Correlation between choline containing metabolites and choline kinase in untreated pediatric brain tumors

D. E. Schofield[1], A. Panigrahy[2], M. D. Krieger[3], M. D. Nelson[2], F. H. Gilles[4], I. Gonzalez-Gomez[4], S. Bluml[2,5]

[1]Pathology, Childresn Hospital Los Angeles, Los Angeles, CA, United States, [2]Radiology, Childrens Hospital Los Angeles, Los Angeles, CA, United States, [3]Neurosurgery, Childrens Hospital Los Angeles, Los Angeles, CA, United States, [4]Neuropathology, Childrens Hospital Los Angeles, Los Angeles, CA, United States, [5]Rudi Schulte Reserach Institute, Santa Barbara, CA, United States

Introduction: Childhood neuroglial tumors of the same diagnostic name or grade contain histologic subsets that differ markedly in survival expectation (1,2). Generally, in cell lines and in animal models of cancer, elevated intracellular phosphocholine (PC) pools and an increased PC to glycerophosphocholine (PC/GPC) ratio have been associated with increased malignancy (3-7). Also elevated choline kinase (CHK) activity, facilitating the synthesis of PC from free choline, and a possible cause for large PC pools, has been associated with increased malignancy (8,9). The goal of this study was to characterize the relationship between CHK expression and total choline (tCho), PC/GPC, and PC/ATP in pediatric brain tumors.

Methods: Untreated tumors from eight children (4 medulloblastoma, 2 ependymoma, 1 astrocytoma, 1 fibrous histocytoma) were studied with *in vivo* ^{1}H and proton-decoupled ^{31}P MRS. Short echo time (TE=35ms) single voxel ^{1}H spectra were acquired using a PRESS sequence and metabolite concentration were quantified in mmol/kg tissue using LCModel (10). Slice selective chemical shift imaging (6 × 6 phase encoding steps) with a self-refocusing RF pulse was used for ^{31}P acquisitions and spectra were processed as described in detail in Ref. (11). Patients underwent brain surgery within 3 days of MRS studies. Tissue samples were analyzed with the Affymetrix (Santa Clara, CA) human genome U133 Plus 2.0 chip. Frozen tumor specimen were cut and reviewed with the microscope. Samples with a fraction of > 90% of tumor cells were chosen. Total RNA was extracted and processed following the manufacturer recommended procedures.

Results: PC was prominent whereas GPC was hardly detectable in medulloblastoma and PC/GPC was thus not evaluated (**Fig. 1**). PC/ATP (GPC/ATP) was significantly higher (lower) in medulloblastoma when compared with other tumors. tCho was not significantly different in this small group of patients. When all data were pooled a positive correlation was found between PC/ATP and the CHK expression index whereas there was no correlation between CHK and tCho (**Fig. 2**). The mean CHK expression index was 70% higher in medulloblastoma than in other tumors but did not reach significance due to considerable variations in individual tumors.

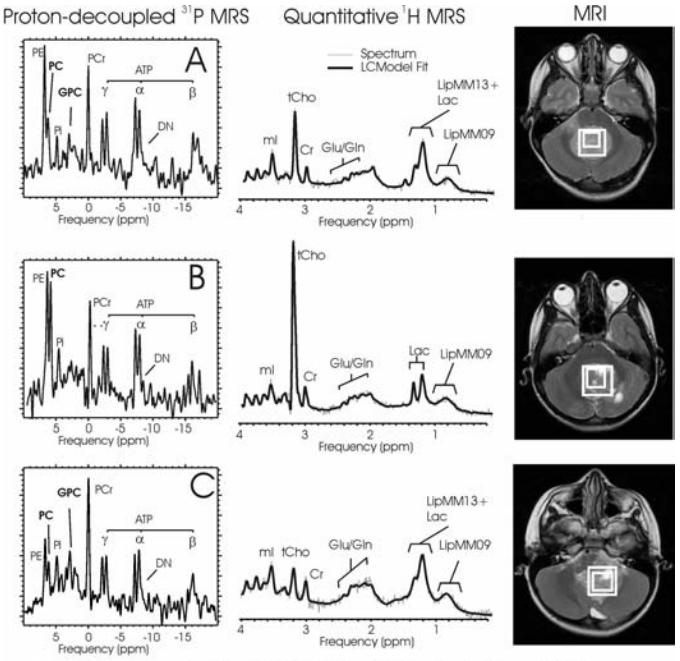

Fig. 1: Proton-decoupled ^{31}P MRS, ^{1}H MRS, and MRI of cerebellar medulloblastoma (A+B) and ependymoma (C). ^{31}P spectra are scaled to ATP as an internal reference. ^{1}H spectra are scaled to measured absolute concentrations to allow direct comparison with each other. Areas or amplitudes of ^{31}P and ^{1}H spectra can not be directly compared. All spectra were acquired on a clinical 1.5 T scanner. A custom-designed dual tuned head coil was used for clinically indicated MR imaging, proton MRS, and for experimental ^{31}P MRS. The total examination time was approximately 70 min. ROIs for ^{1}H (small box) and ^{31}P are marked on MRI, respectively.

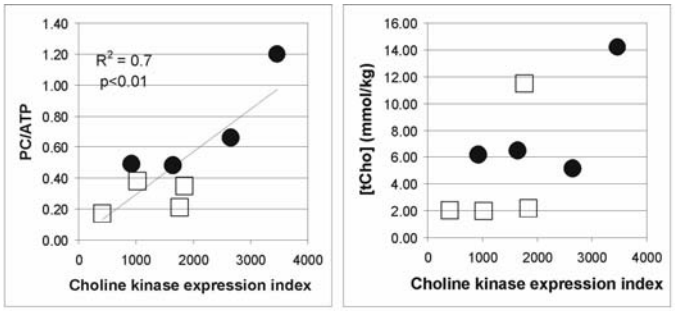

Fig. 2: Correlation between choline containing compounds measured *in vivo* pre-operatively with proton-decoupled ^{31}P MRS and ^{1}H MRS and the choline kinase expression index. Solid circles = medulloblastoma.

Discussion: Choline containing compounds are involved in the synthesis and breakdown of phosphatidylcholine (PtdCho). PtdCho is the major phospholipid component of eukaryotic cells accounting for approx. 60% of total phospholipids. Rapid synthesis of PtdCho is a feature of fast growing tumors. The data indicate that *in vivo* PC/ATP rather than tCho may be useful as an *in vivo* surrogate marker for CHK activity. This is unfortunate because ^{1}H MRS is readily available on clinical MR systems whereas proton-decoupled ^{31}P MRS is time consuming and requires special hardware. The identification of surrogate *in vivo* markers could be important for monitoring of the efficiency of anticancer drugs that specifically aim to inhibit choline kinase (12).

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