Total choline and water diffusion of C-*neu* /HER2 mammary carcinomas in Oncomice® assessed by *in vivo* ^{1}H MRS

B. Madhu[1], L. M. Rodrigues[1], J. R. Griffiths[1]

[1]Cancer Research UK Biomedical Magnetic Resonance Research Group, St. George's Hospital Medical School, London, United Kingdom

INTRODUCTION: Cancerous breast tissue contains higher concentrations of choline metabolites than normal breast tissue or benign lesions, possibly because products of increased membrane choline lipid metabolism can contribute to mitogenic signal transduction. The ^{1}H MRS choline signals have been used as a diagnostic marker for malignancy, and phosphomonoesters (PMEs, mainly phosphoethanolamine and phosphocholine (PC)) measured by ^{31}P MRS have been found to increase with tumour progression[1]. Another marker of pathophysiological change associated with tumour growth, heterogeneity and damage is the diffusion of water which will be affected by the cellular architecture of a tissue to include cell shape and size, membrane permeability, vascularity and cellular volume fraction. Transgenic oncomice expressing the c-*neu* oncogene develop aggressive mammary carcinomas in the mammary gland area[2] and show increasing PMEs with tumour progression by *in vivo* ^{31}P MRS[3]. Amplification and overexpression of oncogenes such as C-*neu*/HER2 is important in the progression of breast cancer. Here we monitor the total choline (PC+GPC (glycerophosphocholine)+choline) and the apparent diffusion coefficient (ADC) of water in these tumours during growth, by *in vivo* ^{1}H MRS.

METHODS: Female oncomice carrying the C-*neu* oncogene develop tumours in the mammary gland area between 18-20 weeks of age. Mice (n=4) were anaesthetised with hynorm/hypnovel, and ^{1}H MRS with a 2 turn RF coil was performed on a Varian 4.7T spectrometer. Voxels encompassing most of the tumour were selected from scout gradient echo images and for the same voxel two protocols were used: 1) total choline was measured using PRESS localisation with water suppression, TR=2s, 64 transients and TE=20, 68, 136, 272 and 408 ms. Unsuppressed water was measured with the same parameters except nt=16 and a lower receiver gain. Choline concentration was calculated using tumour unsuppressed water as a reference[4]. 2) ADC was estimated using Diffusion Weighted ^{1}H MRS and a localized STEAM sequence with diffusion-sensitising gradients in echo-time periods, TE=24ms, TM=100ms,Tr=3sec, δ = 6ms, Δ = 112 ms and diffusion gradients increased from 0 to 13 G/cm with 1 G/cm intervals. The diffusion weighted ^{1}H MRS data analysis with plots of the natural logarithm of normalised water signal [Ln(S/So)] against the b-values [$\gamma^2G^2\delta^2(\Delta-\delta/3)$s/cm^2] resulted in fitting two linear regression lines giving rise to a fast and slow component for ADC of water.

RESULTS: 3 out of 4 tumours showed increased total choline (3.23ppm) with increase in tumour volume, and a scatter plot gave a correlation coefficient of 0.86 ($p<0.001$) (*Figure 1a)*. This fits with our ^{31}P *in vivo* MRS study of these oncomouse mammary carcinomas which showed an increase in total PME, expressed as a ratio of PME/ total P (●), with increase in tumour volume ($r=0.99$, $p=0.012$) (*Figure 1b*). There was no correlation between either water T_2 or choline T_2 (data not shown) and tumour volume. The diffusion weighted ^{1}H MRS data showed a fast (range of 0.63 - 1.9 x10^{-9}m^{2}s^{-1}) and slow (range of 0.22 -0.68 x10^{-9}m^{2}s^{-1}) component diffusion coefficient for water. However, when either ADC component was plotted against tumour volume there was no correlation (data not shown).

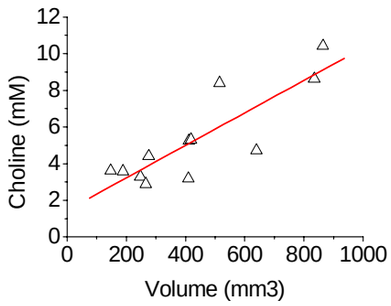

***Figure 1a**:* Choline (mM) *vs* tumour volume by *in vivo* ^{1}H MRS (n=4)

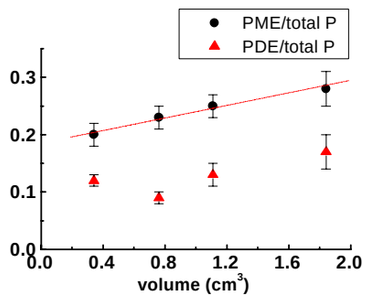

***Figure 1b**:* PME/total P (●) and PDE (phosphodiesters) /total P(▲) during growth by *in vivo* ^{31}P MRS[3]

DISCUSSION: The total choline concentration of adenocarcinomas in oncomice was linearly related to tumour size (*Figure 1a*), perhaps reflecting increased membrane choline metabolism in proliferating cells. This finding correlates with previous *in vivo* ^{31}P MRS studies on these tumours[3], where PMEs also increased linearly with tumour progression (*Figure 1b*). There was no significant association of water T_2 or of either the fast or slow ADC components with increase in tumour volume, suggesting that there were no major changes in tissue morphology or necrosis. Since overexpression of the c-*neu*/HER2 gene activates a growth-control pathway, these tumour growth-associated changes in resonances related to membrane choline phospholipid metabolism might suggest a role for choline metabolites in intracellular signal transduction processes through the activated tyrosine kinase c-*neu*/HER2 cascade.

REFERENCES: (1) Negendank, W *NMR in Biomed*. **5**, 303-324, 1992. (2) Muller et al, *Cell* **54**, 105-115, 1988. (3) Rodrigues et al, *Magma* (in press) 2004. (4) Madhu B *et al*, Proc ISMRM 1287, 2003.

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