A metabolomic study of HIF-2a over-expressed and empty vector 7860 renal tumours using in vivo and in vitro 1H MRS.

H. Troy[1], Y. L. Chung[1], B. Madhu[1], A. L. Harris[2], S. Wigfield[2], S. Biswas[2], M. Stubbs[1], J. R. Griffiths[1]

[1]St. George's University of London, London, United Kingdom, [2]John Radcliffe Hospital, Oxford, United Kingdom

INTRODUCTION:- HIF-2 is a transcription factor believed to be responsible for the responses of tissues to an inadequate oxygen supply. HIF-2α is expressed in cells of most organs post-systemic hypoxic exposures and its expression is involved in tumourigenesis [1]. The HIF-2 complex activates transcription from DNA recognition sites such as the hypoxia-response element of the erythropoietin and VEGF [2]. In hereditary von Hippel-Lindau (VHL) disease, elevated levels of VEGF and HIF-2α mRNA have been found in stromal cells of tumours [3]. Mutations of the VHL tumour suppressor gene are associated with a variety of tumours such as renal cell carcinomas [4]. We studied the metabolite profiles of HIF-2α over-expressed 7860 renal cancer cells grown as xenografts, and compared them with controls grown from the empty vector (EV) 7860 renal cancer line, both *in vivo* by MRS and *in vitro* by ^{1}H MRS of tumour extracts, in order to study the role of HIF-2 in tumour metabolism.

EXPERIMENTAL METHODS:-

^{1}H MRS *in vivo*: HIF-2α over-expressed and EV 7860 renal cancer cells were implanted subcutaneously in MF1 nude mice. Tumour size(l x w x d x π/6) was monitored and ~500mg tumours were studied by a localised ^{1}H MRS PRESS sequence to measure choline levels at 37 °C on a Varian 4.7T spectrometer with a 15mm 2-turn surface coil; lactate signal was edited by a localised modified SSEL-MQC sequence and the *in vivo* lactate/water ratio and choline peak were quantified [5].

^{1}H MRS *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 600 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.

RESULTS:- HIF-2α over-expressed tumours grew at a significantly faster rate than the EV tumours (Figure 1). A significantly ($p < 0.04$) higher total choline level (12.73 ± 1.11 mM (n=7)) was observed in the HIF-2α over-expressed tumours *in vivo* when compared to the empty vector tumours (10.18 ± 0.44 mM (n=8)). This was mirrored by the significantly higher level of free choline and phosphocholine (PC) in the extracts. Taurine, creatine and glucose were also significantly higher in the HIF-2α over-expressers whereas surprisingly lactate levels were significantly lower in the tumour extracts (Table 1)

Table 1. **^{1}H MRS of the tumour extracts**

Metabolite	EV (n= 5)	HIF-2α (n=4)
Leucine	0.13 ± 0.01	0.17 ± 0.01 [a]
Isoleucine	0.06 ± 0.01	0.08 ± 0.01
Lactate	5.13 ± 0.85	2.54 ± 0.58 [a]
Alanine	0.84 ± 0.06	0.63 ± 0.04 [a]
Choline	0.17 ± 0.02	0.29 ± 0.05 [a]
PC	0.73 ± 0.09	1.14 ± 0.05 [a]
Taurine	13.96 ± 1.55	16.70 ± 0.66 [a]
Creatine	1.26 ± 0.14	2.00 ± 0.17 [a]
Glucose	0.63 ± 0.10	1.19 ± 0.29 [a]

Metabolites expressed as μmol/g wet weight, mean +/- S.E.M. $P < 0.05$ for significance. [a] denotes significance comparing EV with HIF-2α over-expressed tumours.

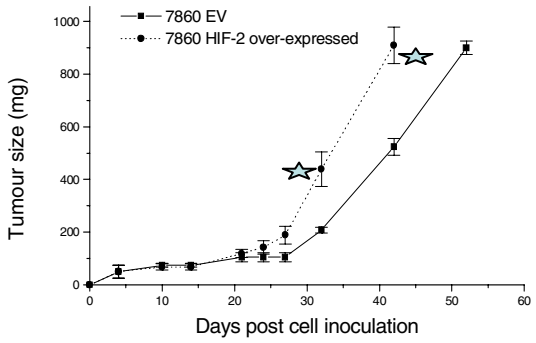

Figure 1. Renal 7860 tumour growth curve
☆ denotes significance comparing EV with HIF-2α over-expressed tumours.

DISCUSSION:- The HIF-2α over-expressing renal tumours had significantly higher levels of choline and PC than the EV tumours. Elevated levels of choline are often associated with faster tumour growth. They also showed lower lactate and alanine levels (products of anaerobic metabolism) and higher glucose. These findings suggest that the faster growth rate of HIF-2α over-expressing tumours was sustained by the more effective metabolism of glucose, i.e., oxidative metabolism which produces 38 ATP molecules for each glucose molecule whereas glycolysis produces only 2 ATP molecules. The lower lactate and alanine levels were unexpected results, since (by analogy with the role of HIF-1α in tumours) one would expect HIF-2α over-expression to induce a state of pseudo-hypoxia in which anaerobic metabolism would be upregulated. Sowter *et al*., 2003 [6] found that GLUT-l was under HIF-2 control (which would explain the elevated glucose we observed) but did not report any effect on glycolytic or oxidative enzymes.

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