

## Creatine and N-acetylaspartate concentrations are associated with p53 status in astrocytoma

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**Introduction:** Mutations in the TP53 gene that encodes the p53 protein occur in approximately 50% of Grades 2 and 3 astrocytoma (AS2 and AS3). Positive staining on immunohistochemical assays (IHC) indicates abnormal function of p53 protein and may reflect poorly functioning DNA repair mechanisms and reduced apoptosis leading to increased tumor density and resistance to DNA-damaging therapies. More recent studies have suggested that, counterintuitively, inhibiting p53 actually enhances the effect of the chemotherapeutic agent temozolomide<sup>[1]</sup>. Due to its influence on tumor growth, we hypothesized that a downstream function of p53 might be an alteration in cell metabolism. In the current study, we compared the HRMAS MRS profile of astrocytoma that were immunonegative (p53wt) and immunopositive (p53ab) for p53. We then compared the metabolites that were found to differ between the two groups with the cell density, proliferation, and apoptotic status of the biopsies to determine whether the p53 associated metabolites were associated with tumor growth. **Methods:** We studied 23 patients harboring non-enhancing lesions (10AS2 and 13 AS3). During surgical resection, paired biopsies were retrieved from one or more tumor locations, one was flash frozen in liquid nitrogen and the other was immediately stored in ethanol and later processed for IHC. A total of 39 biopsy-pairs were collected. We used a Varian 500MHz spectrometer, equipped with a gHX gradient nanoprobe to obtain the HRMAS spectrum from the frozen biopsies. Samples were evaluated at 1° C while the tissue was spun at 22.5 kHz at the magic angle. The fully relaxed ERETIC-referenced water presaturation sequence parameters were pulse width = 7.8us, transients = 128, sweep width = 40kHz, and 40,000 points. The absolute concentrations of the following metabolites were quantified using software developed in-house: Ala, Cre, GPC, PC, fCho, Gln, Glu, NAA, Tau, and mI. The Ki-67 proliferation index, caspase-3 apoptotic index, cell density, and p53 status of the paraffin-embedded sister biopsies was assessed with IHC. Student's t-tests were used to compare results from p53wt and p53ab biopsies. Pearson correlations were performed to identify associations between MRS and IHC parameters. A significance level of p <0.05 was used for all tests. **Results:** In total there were 15 p53wt and 24 p53ab biopsies. Of the 10 metabolites studied, only Cre and NAA differed

	Cre	NAA	Casp-3	Ki-67	CD
p53 wt	5.26+/- 2.46	0.39+/- 0.41	0.03+/- 0.02	2.51+/- 1.78	1852+/- 814
p53 ab	3.74+/- 1.6	0.15+/- 0.11	0.04+/- 0.05	2.14+/- 1.59	2809+/- 1223
p	0.02*	0.01*	0.37	0.51	0.01*

**Table 1** Mean +/- SD of metabolic and growth measures in p53ab and p53wt astrocytoma

between the p53wt and p53ab groups (Table 1). Both were lower and more uniform in the p53ab

tumors compared with those with normal p53 function (Figure 1). Further, the cell density of the p53ab tumors was higher than that of the p53wt tumors. No correlations were observed between any of the IHC and metabolic parameters.

**Discussion:** This is the first observation of a relationship between p53 and any MRS marker in astrocytomas. Our results suggest that p53 may regulate creatine kinase activity; indeed a p53 binding domain has been identified in the promoter region for creatine kinase<sup>[2]</sup>. The reduction in NAA may result from the displacement of normal functioning neurons by tumor cells in the high cell density p53ab tumors. **Conclusion:** These findings suggest that p53 may influence the energy production pathway and cell density of astrocytoma; however, mechanism governing the increased cell density in p53ab tumors remains unclear. **Acknowledgements:** R01 CA116041, UCSF Neurosurgery Tissue Bank, Jan Wooten, Hikari Yoshihara **References:** 1.Dinca, E.B., et al., Cancer Res, 2008. 68(24): p. 10034-9., 2.Weintraub, H., et al Proc Natl Acad Sci U S A, 1991. 88(11): p. 4570-1.

