causes an increase in ROS in mesenchymal GBM CSCs.
Discussion

- Glioblastoma (GBM) has a 9.8% 5-year survival rate given the first-line temozolamide + radiation therapy, necessitating better treatment options.
- The Warburg Effect, a shift from oxidative phosphorylation to glycolysis for energy derivation, has become a recent target of interest in reversing the GBM phenotype.
- GLUT1 is a primary mediator of glycolysis and its availability is rate-limiting in the influx of glucose into cells.
- Our studies indicate that the upregulation of GLUT1 is upregulated in GBM, and this phenomenon is facilitated in part by tubulin-dependent membrane trafficking.
- Because preliminary data demonstrates a strong association between GLUT1 & TUBB4, there is a strong possibility that GLUT1 can be downregulated via tubulin-mediated trafficking inhibition.
GLUT1 is significantly upregulated in GBM compared to lower grade gliomas

Patients with GBMs that express high levels of GLUT1 have poorer prognoses relative to tumors with low GLUT1 expression

TUBB4 has significant binding affinity with GLUT1 and is confirmed to be associated with GLUT1 isolated from mesenchymal GBM cell cultures

Colony formation of mesenchymal CSCs is inhibited on a dose-dependent basis with treatment fasentin and 2nM of CR-42-24 colchicine derivative in vitro

Treatment with fasentin shifts metabolism toward a more oxygen-dependent state in GBM CSCs

GLUT1 targeting via TUBB4 may serve as a novel therapeutic target in GBM