

MOBILE CHOLESTEROL COMPOUNDS IN EXPERIMENTAL GLIOMAS BY ^1H MRS *IN VIVO*: EFFECTS OF GENE-THERAPY-INDUCED APOPTOSIS ON LIPIDS

Timo Liimatainen,^{1,2*} Kimmo Lehtimäki,¹ Juhana Hakumäki,³ and Mika Ala-Korpela^{4*}

¹A. I. Virtanen Institute for Molecular Sciences, University of Kuopio; ²Department of Chemistry, University of Kuopio; ³Department of Clinical Radiology, Kuopio University Hospital; ⁴Laboratory of Computational Engineering, Systems Biology and Bioinformatics, Helsinki University of Technology, Finland

*Corresponding authors: timo.liimatainen@uku.fi, mika.ala-korpela@hut.fi

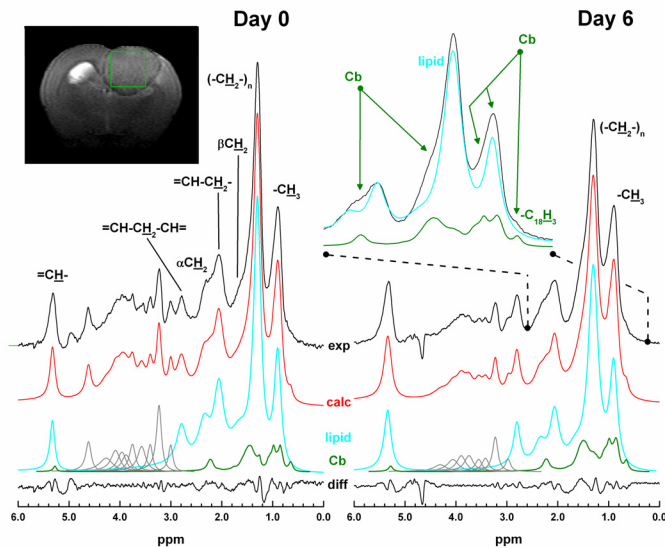


Fig. 1. Characteristic *in vivo* STEAM single voxel ^1H NMR spectra and lineshape fitting analysis for a single animal at Day 0 and after gene therapy induced apoptosis (Day 6). The lipid model signal is shown in cyan, the model signal for the cholesterol backbone of mobile cholesterol compounds (Cb) in green and other signals in grey. The inset in the upper left corner shows a T2-weighted MR image of rat brain containing a tumour and the voxel of interest in STEAM spectroscopy. The PERCH NMR software was used for all the lineshape fitting analyses of the spectra.

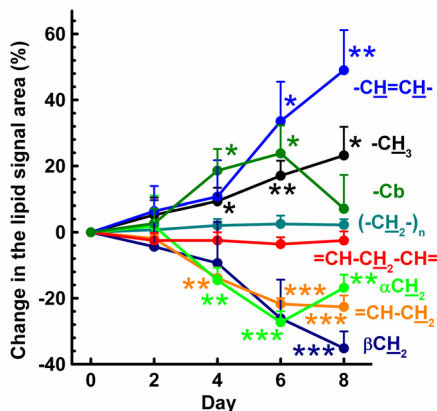
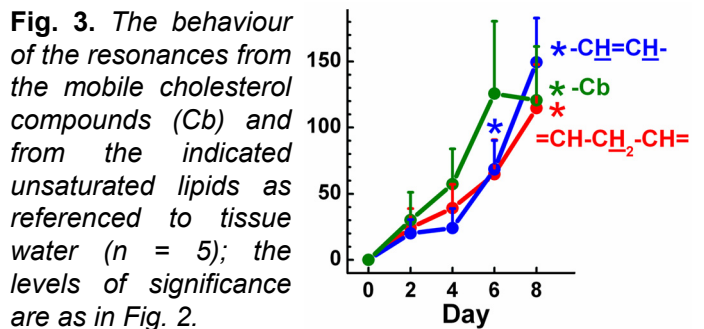


Fig. 2. The behaviour of all lipid resonances as observed by ^1H MRS *in vivo* during the 8-day programmed cell death period (per total lipid; $n = 10$). The values are mean \pm SEM and given as percentage changes in relation to Day 0. * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) between the treatment day and nontreated animals (day 0), Student's paired *t*-test.

- Apoptosis is a natural way of cell death. It is often induced by chemotherapeutic cancer treatments and is also dominant in many other non-surgical anti-tumour therapies.
- Here we present a novel identification and analysis of mobile cholesterol compounds in an experimental glioma / apoptosis model by ^1H MRS *in vivo*. As far as we are aware, this is the first study to identify ^1H MRS detectable mobile cholesterol compounds during apoptosis *in vivo*.
- The inclusion of the multi-component model lineshape of cholesterol backbone into spectral analyses leads to novel biochemical information on cholesterol compounds, and provides a more reliable analysis of the overlapping fatty acid resonances.



- The data analysis revealed that the resonance for the cholesterol backbone of mobile cholesterol compounds (Cb) can cause a remarkable signal below the fatty acid resonances *in vivo*; comprising 12.3 ± 0.8 % of the total MRS visible lipid signal in non-treated tumours. This value corresponds to 16.4 ± 1.2 % cholesterol compounds in molar terms (with triglycerides as the main fatty acid pool).
- The results also imply partly associated accumulation of ^1H MRS detectable cholesterol compounds and unsaturated lipids during gene therapy-induced apoptosis, and indicate that the contribution of cholesterol compounds can not be bypassed in spectral lipid analysis. Apparent biochemical process facilitating such lipid accumulation during apoptosis would be enhanced uptake of serum lipoproteins and degradation of mitochondrial membranes.
- The introduced ^1H MRS approach facilitates a non-invasive follow-up of mobile cholesterol compounds, paving way for studies of tumour cholesterol metabolism *in vivo*.