DEFINING THE ROLE OF PCK2 IN T CELL METABOLIC PLASTICITY IN GliOBlastoma

LaTambria Hampton*, Lora J Rogers, Michael A Bauer, Daniel Fil, Jessica L Kellifer, Sydney I. Shuttleworth, Emilio Danignes, Analiz Rodriguez, Brian Koss. University of Arkansas for Medical Sciences, Little Rock, AR, USA

Background Glioblastoma (GBM) is the most common malignant brain tumor in adults and remains incurable.1 There is an urgent need to develop new therapeutic approaches to combat this aggressive tumor. Adoptive T-cell therapies, including genetically modified T-cells with a chimeric antigen receptor (CAR) for treatment of GBM, can significantly expand patient lifespans.2 However, a major cause of treatment failure is the inability of T-cells to persist within a metabolically immunosuppressive GBM environment.3 4 Glucose availability in the GBM is a major metabolic factor restricting T-cell function as they canonically depend entirely on neuronal glucose transporter, GLUT1 for glucose uptake. In this environment both neurons and cancer cells outcompete T-cells, while expressing GLUT3, for the already restricted extracellular glucose (20% of blood levels) in the brain. As a result, new approaches are needed to overcome the lack of nutrient availability to adoptive T-cells in brain tumors. Here we describe discovery and utilization of mitochondrial gluconeogenic enzyme phosphoenolpyruvate carboxykinase, PCK2 to circumvent T-cell dependency on glucose thereby reducing metabolic exhaustion in glioblastoma.

Methods Published single cell RNA sequencing data consisting of 201,986 aggregate single cells from 18 human glioma patients was analyzed for cell type clustering. Additionally, 10X v2 Chromium RNA sequencing was utilized to profile the composition of 11 primary glioblastoma tumors at the University of California Santa Cruz. Proteomics was done to determine the differentially expressed genes downstream of CD28 co-stimulation. Western blots were done to validate PCK2 expression alongside CD28 co-stimulation in mice and human CD8+ T cells.

Results Single cell RNA sequencing confirmed the divergence in glucose transporter expression amongst neurons, cancer cells, and T cells.5 6 Proteomics revealed that PCK2 was the most significantly upregulated gene downstream of CD28. Western blots carried out validated that PCK2 is upregulated only in the presence of CD28 co-stimulation. T cell functional data will be presented using T cells from PCK2 KO mice and overexpression of PCK2 in human CAR T cells.

Conclusions Single cell RNA sequencing revealed glioma cells adapt in the nutrient deprived tumor microenvironment by upregulating the highly efficient neuronal glucose transporter, GLUT3. Through proteomic interrogation of T-cell activation, we have identified, in addition to GLUT1, the mitochondrial gluconeogenic enzyme phosphoenolpyruvate carboxykinase, PCK2 as a critical factor in T-cell energy metabolism specifically downstream of CD28 co-stimulation. We are actively exploring the role of PCK2 for T cell activation and metabolic persistence in nutrient deprived environments with PCK2 knockout mice. 

Acknowledgements The authors would like to thank the UAMS Biochemistry and Molecular Biology Department and the Winthrop P. Rockefeller Cancer Institute (WPRCI). We acknowledge support from VCRI Startup funds (B. Koss), WPRCI Startup funds (B. Koss), and NIH grant DP5OD031863 (B. Koss).

REFERENCES

Ethics Approval This study was approved by the University of Arkansas for Medical Sciences’ institutional review board (IRB) and the animal care and use committee (IACUC).