MRI AND MRS CHARACTERIZATION OF TWO EXPERIMENTAL MODELS OF OVARIAN CANCER WITH DIFFERENT GLYCOLYTIC PHENOTYPES

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Introduction

In a previous study, two ovarian cancer cells endowed with different glycolytic phenotypes and different resistance to severe hypoxia *in vitro* have been identified (1). The various intensities of the Warburg effect observed in tumor cell lines possibly reflect their genetic heterogenicity and, importantly, this has also been observed in patients affected by different tumor entities or among individuals affected by the same tumor type, mainly by 18 FDG PET studies (2,3).

Proper animal models would greatly contribute to evaluate the significance and biological role of these and other alterations during tumour growth or following treatment in ovarian cancer. In this work two models of human ovarian carcinoma were characterised by quantitative MRS and ADC analyses.

Methods

Based on measurements of glucose consumption and lactate production rates *in vitro* as well as assessment of expression levels of glycolysis-associated genes, we recently identified OC316 and IGROV1 cells as prototypes of highly and poorly glycolytic ovarian cancer cells, respectively (1).

In vivo MRS measurements were performed on a Varian Inova system, equipped with a 200/183 horizontal magnet at 4.7 T. MRI/MRS analyses were performed on tumour xenografts 11-35 days following s.c. implantation in the dorsum of OC316 and IGROV-1 cells in SCID mice. MRI evaluation was performed by T1W (TR/TE=500/20ms), T2W (TR/TE=3000/70ms) and PD (TR/TE=3000/20ms) multislice spin echo images with an in plane resolution as high as 47 x 94 μm^2 and a thickness of 600 μm. ADC measurements were performed by acquiring DW images (TR/TE=2000/50 ms, b ranging from 123 to 1105 s/mm^2). Quantitative ^{1}H MRS analyses were performed according to a protocol described in (Canese et al, submitted, 4) by using a PRESS sequence (TR =4000 ms, in order to minimise T1 relaxation losses and TE ranging from 23 to 272 ms) and assuming 80% of tumour water content. LCModel was used for the spectral fitting. Histological analysis of OC316 and IGROV-1 tumor sections following hematoxylin/eosin staining was performed according to standard methods (1).

Results and Discussion

High resolution (9.4 T) quantitative MRS analyses on cell extracts showed major differences in the intracellular concentration of lactate (Lac) and the pool of glutamate and glutamine (Glx) in the OC316 cells compared with the IGROV1 cells. Metabolic differences have also been detected by in vivo localised MRS between the OC316 and IGROV1 xenografts as shown in Fig. 1 – lower panel. Lactate was observed in all the OC316 tumours (n=3), and in 50% of the IGROV1 xenografts (n=4) analyzed. Lactate concentrations were 38.2 ± 8.3 and 1.6 ± 0.1 mM in the OC316 and IGROV1 tumours, respectively.

Region of necrosis or haemorrhage could be easily identified as hyperintense or hipointense areas, respectively, in the T2-weighted images (Fig. 1- upper panel). MRI revealed differences in tumour morphology and internal composition between the two models: OC316 were characterized by a prominent central necrotic core, while IGROV1 had several small and diffuse necrotic areas. Furthermore, by using MR images it was possible to perform volume analysis during growth with higher accuracy than the conventional methods based of calipers measurements and approximate formula.

The ADC value is often correlated with tumour cellularity, as observed in histological sections and an elevated ADC value has been correlated with the necrotic fraction of animal tumour models (5). Quantitative MRI analysis showed ADC values of 6.5± 4.0 and 7.3 ± 4.1 x $10^{-4}mm^2/s$ for the OC316 and IGROV1 model, respectively, which were consistent with those measured in ovarian carcinoma patients (6). The large spread in the ADC values highlights tumour heterogeneity due to the presence of necrotic or haemorrhagic areas.

Areas of elevated ADC were found to merge with the necrotic regions detected by histological analysis of specimens excised one week after the end of treatment (at late stages of growth) in both models. In particular it was possible to find a correspondence between the percentages of necrosis in the OC316 and IGROV1 models measured by histology and those found by using ADC maps.

Conclusions

Differences in the spectral profile could reflect changes in tumour environment (i.e. pH) and/or altered metabolism. Appropriate experimental set up will be required to identify the nature of these alterations.

These results could highlight a link between the Warburg effect and the response to anti angiogenic therapy in ovarian cancer.

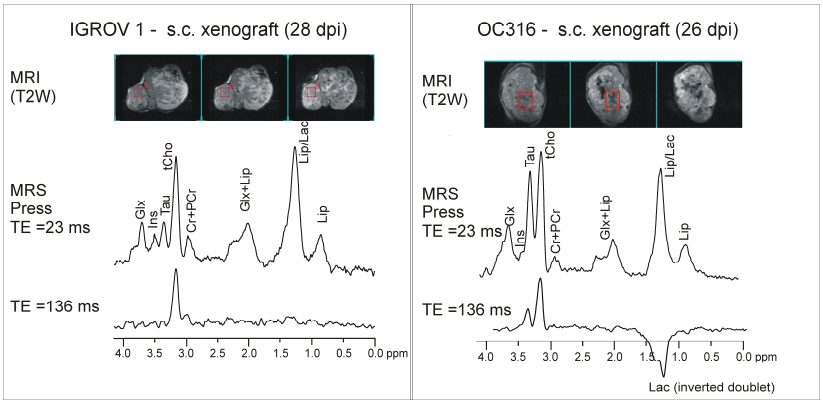

Fig – 1

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