Spatial dependence of metabolite concentrations in wild type and *Apc*$^{Min/+}$ mouse GI tissues: An *ex-vivo* HR-MAS ^{1}H NMR spectroscopic study

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INTRODUCTION: The *Apc*$^{Min/+}$ mouse is a genetically engineered model for gastrointestinal (GI) tumourigenesis since it spontaneously develops tumours in the GI tract (1). 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 tumour biopsies from ApcMin/+ mice, and also from the normal small intestines of the *Apc*$^{Min/+}$ mice and their wild type littermates. The normal intestinal tissues were collected at regular distances from stomach to colon, in order to investigate spatial dependence of metabolite concentrations along the small intestine. We also compared the tumour metabolic data with that from adjacent normal tissues in *Apc*$^{Min/+}$ mouse intestines, in order to test for any "field cancerisation" effect.

METHODS: Animals and Sample collection: *Apc*$^{Min/+}$ mice were bred and maintained by backcrossing with a colony of C57BL/6J mice. HRMAS ^{1}H NMR spectroscopy of *ex vivo* tumour and normal adjacent (NA) tissues from the GI tracts of *Apc*$^{Min/+}$ mice and normal tissues from wild type littermate 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 concentrations. 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 and then student's t-test was carried out. The methodology for estimation of metabolite concentrations was validated with phantoms containing known concentrations of metabolites. In this preliminary study, NMR data from wild mice (n=2) and *Apc*$^{Min/+}$ mice (n=2) were obtained; normal tissue (n=22) tumour (n=19) and NA (n=21) tissue were sampled in regular distances from stomach to colon.

RESULTS: Figure 1 shows the plots of alanine, Glx (glutamate + glutamine) and t-Cr (creatine + phosphocreatine) concentrations of GI tissues of *Apc*$^{Min/+}$ and wild type mice as a function of distance from the stomach. The concentrations of these three metabolite groups show smooth gradients along the normal small intestines of both mouse genotypes, whereas the concentrations are very different in *Apc*$^{Min/+}$ tumour tissues. Figure 2 shows the plot of taurine, glycine and t-choline (choline + phosphocholine + glycerophosphocholine) concentrations of GI tissues of wild type and *Apc*$^{Min/+}$ mice. Taurine (p=0.0035), glycine (p=0.0693) and t-choline (p=0.0423) show higher concentrations in normal tissues from *Apc*$^{Min/+}$ mice compared to wild type mice. Tumour samples showed consistently higher levels of all the metabolites. The magnitude in change of many of the gradients is greater with increased distance from the stomach, which correlates with increased tumour frequency in the distal part of the small bowel.

DISCUSSION: Increased level of choline containing metabolites has been observed by NMR in many cancers, both in clinical biopsies and preclinical mouse model tumours, and is usually thought to be associated with proliferation and malignancy (3). The increased glycine we observed in these *Apc*$^{Min/+}$ tumours relative to normal tissues has previously been seen in *ex vivo* HRMAS ^{1}H NMR of human colon cancer tissues (4). Glycine can be formed from the glycolytic intermediate 3-phosphoglycerate, so increased glycine in tumour tissues could result from a (Warburg) glycolytic phenotype (4). 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 (5). Possible explanations for changes in taurine and glycine metabolites in *Apc*$^{Min/+}$ tissues may be due to a "field cancerisation" effect where the normal adjacent tissue is "predisposed" for tumorigenesis (6). The unchanged levels of alanine, Glx, t-Cr suggest that amino acid and energy metabolism is not affected in normal tissues of *Apc*$^{Min/+}$ mice. In contrast increased levels of choline, alanine, glycine, taurine, Glx and t-Cr in *Apc*$^{Min/+}$ mouse tumours would be consistent with transformed phospholipid, amino acid and energy metabolism.

CONCLUSIONS: Normal gut tissues from wild type and *Apc*$^{Min/+}$ mouse showed spatial variation of metabolite concentrations along the small intestine. Increased levels of taurine and glycine metabolites in *Apc*$^{Min/+}$ mouse small bowel samples can be attributed to "field cancerisation" effect, which needs further investigation. On the other hand *Apc*$^{Min/+}$ tumour tissue samples showed modified phospholipid, amino acid and energy metabolism.

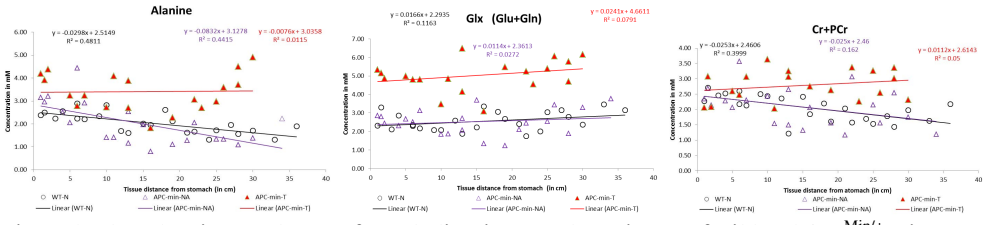

Figure 1: Plots showing no change of metabolites in normal gut tissues of wild and Apc$^{Min/+}$ mice

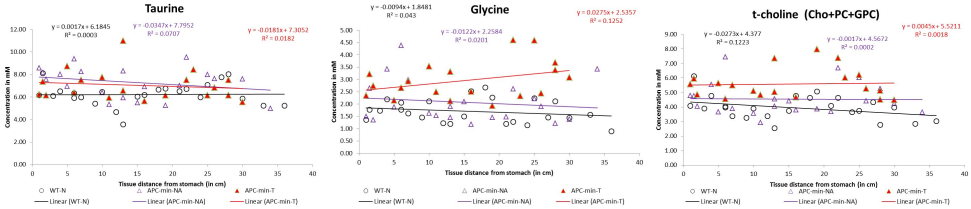

Figure 2: Plots showing changes of metabolites in normal gut tissues of wild and Apc$^{Min/+}$ mice

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