Metabolic Characterisation of Retinoblastoma Tumour Tissue

M. Wilson[1,2], G. Kapatai[1], R. A. Kauppinen[3], T. N. Arvanitis[2,4], C. McConville[1], and A. C. Peet[1,2]
[1]Cancer Sciences, University of Birmingham, Birmingham, United Kingdom, [2]Birmingham Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom, [3]Department of Radiology, Dartmouth College, Hanover, NH, United States, [4]School of Electronic, Electrical and Computer Enineering, University of Birmingham, Birmingham, United Kingdom

Introduction

Retinoblastoma arises in the embryonic neural retina and is a disease rarely seen beyond early childhood (5 years of age), often present at birth. The formation of retinoblastoma is known to be the result of a mutation of the Rb tumour suppressor gene together with other genetic alterations which currently are poorly characterised. Despite the high survival rate (>90%), retinoblastoma is of particular interest since the Rb gene is also known to be relevant to a number of other tumours, including lung, breast and bladder cancers. In this study, the metabolic profile of retinoblastoma is investigated and compared to histological and molecular genetic data, with an aim to improve understanding of the molecular pathways important in this disease.

Methods

^{1}H high-resolution magic angle spinning NMR (HR-MAS) was performed on 12 intact retinoblastoma tumour samples taken from 11 patients. Experiments were performed at field strength of 500MHz using a Bruker TXI hr-MAS probe. Tissue was thawed and inserted into a 25ul rotor and 5ul of 10mM TMSP dissolved into D2O was added. Any remaining space in the rotor was filled with D2O. A pulse acquire acquisition was used, with two seconds of NOESY presat for water suppression. The total acquisition time was either 17 or 34 minutes depending on the sample size. All experiments were performed at probe temperature of 4 degrees Celsius to minimise metabolite degradation. One sample was excluded from further analysis due to its small size (2mg). Spectra were processed using in-house software and principal component analysis (PCA) was performed on the spectral data, binned to a width of 0.01ppm. In addition to HR-MAS the following information was available: histology (N=11), molecular genetics (N=10) and gene-expression (N=10).

Results

The following metabolites were visible in the majority of retinoblastoma spectra: lactate, alanine, leucine/lycine, GABA, acetate, glutamate, glutamine, hypo-taurine (h-Tau), creatine (Cr), choline (Cho), phospocholine (PC), glycerophosphocholine (GPC), taurine (Tau), glycine (Gly) and phospoethanolamine (PEth). Of particular interest was the level of taurine that was notably higher than other tumours of the CNS (Figure 1). Gene-expression analysis showed that the samples could be split into three groups based on genes involved in retinal development and function: A) undifferentiated (N=5), B) differentiated (N=3) and C) highly differentiated (N=1). The clustering of Group C was separated from groups A and B. PCA of the metabolite profiles (Figure 2) revealed that sample 3 had a high value in PC1 and this sample belonged to the highly differentiated group C. PC2 was able to separate the differentiated tumours from the undifferentiated ones with the exception of sample 11. A visual comparison of the spectra showed two samples (8 and 10) had an elevated hypo-taurine (Figure 3) and these samples were also found to be the only undifferentiated tumours without chromosomal alterations.

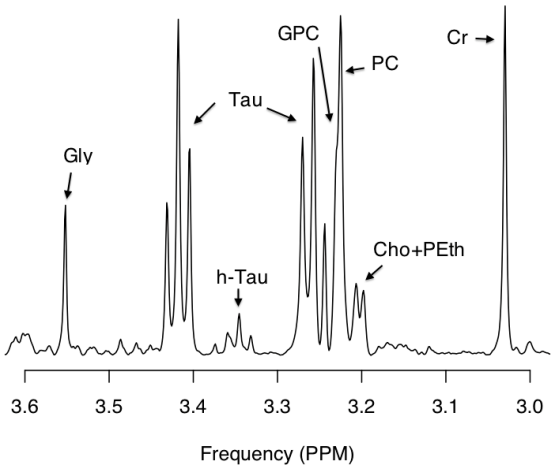

Figure 1 mean spectrum showing high taurine.

Discussion

To the best of the authors` knowledge, this study is the first to report the spectral appearance of retinoblastoma tumour tissue acquired using ^{1}H HR-MAS. Elevated taurine in retinoblastoma may indicate is it general marker of primitive neuroectodermal tumours, since its elevation has also been also been reported in medulloblastoma and neuroblastoma (Wilson et al 2009). Despite the small number of samples investigated, some correlation with gene-expression and molecular genetics was observed, indicating that metabolite profiles may reflect the different genetic subtypes in this disease.

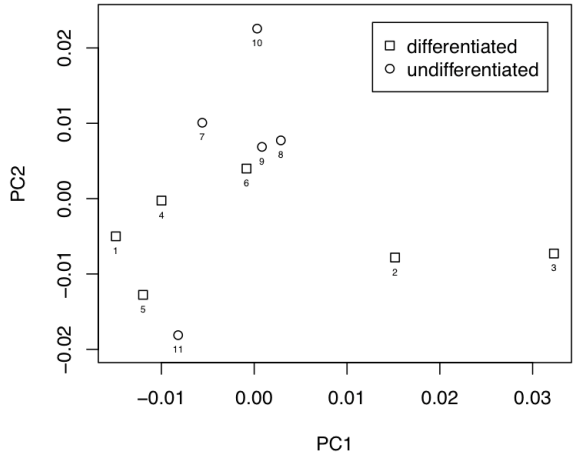

Figure 2 PCA of retinoblastoma tumours.

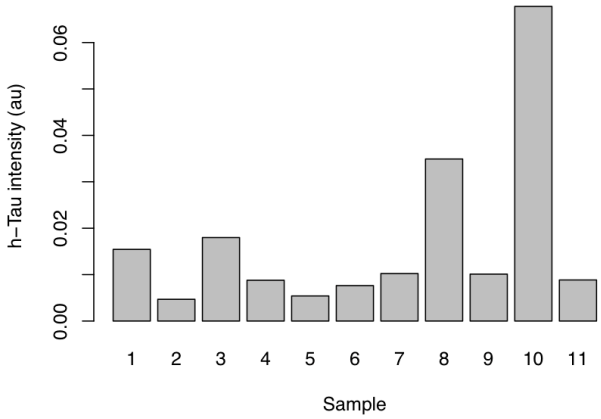

Figure 3 hypotaurine intensity.