

Glioma cells overexpressing isocitrate dehydrogenase 1 (IDH1) mutation produce 2-hydroxy glutaric acid detectable in vivo

Jelena Lazovic¹, Horacio Soto², David Piccioni², Arthur Chou², Sichen Li², Robert Prins², Linda Liau², Timothy Cloughesy², Albert Lai², and Whitney Pope³
¹Radiology, University of California, Los Angeles, CA, United States, ²University of California, ³Radiology, University of California, Los Angeles

INTRODUCTION: The cytosolic isocitrate dehydrogenase 1 (IDH1) enzyme is frequently mutated in gliomas and secondary glioblastoma. The mutation represents point mutation leading to the replacement of arginine at the amino acid residue 132 of the protein (IDH1-R132). The IDH1 mutation appears to be an early event in gliomagenesis and it is estimated that ~70% of grade II and III gliomas have this mutation. Recent reports [1] correlate the presence of this mutation with more favorable outcome including increased survival among glioblastoma patients. In gliomas, IDH1 mutation appears to be "gain of function" mutation, accompanied by production of 2-hydroxyglutaric (2-HG) acid. The significance of increased 2-HG production in gliomas and secondary glioblastoma remains to be determined. In this work U87 glioma cell lines transduced with viral vector that carries IDH1-R132 mutation were injected into the flank of NOD-scid mice to determine if they will produce detectable levels of 2-HG and the impact of this mutation on the rate of tumor growth and tumor metabolism.

METHODS: 6-8 week old male NOD-scid mice were used for all experiments. FuGene HD transfection system (Roche) was used to transfect Plat-A cells with pLPCH-IDH1-ΔR132H-Flag construct (containing mutated IDH1-R132 gene), with pLPCH-IDH1-Flag (containing WT IDH1 gene) and control vector only pLPCH-Flag construct. Three groups of NOD-scid mice were injected with 10⁶ glioma cells into the right flank: 1) wild type (WT) IDH1 U87 cells (N=4), 2) U87 cells transduced with vector-control construct (N=2) and 3) U87 cells transduced with IDH1-R132-construct (N=7). Mice were imaged at 14 and 21 days following glioma cell injections. MR imaging and spectroscopy was performed on a 7T Bruker system (Ettlingen, Germany). To quantify transverse relaxation time (T₂) of different tumors a multi-echo spin echo sequence was used (TR/TE 2000/7.26-101.64 ms, 14 echoes, 78° μm² resolution, 2 NAX). A diffusion-weighted echo planar imaging sequence (TR/TE 3800/22.03, with three b-value=0, 500, 1000, 3 diffusion directions and 156° μm² resolution, 2 NAX) was used to measure apparent diffusion coefficient (ADC). ADC and T₂ were calculated on a pixel-by pixel basis using ImageJ (plugin by Karl Schmidt). MR spectroscopy was done using PRESS sequence (TR/TE 4000/7 ms, 512 NAX, 3 ml voxel within the tumor volume). MRS data was processed using LCModel (Steven Provencher), and 2-HG was modeled based on J-coupling. Due to very low creatine levels in these tumors, metabolites were expressed as ratio to choline. At the end of the MRI and MRS studies, mice were sacrificed and tissue was processed for histology, H&E staining and IDH1-immunohistochemistry. Statistical analysis was performed using ANOVA with Holm-Sidak post-hoc test, where p-value<0.05 was considered significant.

RESULTS: At 21 days post injections, tumors that overexpressed mutated IDH1-R132 had significantly (p<0.05) larger volumes (1028±52 ml) compared to WT (315±97 ml) and vector-only tumors (605±71 ml), **Fig. 1A**. MR spectroscopy of IDH1-R132 U87 tumors revealed detectable levels of 2-HG at 14 days following tumor cell injection and significantly reduced glutamate (p<0.05) compared to WT and vector-control tumors, **Fig. 2, Fig.3**. The additional increase in lactate in IDH1-R132 tumors was not significant compared to WT tumors, but was significant (p<0.05) compared to vector-control tumors. No significant difference in T₂- (**Fig. 1B**) and ADC-values (**Fig. 1C**) were found among WT- and vector-only tumors at 21 days compared to IDH1-R132 overexpressing tumors at 14 days. At 21 days, IDH1-R132 overexpressing tumors had regions with significantly (p<0.05) increased T₂- (91.9±3.6 vs. 62.3±3.5) ms and ADC-values (1.14±0.19 vs. 0.67±0.05)X 10⁻³mm²/s **Fig. 1B, C**, which correlated with necrotic areas on histology. In parallel, MR spectroscopy revealed high levels of lactate in the necrotic areas.

DISCUSSION: In contrast to our expectations based on improved survival of patients with the IDH1 mutations, IDH1-R132 overexpressing tumors grew faster compared to WT tumors. Further study will be required to determine if there is a good correlation between 2HG levels and tumor growth. However, we speculate that increased levels of 2-HG contribute to reduced glutamate levels (as glutamine, via glutamate, has been shown to serve as a precursor for 2-HG production [2]). Metabolic changes including increased lactate and significantly reduced glutamate in IDH1-R132 overexpressing tumors are consistent with reduced oxidative phosphorylation and pseudo hypoxia. The hypoxia is thought to be the key process preceding angiogenesis responsible for tumor progression. It is therefore not clear why necrosis was observed only in IDH1-R132 overexpressing tumors. A possible explanation could be that due to more rapid tumor growth, IDH1-R132 tumors outgrew their blood supply, or alternatively accumulation of 2-HG reached toxic levels in these tumors and consequently caused necrosis. Thus it is yet to be established why patients with IDH1-R132 mutated gliomas have better prognosis compared to patients that do not carry this mutation.

Figure 1.

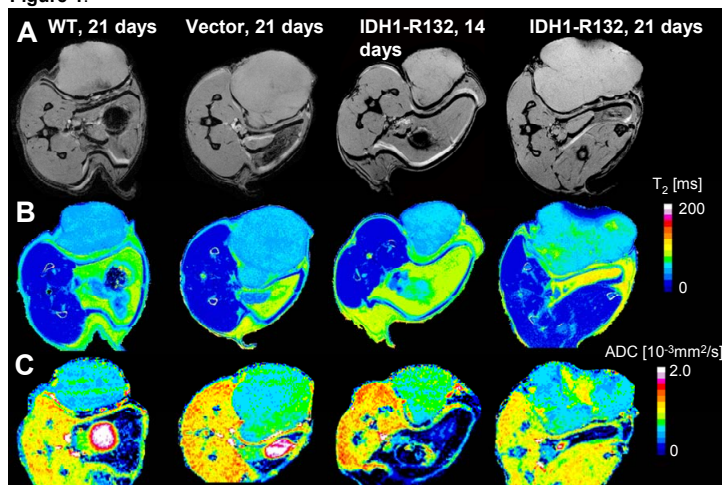


Figure 3. Metabolite quantification for 2-HG, glutamate and lactate as a ratio to choline.

REFERENCES:

1. Yan et al. N Engl J Med 360(8):765-73.
2. Dang et al. Nature 462(7274):739-44.

Figure 2.

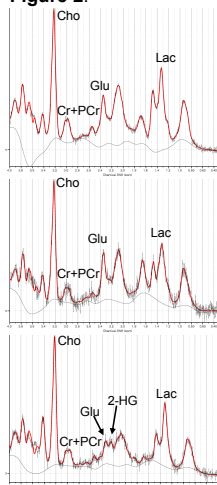


Figure 3.

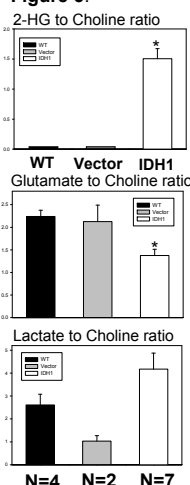


Figure 1. Representative gradient-echo (T₂*-weighted) images of WT and vector-control (at 21 days) and IDH1-R132 tumors (at 14 and 21 days). T₂-maps of the corresponding tumors and slices. Color scale represents different T₂-values. ADC-maps of the corresponding tumors. Notice increased ADC values in IDH1-R132 tumor at 21 day. Color scale represents different ADC values.

Figure 2. Representative MR spectrums acquired within the tumor volume. Notice presence of additional peak corresponding to 2-HG in IDH1-R132 spectrum.