A metabolomic study of wild type and HIF-1α deficient astrocytomas measured by in vivo and in vitro 1H MRS.

H. Troy[1], Y-L. Chung[1], B. Madhu[1], M. Mayr[2], L. Ly[2], B. Blouw[3], R. Johnson[3], J. R. Griffiths[1], M. Stubbs[1]

[1]Basic Medical Sciences, St George's Hospital Medical School, Tooting, London, United Kingdom, [2]Cardiac and Vascular Sciences, St George's Hospital Medical School, Tooting, London, United Kingdom, [3]Molecular Biology, University of California, San Diego, California, United States

INTRODUCTION:- HIF-1, the transcription factor believed to be largely responsible for the responses of tissues to an inadequate oxygen supply, is widely overexpressed across a broad range of cancers [1]. In hypoxia, a common feature of the tumour environment, the α and β subunits complex translocates to the nucleus where it upregulates many pathways including glycolysis and VEGF secretion. Blouw et al [2] found that astrocytomas deficient in HIF-1α (HIF-1ko) grew more slowly in subcutaneous (SC) sites than wild-type tumours (HIF-1wt) and had lower expression of a glycolytic enzyme and lower VEGF secretion in response to hypoxia. We studied the metabolite profiles of these HIF-1wt and HIF-1ko astrocytes grown as SC xenografts, both *in vivo* by MRS and *in vitro* by ^{1}H NMR of the tumour extracts, to study the role of HIF-1α in tumour metabolism.

EXPERIMENTAL METHODS:-

^{1}H MRS *in vivo*: HIF-1wt and HIF-1ko astrocytes were implanted SC in MF1 nude mice. Tumour volume (l x w x d x π/6) was monitored and ~500mg tumours were studied by a localised ^{1}H MRS PRESS sequence at 37 °C on a Varian 4.7T spectrometer with a 15mm 2-turn surface coil; the lactate peak was edited by a localised modified SSEL-MQC sequence and the *in vivo* lactate/water ratio was quantified [3].

^{1}H NMR *in vitro*: After the *in vivo* ^{1}H MRS study, tumours were freeze-clamped and extracted with perchloric acid. Neutralised samples were then freeze-dried and reconstituted in D_2O. Sodium 3-tri-methylsilyl-2-2-3-3-tetradeutero-propionate was added for chemical shift calibration and quantification. *In vitro* ^{1}H MRS of tumour extracts was performed on a 500 MHz Bruker spectrometer [pulse angle 45°, repetition time of 3.5 s]. The water resonance was suppressed by using gated irradiation centred on the water frequency.

Western Blots: Nuclear extracts were prepared on cells exposed to hypoxic conditions and normoxic conditions and tested for HIF-1 α and β.

RESULTS:- The HIF-1ko tumours grew at about 40% of the rate of HIF-1wt tumours. The HIF-1ko cells, as expected, showed no nuclear translocation of HIF-1α or β in response to hypoxia. A significantly ($p < 0.02$) higher lactate/water ratio of 3.67 ± 0.40 (n=9) was observed in the HIF-1wt tumours *in vivo* compared to 2.06 ± 0.44 (n=8) in HIF-1ko tumours. This was mirrored by the significantly higher lactate measured in the extracts. Leucine, lactate, alanine, glutamate, glutathione, total choline and myoinositol (Table 1) were all significantly lower in the HIF-1ko compared with the HIF-1wt astrocytomas, whereas glucose was 2.4 fold higher in the HIF-1ko tumours ($p < 0.05$).

DISCUSSION:- The HIF-1ko cells, as expected, showed no nuclear HIF-1 α or β. The HIF-1ko tumours grew significantly slower than the HIF-1 wt tumours, as seen in several HIF-1 deficient models [2,4]. ^{1}H MRS measurements *in vivo* showed significantly less lactate in the HIF-1ko tumours, consistent with a failure to upregulate glycolysis in the absence of HIF. Similarly, significantly lower concentrations of lactate and several other metabolites were observed by ^{1}H NMR *in vitro* (Table 1). However, these decreases were quite small. Since HIF-1α is considered to be the regulator of 40-60 genes associated with hypoxia [5] it is rather surprising to find that its absence has so little effect on the metabolite profile of these xenografts. Surprisingly too (since HIF-1 is supposed to upregulate glucose transporters) there was 2.4-fold *higher* glucose in the HIF-1ko tumour extracts. We have recently shown enhanced glucose uptake in another HIF-1 deficient model [6]. Blouw et al [2] found much less hypoxia and necrosis in HIF-1wt than HIF-1ko tumours when grown SC, but this is not apparent in the present metabolite profiles. We conclude that there are mechanisms other than HIF-1α transcription that may regulate metabolism in these astrocytomas, which emphasizes the robustness of metabolism *in vivo*.

	HIF-1 wt (n = 5)	**HIF-1ko** (n = 5)
Leucine	0.42 ± 0.02	0.35 ± 0.02*
Lactate	11.89 ± 0.44	10.44 ± 0.36*
Alanine	2.41 ± 0.15	1.87 ± 0.13*
Glutamate	2.96 ± 0.10	2.38 ± 0.16*
Glutathione	1.99 ± 0.12	1.47 ± 0.17*
Total choline	2.04 ± 0.06	1.67 ± 0.13*
Myoinositol	3.84 ± 0.18	2.93 ± 0.34*
Glucose	0.19 ± 0.05	0.46 ± 0.07*

Table 1: Summary of ^{1}H NMR of tumour extract
Data expressed as μmol/g wet weight, mean +/- S.E.M. * statistically significant, $p < 0.05$.

ACKNOWLEDGEMENTS:- This work was supported by the Cancer Research UK.

REFERENCES: [1] Harris, *et al.* Nature Reviews Cancer 2: 38-47 (2002), [2] Blouw, *et al.* Cancer Cell 4: 133-146 (2003), [3] Madhu B *et al*, Proc ISMRM 1287, (2003), [4] Griffiths, *et al.* Cancer Research 62: 688-695 (2002), [5] Semenza, *et al.* Genes and Development Review 14: 1983-1991 (2000), [6] Troy, *et al.* ISMRM Workshop on Advances in Experimental & Clinical MR in Cancer Research (2004).