GLUTAMATE DEHYDROGENASE INHIBITION REDUCES GLUTAMINE CONVERSION INTO 2HG IN IDH1-MUTATED CANCER CELLS AS DETECTED BY ^{13}C MRS

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Introduction: The cytosolic enzyme isocitrate dehydrogenase (IDH1) is involved in $NADP^+$ dependent oxidative decarboxylation of isocitrate into α-ketoglutarate (αKG) while producing NADPH (fig.1). αKG is an important intermediate for lipid synthesis and cell proliferation whereas NADPH is important for biosynthesis of metabolites that protect cells from reactive oxygen species. More than 70% of all low grade gliomas exhibit a heterozygous R132H mutation in IDH1[1] leading to a loss in isocitrate binding capacity and a conversion of αKG into *D*-2-hydroxyglutarate (2HG) by NADPH oxidation instead[2]. Depletion of the αKG and NADPH pools imposes metabolic stress and hampers cell proliferation. Although there are indications for increased dependence on glutaminolysis of $IDH1^{R132H}$ cells[3], multiple compensatory anaplerotic mechanisms that these tumor cells develop to survive are currently being studied[4]. Since it is hypothesized that in $IDH1^{wt/R132H}$ cells most 2HG is synthesized from glutamate-derived αKG[5] our goal was to investigate the effect of glutamate dehydrogenase (GDH1) inhibitor epigallocatechin gallate (EGCG)[6] on the metabolic conversion of glutamine into 2HG in IDH1 mutant human colorectal cancer cells (HCT116) with ^{13}C MRS.

Materials and methods: The heterozygous $IDH1^{wt/R132H}$ genotype was created by a knock-in of a $IDH1^{R132H}$ allele by rAAV targeting technology GENESIS. Parental $HCT116^{wt/wt}$ cells were used as control. Cells were maintained in DMEM containing 10% FBS, 5.6 mM glucose and 4mM glutamine. 17 hours prior to extraction the glutamine was replaced by an equivalent of 1-^{13}C-labeled glutamine. EGCG-treated cells were supplied with 100μM EGCG 2 hours prior to and during ^{13}C-glutamine incubation. Before extraction all cells were placed on ice and washed once with cold PBS. Subsequently cells were scraped in an ice-cold 50:50 MeOH:H_2O solution containing formic acid as reference compound. The cell suspension was centrifuged for 10 minutes at 1000 x g at 4°C. Supernatant was dried in a Savant SpeedVac evaporator and redissolved in deuterated water. All samples contained the equivalents of approximately $18 \cdot 10^7$ cells and were measured on a Bruker Avance III 500MHz spectrometer. Proton-decoupled ^{13}C spectra were acquired with a pulse-acquire experiment (TR = 5s, 90° flip angle, NS = 4150). Additional ^{1}H spectra were acquired to study total metabolite pool sizes (TR = 8s, 90° flip angle, NS = 64). ^{13}C spectra were fitted with a Lorentzian shape using Bruker Topspin software. Fits were corrected for cell number and reference compound concentration, and spectra were further analyzed with SpinWorks 4 (University of Manitoba, Winnipeg, Canada). For a proliferation assay HCT116 cells were seeded in 6-wells dishes at low density (60 cells/well) and subsequently treated with EGCG (20 μM). After two weeks colonies were stained and counted with an inverted microscope. All counts were normalized to the control groups.

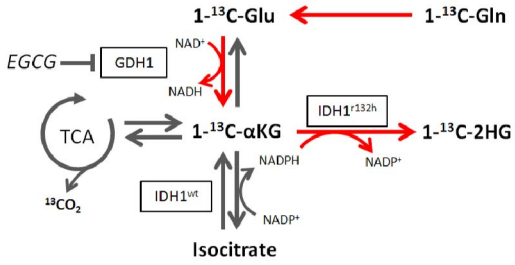

***figure 1** I schematic overview of the observed metabolic pathway. IDH1 'mutabolism' is shown in red.*

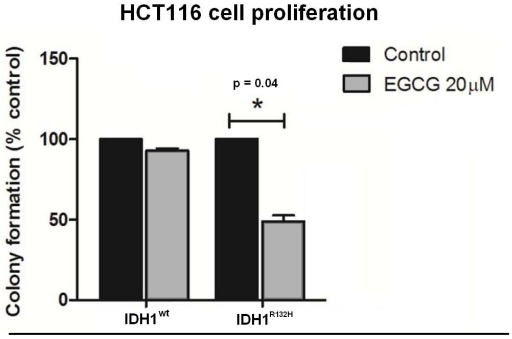

***figure 2** I cell proliferation in $IDH1^{wt/wt}$ and $IDH1^{wt/R132H}$ cells, with and without EGCG treatment (n=2).*

Results: From the ^{13}C MR spectra it follows that 1-^{13}C-glutamine was converted via glutamate and αKG into 1-^{13}C-2HG (fig.1 and 3). Labeled 2HG was only detected in the $IDH1^{wt/R132H}$ cell line (181.35 ppm). GDH1 inhibition with EGCG resulted in a significant decrease in $IDH1^{wt/R132H}$ HCT116 cell proliferation compared to the non-treated control cells (fig.2) and reduced the production of 1-^{13}C-glutamine derived 2HG with almost 50% (fig.3). The pool sizes of 1-^{13}C-labeled glutamine and glutamate were large in all samples whereas their 2-5C resonances were in the range of intensities that originate from natural abundant ^{13}C, implying no label scrambling occurred. Label that entered the TCA cycle via 1-^{13}C-αKG was removed by oxidative decarboxylation. Furthermore, in both parental and mutated cell lines EGCG induced a decrease in total glutamate and an increase in total glutamine concentrations as observed in the ^{1}H spectra (fig.4).

Discussion: We conclude that the metabolic pathway from glutamine to 2HG is important for the generation of 2HG in $IDH1^{wt/R132H}$ HCT116 cells since the amount of glutamine-derived 2HG was considerably reduced due to the administration of EGCG. Even though multiple enzymes are co-responsible for the conversion of glutamate into αKG (e.g. BCAT1, ALT1 and AST1), we showed that the inhibition of GDH1 has a severe effect on cell proliferation. Therefore, EGCG could be a promising therapeutic agent for IDH1-mutant tumors that inhibits glutamate-dependent metabolism and exposes tumor cells to reactive oxygen species.

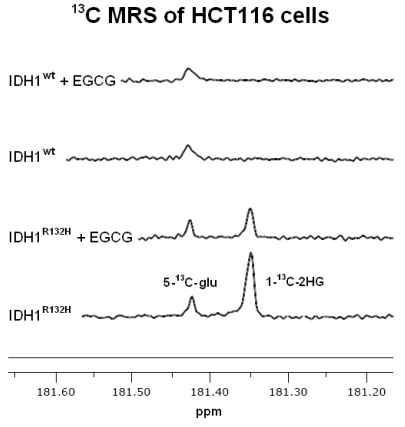

***figure 3** I proton-decoupled ^{13}C spectra showing decreased accumulation of 1-^{13}C-2HG after EGCG treatment.*

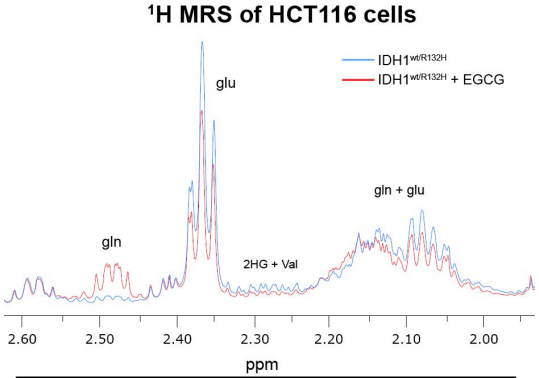

***figure 4** I ^{1}H spectra showing glu and gln total pool size alterations caused by EGCG treatment.*

References

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