Characterization of specific metabolic profiles associated with malignant transformation in cultured human astrocytes using 1H HR-MAS spectroscopy

S. jarso[1], J. kurhanewicz[2], A. Zektzer[3], T. R. Mcknight[3]

[1]Biophysics, uc Berkeley, berkeley, Ca, United States, [2]ucsf, San Francisco, CA, United States, [3]ucsf, San Francisco, ca, United States

Introduction.

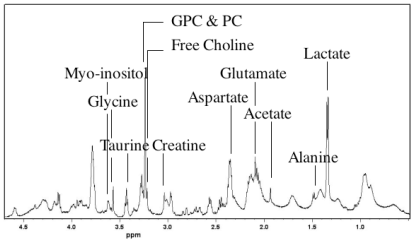
Gliomas are the most common human brain tumors. Astrocytomas, gliomas of astrocytic origin, are believed to develop as the result of stepwise accumulations of genetic lesions. The primary molecular characteristic of low grade astrocytoma (Grade II) is mutation of the p53 tumor suppressor gene. In addition to the loss of p53 function, Grade III astrocytomas exhibit loss the of a functional p16/pRb pathways, Ras pathway activation by means other than Ras mutation, and telomerase reactivation. In addition to the genetic alterations seen in grade III astrocytomas, Grade IV astrocytomas are marked by amplification and expression of rearranged EGFR, as well as alterations in PTEN leading to activation of the Akt pathway. Although grades III and IV are both malignant, the prognoses for these tumors are quite different. The two year survival rate for grade III gliomas is ~50% where as for grade IV it is <20%. The goal of this study is to use high resolution magic angle spinning (HRMAS) ^{1}H spectroscopy to investigate and identify statistically significant differences in the concentrations brain tissue metabolites that correlate with the genetic features of astrocytic malignancy.

Methods.

Cell culture: The three cell lines used in this study were the gift of Russell O. Peiper and had the following genetic alterations: (1) Normal human astrocytes were immortalized with hTERT which results in telomerase expression (NHA); (2) the phenotypically grade 3 genetically engineered astrocytes (GEA3) had additional mutations in E6, E7, and H-Ras resulting in inhibition of p53 and pRb, and activation of the Ras pathway; and (3) the grade 4 model (GEA4) had an additional mutation leading to the expression of Akt. The cells were grown in DMEM-H21 with 10% fetal bovine serum and 5% penicillin/streptomycin and passaged 1:4 at 75% confluency. Once the cells reached 90% confluency, they were fed and harvested the next day for NMR analysis.

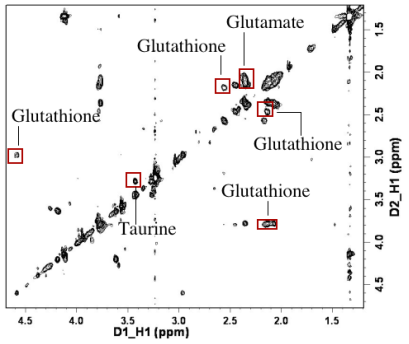
Twenty-one batches of cells (n=10 GEA 3, n = 6 GEA4, and n = 5 NHA) were washed in 30ml of phosphate buffered saline, trypsinized for 2 mins at 37° and spun at 5,000 rpm in 10mls of growth media, at 4°C for 5mins. The media was decanted, the cells were resuspended in 1 ml cold PBS and 100 µl aliquots were taken for Trypan Blue cell count. The cell suspension was spun again at 10 to 14,000 rpm at 4°C for 10 min. The PBS was aspirated and the pellet was transferred to a 1.5 ml cyrotube and stored in liquid nitrogen.

HRMAS: HRMAS 1- and 2-D spectra were acquired using a Varian Inova 500Mhz spectrometer equipped with a gHX gradient nanoprobe, at 1°C. The sample was spun at 2250hz, at the magic angle (θ=54.7°). Acquisitions were obtained using the standard one pulse (proton) sequence, and a rotor synchronized Adiabatic TOCSY pulse sequence. Metabolite peak areas were identified using one- and two-dimensional spectra, and quantified from the proton spectra using Felix 2002, Accelrys Inc. Metabolite levels were reported as ratio of the metabolite peak area to the total metabolite peak areas from 2.0ppm-4.0 ppm and compared between the NHA, GEA3 and GEA4 cell lines. The total metabolite peak area was correlated with cell density measurements.



Results

Figure 1 is a HR MAS H[1] spectrum of GEA3 cells. Table 1 summarizes comparison of normalized metabolite levels in the three cell lines. Significant differences (P<0.05) are highlighted in tan and trends (P<0.08) are in blue. Increases in alanine and glutathione were observed for the transformed cell lines (GEA3 and GEA4) relative to NHA cells. Additionally, taurine tended to be higher in GEA3 cell line relative to the GEA4 cell line. Surprisingly, there were no differences in total Cho, free Cho, or combined PC/GPC among the 3 cell lines. A linear regression analysis demonstrated that the total metabolite peak area from 2.0-4.0ppm correlated with cell density (r = 0.5264, p = 0.0265). This implies that, the observed differences in metabolite levels can be attributed to metabolic changes associated with cell transformation and not cell density. Three sets of adiabatic TOCSY crosspeaks were used to identify the presences of glutathione in the cultured cells. Figure 2 shows an adiabatic TOCSY from the GEA4 cells exhibiting crosspeaks at (2.15,3.77), and (2.20,3.77) that were identified as coming from coupling between methine and methylene protons from a glutamate moiety in glutathione. A second crosspeak at (2.55,2.15) was identified methylene proton couplings in the glutamate moiety of glutathione. A third cross peak at (4.46,2.93) was identified as coming from methylene coupling in the cysteine side chain of glutathione. The coordinated changes in these crosspeak intensities suggest that they came from glutathione, and not the free amino acids.



Discussion

The primary goal of this study was to investigate differences in the metabolic signature of immortalized and transformed astrocytes using 1-D and 2-D HRMAS MRS. The most striking finding was the lack of a significant difference in the free Cho or GPC/PC levels that are typically used to distinguish normal brain from astrocytoma. This may indicate that the common feature of all the cell lines, namely hTERT expression, affected phosphatidylcholine metabolism in a similar manner. Curiously, the metabolites that were elevated in the transformed cells – glutathione and alanine – are more characteristic of spectra from meningioma than astrocytoma. Taken together, these results suggest that the differences in our HRMAS spectra may be more indicative of malignant transformation than cellular origin. Control experiments using glutathione solutions and cultured cell models of meningioma should be performed to verify these findings.

	Lactate	Alanine	Acetate	Glutamate	Aspartate	Creatine	total Cholin	free Cholin	GPC + PC	Taurine	Glycine	Myo inosito	Glutathione
NHA	0.092	0.004	0.028	0.089	0.006	0.016	0.070	0.009	0.061	0.021	0.011	0.028	0.045
GE3	0.171	0.030	0.034	0.111	0.016	0.038	0.081	0.017	0.064	0.028	0.017	0.023	0.090
GE4	0.121	0.010	0.019	0.087	0.004	0.030	0.074	0.012	0.061	0.015	0.013	0.019	0.071

References

1) Govindaraju V, Young K, Maudsley AA, *NMR Biomed*, 13,2000,129-202. 2) Sonoda Y, Ozawa T, Aldape KD, Berger M, Pieper RO, *Cancer Research* 61,2001, 6674-6678. 3) Sonoda Y, Ozawa T., Hirose Y., Aldape K.D., McMahon M., Berger M.S., Pieper R.O., *Cancer Research* 61,2001 4956-60.

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