Correlation between tCho peak at H-MRS and gene expression of choline kinases and transmembrane choline transporters: an experimental study on a rodent rhabdomyosarcoma model using a standard 3T clinical imager

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Introduction:
Neoplastic processes are known to exhibit increased total choline (tCho) peak at H-MRS [1] together with increased transcription of genes encoding for transmembrane choline transporters and choline kinases [2, 3]. Authors have highlighted that choline kinase activity was unregulated contrarily to downward phosphocholine cytidyl transferase which may constitute a block allowing upward accumulation of phosphocholine [4]. We investigated which from transmembrane choline transporters and choline kinases had the most prominent role in the elevation of the tCho peak at H-MRS using a rodent rabdomyosarcoma model. We voluntarily performed this investigation on a clinical 3T system to ensure the feasibility of such study on a routine system for further translational purposes.

Materials and methods:
Micro-fragments of a rodent rhabdomyosarcoma were subcutaneously implanted in both thighs of male WAG/RijHsd rats. After 2 to 3 weeks, the tumors had reached a sufficient size (ranging from 1 to 3 cm in diameter) for efficient MR imaging. All animals underwent a similar MR protocol on a routine 3T system equipped with 80mT/m gradients (Achieva, Philips Health Care, Best, The Netherlands) using a 4 channel wrist coil (In Vivo, Gainsville, Florida). High-resolution axial T2-weighted FSE 2-mm-thick slices were acquired together with Single Voxel spectroscopy using a water suppressed PRESS-sequence with TR/TE=2000/144 ms and a spectral resolution of 2 Hz. Two to three 1 cm³ Volumes of Interest (VOIs) were drawn within the tumors to cover the entire great axis of the tumor (Fig 1). Whole spectra were fitted and the tCho concentration was calculated relative to the reference water data using the free jMRUI software (Fig 2) [5]. Immediately after completion of the MR procedure, anesthetized rats were sacrificed and sub-regions of the tumors corresponding to spectroscopic VOIs were excised and frozen in liquid nitrogen. From 44 tumors (89 H-MRS voxels), only 39 H-MRS voxels were included into final statistical analysis because the 50 remainders corresponded to necrotic tissue at excision which were therefore not proper for biological analysis. RNA was extracted from all the 39 analyzable samples and was then reverse-transcribed into cDNA. The PCR reaction was performed using a Real-Time PCR System (Applied Biosystem Inc, CA, USA). Choline kinase α and β genes were studied as well as genes of transmembrane carriers OCT1, OCT2, OCT3, CTL1, CTL3, CTL4 and CHT1. The expression level of each gene ('ΔCt'), was referenced to that of the RPL19 gene, according to the equation: $\Delta Ct = 2^{-(Ct\ interest\ gene\ -\ Ct\ reference\ gene)}$ where Ct is the cycle number. The expression level referenced to RPL19 gene was then averaged for each gene. Spearman rank correlation coefficient was used for analysis between variables.

Results and discussion:
High quality H-MR spectra were obtained for each H-MRS VOI with well delineated tCho peaks of which relative concentration was measured as the area under the curve (Fig 2). Expression level of each gene is displayed in figure 3. OCT1 was the more expressed gene but interestingly was not significantly correlated with the tCho peak at H-MRS (p=0.53). In turn, the less expressed CTL1 transporter gene was slightly correlated with the tCho peak (p=0.02; r=0.39). An explanation for the discrepancy could be that OCT1 is only a low affinity polyspecific organic cation transporter on which the extracellular choline less preferentially binds than on an intermediate-affinity choline transporter-like protein like CTL1. When studying the choline kinase gene expression, a moderate but more significant correlation between tCho concentration at H-MRS and the choline kinase α sub-type expression (p=0.002; r=0.51) (Fig 4) was demonstrated. This suggests the prominence of the choline kinase α sub-type on the β one which did not correlate significantly with the tCho peak (p=0.12). Similarly to what was speculated for transmembrane carriers, maybe differences in affinity between the two kinases were responsible for differences in correlation, even though a similar gene expression of α and β is shown on figure 3. That both choline (the substrate of choline kinases) and phosphocholine (their product) were mixed into tCho peak measurements because of insufficient spectral resolution at 3T had low impact on data interpretation. No other significant relationship was observed between tCho peak at H-MRS and the expression of the other genes being investigated, however strong correlations were obtained between some genes: e.g. between CHT1 and OCT3 (p<0.001; r=0.84).

Conclusion:
The feasibility of such experimental study on a clinical 3T system was demonstrated. Although r values remained moderate, statistical analysis suggested in our model the prominence of the choline kinase α expression versus that of the transmembrane choline transporters in the generation of elevated tCho peak at H-MRS.

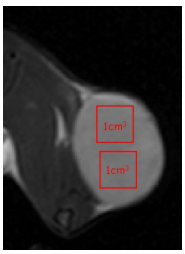

Figure 1

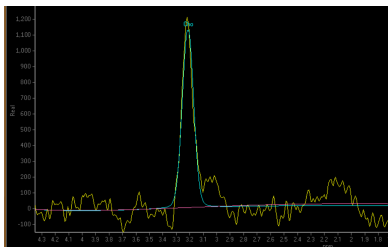

Figure 2

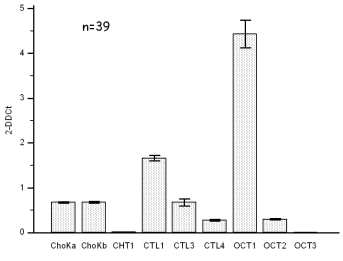

Figure 3

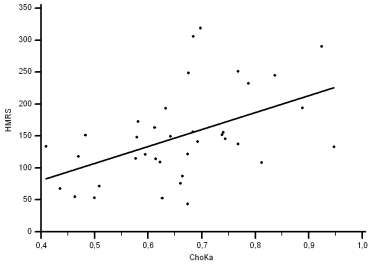

Figure 4

Figure 1: Axial T2-weighted image of sub-cutaneous graft of rhabdomyosarcoma within left thigh. In this case, two 1 cm³ H-MRS VOIs could be placed within tumor.
Figure 2: SV-PRESS fitted proton MR spectrum with well delineated tCho peak at 3.2ppm.
Figure 3: bar graph displaying the expression of the studied genes
Figure 4: regression plot demonstrating significant correlation between tCho at H-MRS and choline kinase α expression

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