

Glycerolphosphocholine is the predominant choline-containing compound and is correlated with proliferation in non-enhancing astrocytoma

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Introduction: We recently reported that the concentrations of both glycerolphosphocholine (GPC) and phosphocholine (PC) were higher in biopsies from non-enhancing Grade 3 astrocytoma (AS3) versus Grade 2 astrocytomas (AS2)^[1]. During that study, we noted that the relative size of the GPC peak in the 1D high-resolution magic angle spinning (HRMAS) spectrum was higher than the PC peak in all cases irrespective of tumor grade. This observation was surprising in light of work by other groups that showed that PC was higher than GPC in high grade glioma^[2,3]. We hypothesized that non-enhancing AS3 are at an earlier stage of malignant progression where proliferation has increased but the balance toward higher PC than GPC production has not occurred. In the current study, we confirmed our observations on a larger cohort of patients by (1) performing 2D total correlation spectroscopy (TOCSY) to verify that that GPC was indeed the larger of the two peaks, and (2) quantifying the GPC:PC concentration ratio for each biopsy. In addition, we looked for associations with proliferation and cell density. **Methods:** We studied 25 patients with treatment-naïve non-enhancing lesions (11AS2 and 14 AS3). Paired biopsies were obtained during surgery, one was flash frozen in liquid N2 and the other was immediately stored in ethanol. A total of 41 biopsy-pairs (18 AS2 and 23 AS3) were collected. HRMAS and TOCSY MRS was performed on the frozen biopsies using a Varian Inova 500MHz spectrometer, equipped with a gHX gradient nanoprobe. Samples were evaluated at 1°C while the tissue was spun at 2250 Hz. The fully relaxed, ERETIC-referenced water presaturation sequence parameters were: PW = 7.8us, NT = 128, sweep width = 40kHz, and 40,000 points. The parameters for the TOCSY experiment were: TR = 1.24s, tm= 40ms, NP=4096 and spectral width of 20,000hz in F2; Ni=64 and spectral width of 6000Hz in F1. Absolute concentrations of GPC, PC, and free choline (fCho) were quantified (CRB < 10%) using software developed in-house. Total choline (tCho) was calculated by the summing PC + GPC + Cho. The proliferative activity and cell density of the fixed biopsies were assessed with immunohistochemistry. Student's t-test with alpha = 0.05 was used to compare results from AS2 and AS3.

Results: Table 1 Shows the mean +/- SD concentrations (mg/kg) of PC, GPC, fCho, tCho, GPC:PC, Ki-67 proliferation index, and cell density (CD) in non-enhancing AS2 and AS3. There was no difference in the ratios of PC:tCho, GPC:tCho, or fCho:tCho between the two groups. The

	PC	GPC	fCho	tCho	GPC:PC	Ki-67	CD
AS2	0.37+/- 0.21	1.73+/- 0.87	0.23+/- 0.15	2.31+/- 0.85	7.7+/- 9.0	1.3+/- 1.4	1968+/- 854
AS3	0.64 +/- 0.28	2.08+/- 0.90	0.32+/- 0.16	2.99+/- 1.12	3.8+/- 1.5	2.8+/- 1.6	2700+/- 1304
P	0.005*	0.19	0.14	0.03*	0.04*	0.002*	0.04*

GPC:PC ratio was >1.0 in all but one of our biopsy samples. Example 1D and 2D spectra showing that the GPC peak predominates in both AS2 and AS3 are shown in Figure 1. There was a positive correlation between Ki-67 and GPC (r=0.38, p=0.01) and tCho (r=0.41, p<0.01) and between CD and tCho (r=0.30, p=0.05). **Discussion:** Our results show that although PC is higher in AS3 than

AS2, GPC is the predominant choline-containing compound in non-enhancing astrocytoma irrespective of grade. The positive association between Ki-67, tCho, and GPC, but not PC, may indicate that the tCho peak in non-enhancing gliomas primarily reflects the increased metabolism of phosphotidylcholine that occurs during membrane remodeling rather than an increase in choline kinase activity and/or choline transport into the cells.

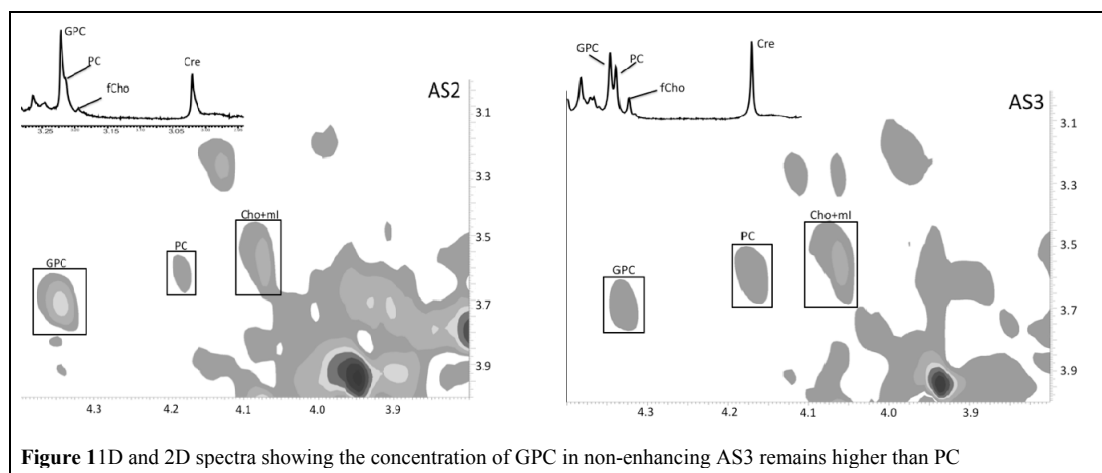


Figure 1 1D and 2D spectra showing the concentration of GPC in non-enhancing AS3 remains higher than PC

However, the lower GPC:PC ratio in AS3 does suggest that the PC-producing processes in the Kennedy pathway are more active in those tumors, but not to the degree observed in contrast-enhancing high grade gliomas where the GPC:PC ratio is typically less than 1.

Conclusion: These results suggest that the presence of contrast-enhancement is associated with choline metabolism in astrocytoma. More studies are needed to determine if the presence of vascular permeability factors such as VEGF can influence the relative levels of GPC and PC in these tumors.

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