An *ex-vivo* HR-MAS ^{1}H NMR metabolic characterization of APC$^{Min/+}$ mouse GI tumours

Basetti Madhu[1], Santiago Uribe Lewis[2], Adele Murrell[2], and John R Griffiths[1]

[1]*Molecular Imaging, Cancer Research UK Cambridge Research Institute, Cambridge, England, United Kingdom,* [2]*Epigenetics and Imprinting Laboratory, Cancer Research UK Cambridge Research Institute, Cambridge, England, United Kingdom*

INTRODUCTION: The *Apc*$^{Min/+}$ mouse is a genetically engineered model for gastrointestinal (GI) tumourigenesis since it spontaneously develops tumours in the GI tract (1). The mutation in the *Apc* gene results in accumulation of β-catenin in the cytosol, and its increased translocation to the nucleus activates the targeted genes. This mouse model is widely used to study GI tract tumour biology, but the metabolism of these spontaneous neoplasms is not well understood. We performed HR-MAS ^{1}H NMR spectroscopic analysis of gut tumour biopsies from *Apc*$^{Min/+}$ mice in order to investigate their metabolic characteristics.

METHODS: Animals and Sample collection: *Apc*$^{Min/+}$ mice were bred and maintained by backcrossing with a colony of C57BL/6J mice (Cancer Research UK Cambridge Research Institute, Biological Resources Unit, Cambridge, UK). HRMAS ^{1}H NMR spectroscopy of *ex vivo* tumours and normal adjacent (NA) tissues from the GI tracts of *Apc*$^{Min/+}$ mice was performed on a Bruker 600MHz, with a 4mm HRMAS probe. All the spectra were obtained at a spin rate of 3000Hz and a sample temperature at 4°C. LCModel software was used on water-suppressed spectra to estimate the metabolite concentration. A modified LCModel basis set was used (2). Since these were not brain tumours, NAA & NAAG were omitted from the analysis. The phosphocreatine (PCr) signal was also simulated. The absolute metabolite concentrations were quantified relative to the water signal observed in each individual experiment. The methodology for estimation of metabolite concentrations was validated with phantoms containing known concentrations of metabolites. In this preliminary study, NMR data from tumour (n=17) and NA (n=11) tissue were averaged per mouse (n=5) and then student's t-test was carried out.

RESULTS: Figure 1 shows the metabolite concentrations of GI tumours from the ileum region of *Apc*$^{Min/+}$ mice, relative to NA tissue. Glycine was significantly increased and taurine was significantly decreased in tumour tissue compared to normal adjacent tissues. Choline-containing compounds (t-Cho) were increased in tumour tissues but the difference was not statistically significant. There was no change in the content of the energy metabolites creatine and phosphocreatine.

DISCUSSION: Taurine, a common osmolyte in animal organs, helps maintain cell volume under short-term hypo-osmotic stress (3). It is also conjugated to cholesterol in the liver, forming the bile acid taurocholate. The ileum actively absorbs and de-conjugates bile acids, and high level of taurine is found in normal ileal tissues (4). The reduced taurine in these tumour tissues might be due to modified cellular osmo-regulation or reduced de-conjugation of taurocholate. The increased glycine we observed in these *Apc*$^{Min/+}$ tumours when compared to normal tissues has previously been seen in *ex vivo* HRMAS ^{1}H NMR of human colon cancer tissues (5). Glycine can be formed from the glycolytic intermediate 3-phosphoglycerate, so increased glycine in tumour tissues could result from a (Warburg) glycolytic phenotype (5). Glycine is also an important source of the one-carbon units for *de novo* purine synthesis, so the increased glycine could reflect enhanced nucleotide synthesis (6). Increased t-Cho has been observed by NMR in many cancers, both in clinical biopsies and preclinical mouse model tumours, and is usually attributed to proliferation and malignancy. The increased t-Cho in the present study - primarily increased GPC - was not statistically significant. Possible explanations for the moderate increase in choline metabolites are (i) that these tumours are more like adenomas than carcinomas or (ii) a "field cancerisation" effect where the normal adjacent tissue is "predisposed" for tumorigenesis (7). The "field cancerisation" effect needs to be further investigated to determine whether the metabolism of the normal adjacent tissue is modified due to the *Apc*$^{Min/+}$ mutation. To this end, the investigation of normal adjacent ileal tissue is under way. The unchanged levels of creatine + phosphocreatine suggest that energy metabolism is not affected in these tumours.

CONCLUSIONS: An HRMAS ^{1}H NMR investigation of tumour tissues of *Apc*$^{Min/+}$ mice showed significantly increased glycine and decreased taurine compared to normal adjacent tissue. Total choline was increased – though not statistically significantly. Energy metabolites (t-Cr) remained unchanged. HRMAS ^{1}H NMR spectroscopy of *Apc*$^{Min/+}$ mouse tumour tissues recapitulates some aspects of the metabolic states observed in human tumours.

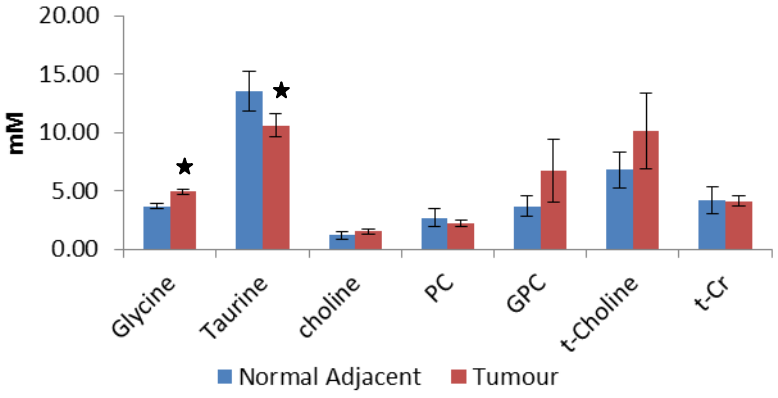

Figure 1: Metabolite concentrations (in mmoles/L tissue water) estimated for tumour and normal adjacent tissues of Apc$^{Min/+}$ mice distal tumour tissues. (Values are mean ± s.e.m.). * $p<0.05$.

This work was supported by Cancer Research UK.

REFERENCES:

1. Giles RH, van Es JH, Clevers H. Biochim Biophys Acta 2003;1653(1):1-24.
2. Opstad KS, Bell BA, Griffiths JR, Howe FA. Magn Reson Med 2008;60(5):1237-1242
3. Wang Y, Holmes E, Comelli EM, Fotopoulos G J Proteome Res. 2007; 6(10):3944-51
4. Wang Y, Tang H, Holmes E, Lindon JC, et al. J Proteome Res. 2005; 4(4):1324-9
5. Chan, E. C.; Koh, P. K.; Mal, M.et al J. Proteome Res. 2009, 8 (1), 352–61
6. Fu TF, Rife JP, Schirch V. Arch Biochem Biophys. 2001 393(1):42-50.
7. Backshall A, Alferez D, Teichert F, et al. J Proteome Res. 2009, 8(3):1423-30.