Early non-invasive prediction of response to Temozolomide in low-grade glioma.

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Abstract

Newly diagnosed low grade glioma (LGG) patients have a relatively long-term survival, but these tumors always recur. One promising approach is treatment with Temozolomide (TMZ), which was previously used only in the management of glioblastoma. However, early indicators of LGG response to TMZ are currently not available. The goal of this study was therefore to use ^{1}H MR spectroscopy (MRS) to identify potential metabolic biomarkers that can predict therapeutic response to treatment with TMZ in a LGG mouse model.

A patient-derived mutant IDH glioma cells, BT142, were intracranially injected into SCID Fox mice. Animals were treated daily p.o. with Ora-plus (4ml/kg) or TMZ (5 mg/kg, 4ml/kg)). All MR studies were performed in a vertical wide-bore Agilent 600MHz scanner. Axial T_2-weighted images were acquired using SEMS (TE/TR=20/1200ms, FOV=30x30mm^2, 256x256, thickness=1mm, NA=2). ^{1}H MRS spectra were recorded using PRESS (TE/TR=20/4000ms, 512) and analyzed using LCmodel. The obtained values were normalized to total signal. At the end of MR studies tumor tissues were extracted and ^{1}H NMR spectra were acquired on a Bruker 500MHz spectrometer. Metabolite concentrations were determined using PULCON and normalized to wet tissue weight.

Once BT142 xenografts reached a size of 81±28mm^3 (124±5 days post-implantation), mice were randomly divided into control and treatment groups. Figure 1A illustrates anatomical T_2-weighted images of control and TMZ-treated BT142 tumor-bearing mice at D0, D6±1 and D15±1 of treatment. Comparison of average tumor volume in each treatment group at D6±1 and D15±1demonstrated a significant tumor shrinkage observed at D15±1 (p-value=0.013). Figure 1B shows the *in vivo* ^{1}H MRS spectrum from the tumor voxel. Metabolites from spectra acquired at D6±1 and at D15±1 were quantified. Significant changes were observed at D6±1 of treatment: tCho decreased by 22.4% (p-value=0.045), GLN increased by 48.5% (p-value=0.020) and GLX increased by 16.6% (p-value=0.040). Interestingly at D15±1 the reduction in tCho was comparable to D6±1 value (26.6%, p-value=0.038), while GLN and GLX levels were similar to those observed in the TMZ-treated tumors (p-value=0.349 and p-value=0.457, respectfully). We further assessed our findings by analyzing ^{1}H NMR spectra of extracted control and TMZ-treated tumors. At D6±1 of TMZ treatment there was a significant 28.5% reduction in tCho (p-value=0.05), a significant 103.0% increase in GLN (p-value=0.036) and a significant 51.5% increase in GLX (p-value=0.043) in line with the *in vivo* observations, whereas data acquired at D15±1 no significant differences in either tCho (p-value=0.316), GLN (p-value=0.881) or GLX (p-value=0.486) between control and treated tumors.

In this study we observed that a decrease in tCho and increase in GLN and GLX occur in LGG model following TMZ treatment prior to visible changes in tumor volume. The decrease in tCho concentration might reflect a decrease in membrane synthesis and cellularity. The increase in GLN/GLX remains to be explained, but, importantly, was previously reported by our group in LGG cell models treated with TMZ. Further studies are needed to confirm the generality of our findings, but our study identifies the increase in GLN/GLX as potential early metabolic biomarkers of LGG response to TMZ treatment.

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Temozolomide treatment of LGG mouse model showed a significant decrease in total choline and increase in both glutamine and glutamine plus glutamate levels prior to visible changes in tumor volume. This identifies potential early MR detectable metabolic biomarkers of LGG response to Temozolomide treatment.
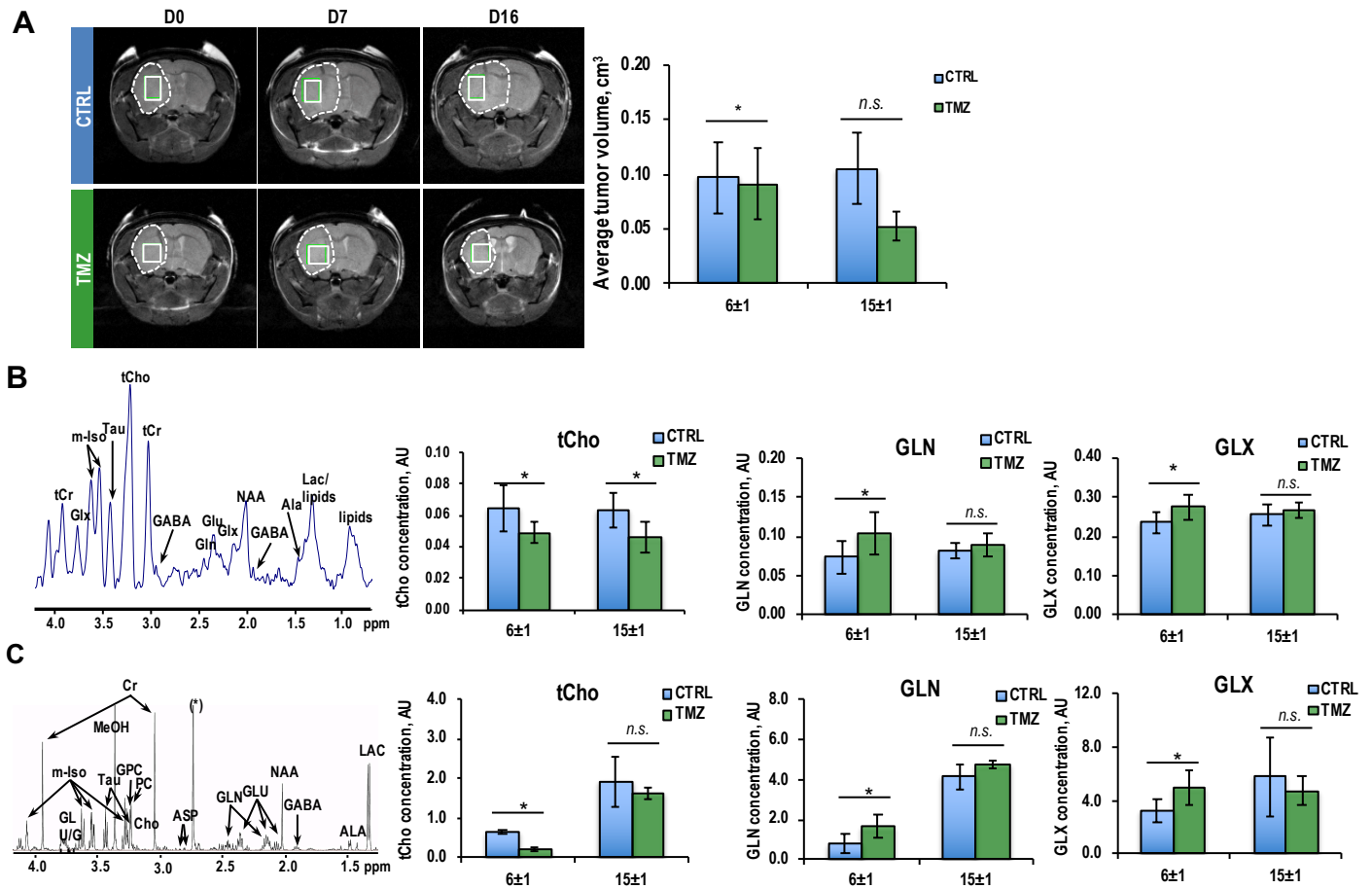

Figure 1: (A) Anatomical axial T_2-weighted images and comparison of average tumor size of control and TMZ-treated BT142 tumor-bearing mice. (B) *In vivo* ^{1}H MRS spectra acquired from the tumor voxel and quantification of tCho, glutamine (GLN) and the sum of glutamine and glutamate (GLX). (C) ^{1}H MRS spectra of the tumor extract and quantification of tCho, GLN and GLX in control and TMZ-treated tumor extracts.