Mutation in isocitrate dehydrogenase 1 (IDH1) leads to increased T2, ADC and decreased lactate and glutamate in glioblastoma model

Jelena Lazovic1, Soto Horacio2, Sichen Li1, Albert Lai1, Linda Liu1, Robert Prins2, Timothy Cloughesy1, and Whitney Pope4

1Radiology, University of California at Los Angeles, Los Angeles, CA, United States, 2Neurosurgery, University of California, Los Angeles, CA, United States, 3Neuro-Oncology, University of California, Los Angeles, CA, United States, 4Radiology, University of California, Los Angeles, CA, United States

INTRODUCTION: It is estimated that ~70% of grade II and III gliomas have mutation in the cytosolic isocitrate dehydrogenase 1 (IDH1) enzyme, where amino acid arginine is replaced at the active site 132 (IDH1-R132) [1]. The significance of this mutation in gliomagenesis and glioma growth is not well understood. The IDH1-R132 mutation is a “gain of function” mutation resulting in the production of 2-hydroxyglutaric acid (2-HG) instead of conversion of isocitrate to α-ketoglutarate. As a consequence, this mutation might have a variety of potential effects that can influence glioma growth, invasion and the treatment outcome. To investigate some of these effects as well as MR imaging and 1H MRS features associated with IDH1 mutation and 2-HG production, an orthotopic glioblastoma mouse model was established using U87 glioblastoma cell line modified to express IDH1 mutation. We tested the hypothesis that U87 cells modified to overexpress IDH1-R132 mutation will have distinct metabolic features detectable by 1H MRS, which may lead to differences in the apparent diffusion coefficient (ADC) and the transverse relaxation time T2 compared to U87 cells with wild type IDH1 gene (IDH1-WT).

METHODS: Immuno-compromised NOD-scid mice (6-8 weeks old) were used for all experiments. A 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 intracranially injected (using stereotactic surgery) with 106 glioma cells: 1) wild type IDH1 overexpressing (WT) U87 cells (N=9), 2) control vector-only transfected U87 cells (N=9) and 3) IDH1-R132 vector transduced U87 cells (N=10). Mice were imaged at 21 days following glioma cell injection. MR imaging and spectroscopy was performed on a 7T Bruker system (Ettlingen, Germany). A multi-echo spin echo sequence was used to quantify T2 (for the tumor volume measurement (TR/TE 2000/7.26-101.64 ms, 14 echoes, 78 mm3 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 resolution, 2 NAX) was used to measure ADC. ADC and T2 were calculated on a pixel-by-pixel basis using ImageJ (plugin by Karl Schmidt). A T2*-weighted gradient echo imaging sequence (TR/TE 500/7ms, 78 mm3 resolution, 4 NAX) was used to determine necrosis. 1H 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 LCMModel (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: Tumors that overexpressed mutated IDH1-R132 had significantly larger volumes (1272±383 ml) compared to WT (183±98 ml) and vector-only tumors (361±83 ml). At 21 days post injection we found significantly increased T2-values in IDH1-R132 overexpressing tumors 64.13±0.822 vs. 61.52±0.71 for IDH1-WT along with significantly increased ADC-values (0.92±0.013) X 10-3/mm/s for IDH1-R132 vs. (0.822±0.036) X 10-3/mm/s for IDH1-WT. Despite the larger volumes IDH1-R132 glioblastoma did not have any MRI visible necrosis compared to WT- and vector-only tumors, Fig. 1 C. We were able to detect a distinct peak at 2.25 ppm chemical shift present only in IDH1-R132 glioblastoma that we assigned to 2-HG, Fig. 1 D (black arrows). 1H MR spectroscopy of IDH1-R132 U87 tumors revealed detectable levels of 2-HG and significantly reduced glutamate and lactate compared to WT and vector-only tumors, Fig. 1 E at 21 days following injection.

DISCUSSION: Our results show that IDH1-R132 overexpression leads to metabolic remodeling that supports enhanced tumor growth, and contributes to less necrosis compared to WT- and vector-only U87 glioblastomas. Reduced lactate in these tumors suggests better preserved Kreb's cycle that can account for less necrotic phenotype. These findings are in the agreement with previously published study in humans with IDH-1 mutation, where these tumors tend to be large at diagnosis and less necrotic [2]. Because IDH1-R132 overexpression caused enhanced tumor growth rate, we hypothesize that improved patient survival might be due to other factors such as differences in cell migratory properties and infiltration, and also different treatment response. Increased ADC- and T2-values in IDH1-R132 overexpressing glioblastoma may indicate decreased cell density of these tumors which could potentially affect adhesion properties of these cells. In conclusion, IDH1-R132 mutation contributes to distinctive 1H MR spectrum, in addition to differences in T2 and ADC values compared to WT or vector-only glioblastoma, all clinically relevant to diagnose and follow up patients with this mutation.