Metabolic reprograming in IDH mutant glioma cells

Jose Luis Izquierdo Garcia[1], Pia Eriksson[1], Myriam Chaumeil[1], Russell O Pieper[2], Joanna J Phillips[2], and Sabrina M Ronen[1]
[1]*Radiology, UCSF, San Francisco, CA, United States,* [2]*Neurological Surgery, Helen Diller Research Center, UCSF, San Francisco, CA, United States*

Background: Metabolic profiling using quantitative nuclear magnetic resonance spectroscopy (MRS) provides a useful tool for biomarker discovery and for understanding the pathogenesis of different conditions. Here, we applied this approach to investigate the isocitrate dehydrogenase (IDH) mutation.

IDH is the enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) whereas mutant IDH catalyzes the conversion of α-KG into 2-hydroxyglutarate (2-HG). Mutations in IDH1 have been reported in over 70% of low-grade gliomas and secondary glioblastomas (GBM). These mutations are associated with the accumulation of 2-HG within the tumor and are believed to be one of the most early events in the development of low grade gliomas.

Objective: To test the hypothesis that the neomorphic activity of mutant IDH1 results not only in production of the oncometabolite 2-HG, but also in a wider metabolic reprogramming.

Material and Methods: Two genetically engineered cell models were investigated: the U87-based model and the E6/E7/hTERT Normal Human Astrocyte (NHA)-based model. For both models, wild-type IDH cells were generated by transduction with a lentiviral vector coding for wild-type IDH1 and mutant IDH cells were generated by transduction with a lentiviral coding for mutant (point mutation in R132) and wild-type IDH1[1]. Cells were extracted using the dual phase extraction method[2] and IDH mutant and wild-type cell extracts were analyzed by ^{1}H NMR spectroscopy on a 600 MHz Bruker spectrometer using a 90° Flip angle and 3 second relaxation delay. Principal Component Analysis was performed using the Metabonomic R package[3] to determine the differences between cells lines. Specific metabolites were quantified by normalizing to the area of the external reference (TSP), correcting for saturation and normalizing to cell number.

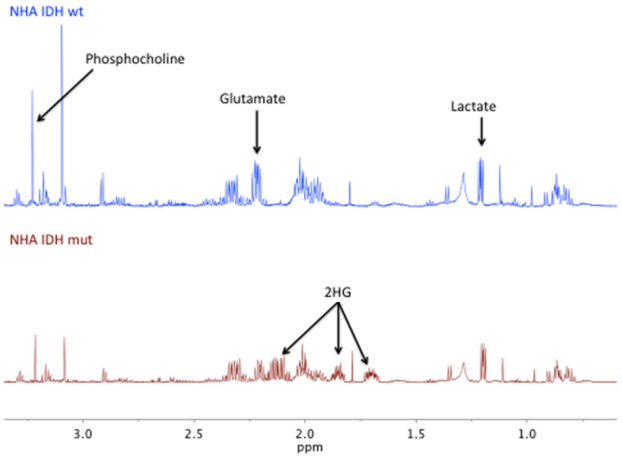

Figure 1: Representative ^{1}H NMR spectra of IDH1 mutant (bottom) and wild-type (top) NHA cell extracts. The figure highlights the metabolic differences between two groups.

Results: Figure 1 illustrates a typical proton spectrum. Metabolic profiling clearly discriminated between wild-type and mutant IDH cells (Figure 2 A&B) When quantifying the metabolites that changed in mutant cells some changes were specific to each cell model: a significant drop in the concentration of acetate, aspartate, glutamine and myo-inositol was observed in U87 mutant IDH cells and lower creatine and lactate concentration was observed in NHA mutant IDH cells when compared to their wild-type IDH counterparts. More importantly however, in both cell models, mutant IDH cells showed a lower concentration of glutamate and phosphocholine as well as the expected elevation in 2-HG (Figure 2 C&D).

Conclusions: This study determined that the IDH mutation leads to a range of metabolic changes. The results are in line with the findings reported by Reitman et al.[4] using a mass spectroscopy metabolomics approach. Understanding this metabolic reprograming is essential for determining the parameters associated with tumor progression and for the potential development of treatments for mutant IDH-expressing gliomas. By providing a snapshot of the steady state levels of cellular metabolites this study also opens future research directions that will help elucidate the mechanisms responsible for the metabolic changes observed and contribute to understanding the disease and its treatment.

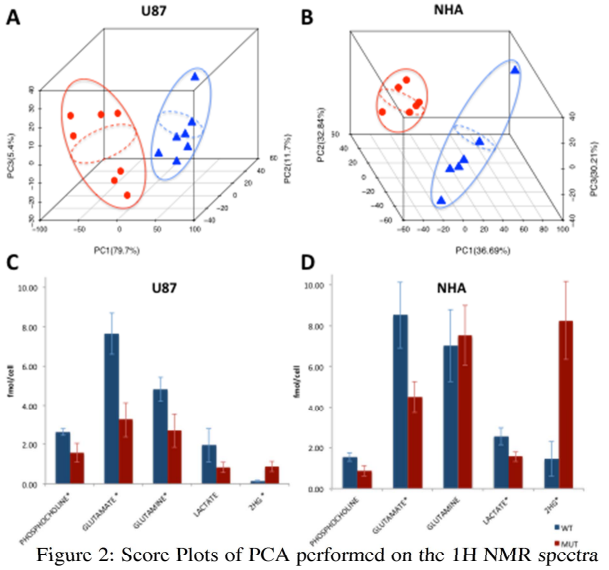

Figure 2: Score Plots of PCA performed on the 1H NMR spectra of U87 (A) and NHA(B) from wild-type (blue) and IDH mutant (red) extract cells. Samples from both groups are clearly separated. The significant variables for the separation between mutant and wild-type cells were quantified in U87 (C) and NHA (D) cells (*p-val<0.05).

Grant Acknowledgments: NIH R21CA161545, NIH R01CA172845, NIH R01CA154915, NIH P41EB013598

References: **1.**Chaumeil, M. M. *et al. Nat Commun* 4, 2429 (2013). **2.**Tyagi, R. K. et al. Magn Reson Med 35, 194–200 (1996). **3.**Izquierdo-García, J. L. et al. BMC Bioinformatics 10, 363 (2009). **4.**Reitman, Z. J. et al. PNAS 108 (8) 3270-3275 (2011).