Spectroscopic imaging of brain metabolism in a mouse model of human glioma-initiating cells: a longitudinal study at 14.1 T

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Introduction Glioblastomas are the most malignant type of human brain tumour. Evidence has been found that a cell subpopulation, called glioma-initiating cells (GIC), is responsible for tumour development [1]. Mouse models of human gliomas have been developed by injection of fresh or cultured GIC, leading to slow infiltrative or fast developing and aggressive brain tumours, respectively [2]. However, although brain tumours have been extensively studied, only a few studies have focused on the metabolic changes during tumour development [3] or on imaging these changes [4]. Therefore, the aim of this study was to longitudinally (i.e. over time) image the metabolic changes occurring during tumour growth using magnetic resonance spectroscopic imaging (MRSI) at ultra-high field 14.1 T.

Methods Tumour cells were obtained from a human glioblastoma multiform of grade IV. Glioma-initiating cells were obtained as in [2] and underwent 11 passages in a culture stem cells medium. Four NOD-scid mice were injected in the striatum with the cultured GIC. MRSI experiments were performed every two weeks after injection until tumour formation on a horizontal 14.1 T magnet (Varian/Magnex) with a custom-built quadrature transceive RF coil. After FASTMAP shimming, MRSI was performed in the striatum region using a modified SPECIAL sequence (TR = 2.5 s, TE = 2.8 ms) with phase encoding in the horizontal plane [5]. The size of the volume of interest (VOI) was 5×7.5×1.5 mm^3. Two averages of 32×32 phase encoding steps were acquired in a FOV of 19×19 mm^2, resulting in a nominal voxel size of 0.6×0.6×1.5 mm^3 (0.5 µl). The *k*-space data were then filtered with a Gaussian function in two spatial directions. The neurochemical profile was determined from the spectra of individual voxels using LCModel. Water signals were used as internal references and were measured using the same protocol without water suppression and with TR=1.5 s. After tumour formation, localized ^{1}H spectra were acquired in the tumour and in the contralateral side using SPECIAL [6] with 512 averages, TE=2.8ms, TR=4s. MM signals were also acquired in the tumour area and processed as described in [7] in order to be included in the LCModel basis-sets for quantification of the tumour spectra.

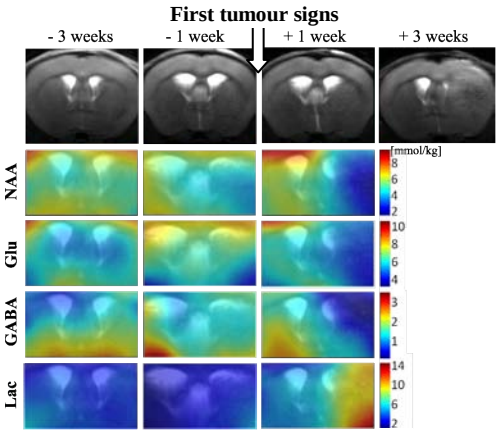

Figure 1 *T_2-weighted images of one mouse during tumour development and corresponding metabolite maps (color) superimposed on anatomical images (grayscale) for NAA, Glu, GABA and Lac, the metabolites showing the earliest alterations during tumour development. Metabolite maps were obtained by spatial interpolation with a Gaussian convolution of the metabolite concentration.*

Results and discussion Fifteen brain metabolites were reliably quantified at each time point. The first signs of tumour development were detected on T_2-weighted images around 5 weeks post-injection. Early metabolite alterations in the tumour region, an NAA decrease and in several mice a glutamate (Glu) decrease were already visually detected on metabolite maps one week prior to the first signs of tumour development on T_2-weighted images (Figure 1). The visual NAA decrease was used to locate the tumour region. In each mouse, four quantified voxels were selected inside the tumour region and in the contralateral side of the brain, resulting in evaluated volumes of 2 µl. Figure 2 shows the time course of the relative metabolite concentrations in the tumour compared to the contralateral side for significantly altered metabolites. Potential metabolite concentration alterations were evaluated at each time point using a paired Student's t-test to compare metabolite levels in the tumour region with those in the contralateral region. NAA was already observed to be significantly decreased 3 weeks prior to the first visual tumour signs in the anatomical images while Glu and GABA were found to be significantly decreased one week prior (resp. after) the first signs. However, it can be observed that those 3 metabolites, which are all linked to the neuronal function, follow a similar decreasing curve. Significantly higher levels of lactate were detected 1 week after the first visual signs, while the Cr+PCr (tCr) level was found decreased only in the final tumour development stage. Moreover, although the total choline (tCho) did not significantly increase over time in the tumour region compared to the contralateral side when evaluated at each time point, it was significantly higher in the tumour region when evaluated for the whole time course. This is consistent with an increased but constant activity over time of the metabolism in the tumour region, which is linked to cell proliferation [8], compared to healthy tissue. In addition, trends to increase were detected in the last stage of tumour formation for glycine and alanine, as well as an increase of mobile lipids between 1.1 and 1.5 ppm (data not shown). These results are in agreement with previous studies performed on high grade glioma models in rodents [3, 9, 10].

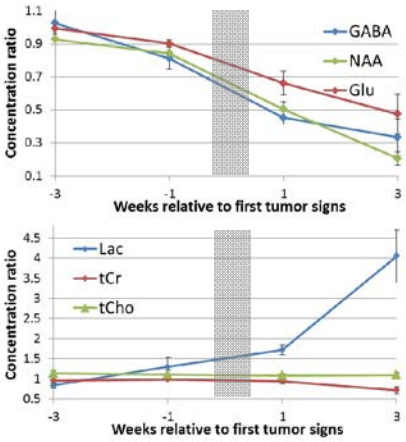

Figure 2 *Time course of the relative concentration of metabolites in the tumour region compared to the healthy contralateral side during tumour development, i.e at 3 and 1 weeks before the first tumour signs and at 1 and 3 weeks after (n=4). Error bars represent the standard error of the mean.*

In conclusion, the use of MRSI in this study allows for sensitive tumour detection prior to any tumour signs on anatomical images, thus insuring an optimal evaluation of the first metabolite alterations, which has to our best knowledge not been published before. Moreover, MRSI also allows for a better evaluation of the tumour rim and potential inhomogeneities, which is not achievable with single voxel spectroscopy. Future work will include evaluating metabolite alterations during tumour growth from fresh GIC, which may uncover links between metabolite alterations and tumour growth, aggressiveness and infiltration after comparison with these results obtained on cultured GIC.

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