^{1}H NMR spectroscopy identifies differences in choline metabolism related to the MYCN oncogene in neuroblastoma

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AIM

To correlate the relative concentrations of choline (Cho), phosphocholine (PCh) and glycerophosphocholine (GPC) with MYCN oncogene status in neuroblastoma cell lines using ^{1}H NMR spectroscopy.

INTRODUCTION

Neuroblastoma is the commonest solid malignancy in children outside the central nervous system. The prognosis for children with the disease is extremely variable and is strongly dependant upon the presence of the oncogene MYCN. For tumours possessing this genetic abnormality, the prognosis is poor and young patients receive intensified treatment. Despite the clinical importance of MYCN our understanding of its mechanism of action remains poor and there are no drugs specifically targeting its pathways in routine clinical use. ^{1}H NMR spectroscopy is a powerful method for probing the biochemical alterations within tumour cells and may be used to help elucidate biochemical pathways. There is increasing evidence from ^{1}H NMR spectroscopy that choline containing metabolites play an important role in cancer cell growth and in particular the concentration ratio GPC / PCh has been found to differ between malignant tumours. In addition, PCh levels are altered in ras oncogene activated cell lines suggesting choline metabolism as a potential pathway for action of other oncogenes.

METHOD

11 neuroblastoma cell lines, 5 MYCN positive and 6 MYCN negative (table 1) were grown in DME/F12 supplemented with L-glutamine, 15% foetal calf serum, penicillin, streptomycin and non-essential amino acid solution. Cells were incubated at 37 ^{0}C in 5% CO_2 and harvested whilst in the exponential phase of growth at about 90% confluence. Between 10^7 and 10^8 cells were harvested 24 hours after a final medium change. The cells were detached from the surface of the flasks using trypsin-EDTA and washed twice in 20ml chilled phosphate buffered saline (PBS) centrifuging in between the washes. Cells were resuspended in 1ml cold PBS and transferred to a cryovial. The sample was then snap frozen in liquid nitrogen before being transferred to a –80 ^{0}C freezer. More than one replicate sample was grown for most of the cell lines. Water soluble metabolites were extracted using perchloric acid and the pH adjusted to be close to pH 7. The resulting solution was freeze dried and then resuspended in D_2O. ^{1}H-NMR spectroscopy of the resulting extracts was carried out using a 90degree pulse and acquire sequence on a Bruker 500MHz spectrometer with a cryoprobe at 293K using 128 scans. Data analysis was performed using Bruker AMIX software and the spectra were referenced to lactate.

Figure 1a. ^{1}H NMR spectrum of SK-N-AS, MYCN negative.

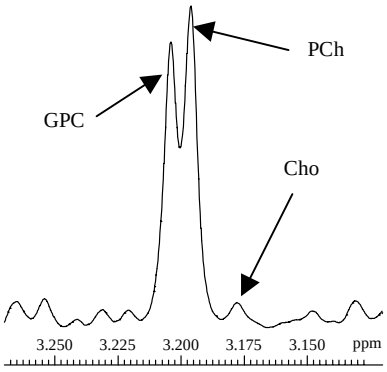

Figure 1b . ^{1}H NMR spectrum of BCH-N-AD, MYCN positive.

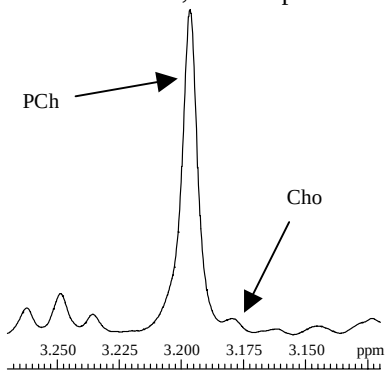

Table 1. A tabulated summary of each cell lines' corresponding choline ratios

Cell Line	MYCN Amp	No of Repeats	GPC/PCh Mean (SD)	Cho/PCh Mean (SD)
KELLY	yes	5	0.00	0.00
BCH-N-AD	yes	1	0.00	0.37
BCH-N-MS	yes	3	0.00	0.08 (0.01)
BE(2)-M17	yes	1	0.00	0.00
BCH-N-KE	yes	3	0.00	0.31 (0.02)
SH-EP1	no	3	0.00	0.37 (0.05)
BCH-N-DR	no	4	0.31 (0.01)	0.11 (0.01)
BCH-N-JW	no	1	1.22	0.13
SK-NAS	no	2	1.52 (0.24)	0.00
SH-SY5Y	no	2	1.44 (0.20)	0.2 (0.04)
GI-M-EN	no	1	0.00	0.08

RESULTS

Spectral resolution was sufficient to quantify choline, PCh and GPC where present and repeat samples from the same cell line showed a high degree of reproducibility for all three choline containing molecules (table 1). A striking feature of the all MYCN positive cell line spectra is that GPC is not present in detectable quantities, with PCh being the predominant metabolite (see fig 1). The GPC / PCh ratio is significantly greater in the MYCN negative cell lines ($p=0.026$ for a one tailed t test) with GPC being present in substantial quantities in 4 of the 6 MYCN negative cell lines.

CONCLUSIONS

^{1}H NMR spectroscopy can determine the relative concentrations of different choline containing metabolites in cell culture extracts with a high degree of reproducibility. Neuroblastoma cell lines with multiple copies of the oncogene MYCN have a significantly decreased GPC/PCh ratio compared with the cell lines not possessing this genetic change. Choline metabolism is altered in MYCN amplified tumours and these pathways present a potential target for new agents.