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

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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 ^{1}H 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 ^{1}H MRS, which may lead to differences in the apparent diffusion coefficient (ADC) and the transverse relaxation time T_2 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 stereotaxic surgery) with 10^5 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 T_2 and for the tumor volume measurement (TR/TE 2000/7.26-101.64 ms, 14 echoes, 78^2 µm^2 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 156^2 µm^2 resolution, 2 NAX) was used to measure ADC. ADC and T_2 were calculated on a pixel-by pixel basis using ImageJ (plugin by Karl Schmidt). A T_2*-weighted gradient echo imaging sequence (TR/TE 500/7ms, 78^2 µm^2 resolution, 4 NAX) was used to determine necrosis. ^{1}H 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: 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), (*p<0.05). At 21 days post injection we found significantly increased T_2-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^2/s for IDH1-R132 vs. (0.822±0.036) X 10^{-3}mm^2/s for IDH1-WT **Fig. 1 A, B**. 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 glioblstoma that we assigned to 2-HG, **Fig. 1 D** (black arrows). ^{1}H 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 ^{1}H MR spectrum, in addition to differences in T_2 and ADC values compared to WT or vector-only glioblastoma, all clinically relevant to diagnose and follow up patients with this mutation.

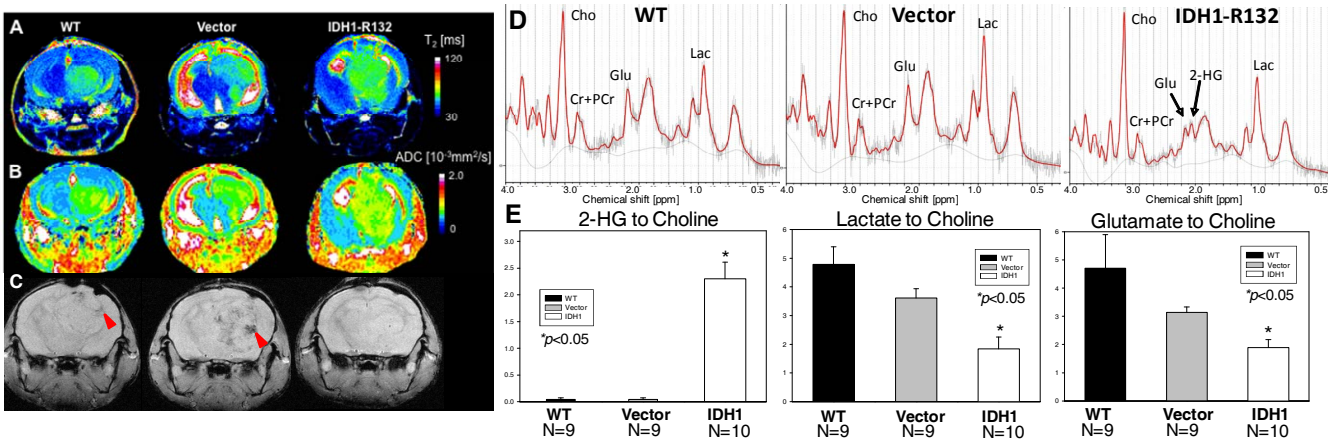

Figure 1. A Representative T_2-maps of NOD-*scid* mice brains injected with WT-, Vector- and IDH1-R132 U87 cells. Color scale represents different T_2-values. Notice the largest tumor volume and increased T_2-values in IDH1-R132 glioblastoma. **B** Corresponding representative ADC-maps, with increased ADC values in IDH1-R132 glioblastoma. **C** Representative T_2*-weighted images with necrosis regions in WT- and Vector- (red arrowhead) and absence in IDH1-R132 glioblastoma. **D** Representative 1H MRS spectrums. Notice well resolved 2-HG from Glutamate in IDH1-R132 glioblastoma (black arrows). **E** Metabolites quantification expressed as ratio to choline for 2-HG, lactate and glutamate (ANOVA, p<0.05).

REFERENCES: 1. Yan H et al. N Engl J Med 360(8):765-73. 2. Yan W et al. PLOS One. 2012 7(1): e30339.