Magnetic Resonance Spectroscopic Imaging of Orthotopic Ovarian Cancer

M-F. Penet[1], K. Glunde[1], D. Artemov[1], F. Podo[2], and Z. M. Bhujwalla[1]
[1]JHU ICMIC Program, Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Department of Cell Biology and Neurosciences, Section of Molecular and Cellular Imaging, Istituto Superiore di Sanità, Rome, Italy

Introduction Epithelial ovarian cancer (EOC) remains the leading cause of death from gynecologic malignancy among women in developed countries. Although the prognosis in cases detected at early stage is quite favorable, the vast majority of cases are diagnosed at an advanced stage when five-year survival rates are only 30-40%. The poor prognosis of ovarian cancer is due to a combination of the aggressive characteristics of the disease and a lack of effective therapy, further compounded by the key problems of late detection of the disease and resistance of most tumors to current treatments. New therapeutic strategies are urgently needed to improve survival rates and to eventually cure patients. Since ovarian cancer cells typically have high choline kinase compared to nonmalignant epithelial cells (1), choline kinase may be a novel target for ovarian cancer treatment. Treatment effects can also be monitored with ^{1}H MRSI. To evaluate treatment strategies it is important to use models that closely mimic tumor growth in humans. We therefore orthotopically implanted ovarian cancer tissue in the ovary of female mice and demonstrated that tumor growth can be followed by MRI using T_1-weighted images combined with diffusion-weighted images (DWI), and tumor metabolism with ^{1}H MRSI. Significant differences were observed between total choline and PC levels in orthotopic *versus* subcutaneous tumors that emphasize the importance of using orthotopic tumors for evaluating metabolic targeting.

Methods We used OVCAR3 cells from ATCC, grown in RPMI-1640 medium supplemented with 10% FBS. The OVCAR3 line was originally established from the malignant ascites of a patient with progressive adenocarcinoma of the ovary after combination chemotherapy (2). We performed surgical orthotopic implantation of OVCAR3 cells by transplanting a piece of tumor tissue in the ovary of severe combined immunodeficient (SCID) female mice. Tumor tissue pieces were obtained from subcutaneously implanted tumors obtained following the inoculation of 2 x 10^6 cells in the flank of female SCID mice. We also performed subcutaneous implantation with pieces of tumor tissue of similar size for comparison. MRI/MRSI studies were performed on a 4.7T Bruker Avance (Bruker, Billerica, MA) spectrometer using a home-built volume coil for the orthotopic tumors and a solenoid coil for the subcutaneous tumors. Mice were anesthetized with an i.p. injection of ketamine and acepromazine. Briefly, T_1-weighted images and DWI were acquired to localize the tumor that appears hyperintense in the images. Metabolic maps of total choline (tCho) and lactate+lipid were obtained from a 4-mm thick slice using a 2D-CSI sequence with VAPOR water suppression. MRSI acquired without water suppression was performed on the same slice for quantification. The acquisition was performed with the following parameters: echo time of 120 ms, repetition time of 1000 ms, field of view 3.2 cm x 3.2 cm (1.6 cm x 1.6 cm for the subcutaneous tumors), 16 x 16 phase encode steps, number of scans of 4, block size of 1024. Reference 2D-CSI images of the unsuppressed water signal were acquired with TE = 20 ms and NS = 1, with all other parameters remaining the same. Quantitative maps of tCho and lipids+lactate were generated according to the method described by Bolan *et al.* (3). High-resolution ^{1}H MRS was performed on tumor extracts obtained using dual-phase extraction, on a 500 MHz Bruker spectrometer.

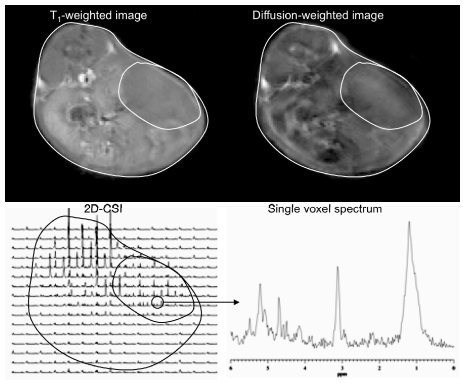

Figure 1: Top row, T_1-weighted image and DWI acquired at 4.7T of a mouse implanted orthotopically with an OVCAR3 tumor. The tumor is highlighted by a white line. Bottom row, 2D CSI and a representative single voxel spectrum with the tCho peak visible at 3.2 ppm.

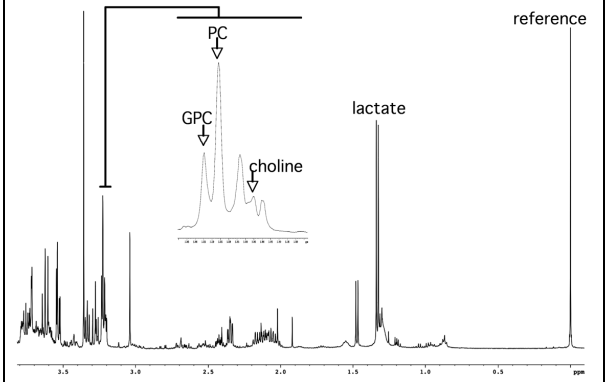

Figure 2: Representative spectrum of the water phase of an orthotopic OVCAR3 tumor extract, showing a high level of phosphocholine (PC). The spectrum was acquired on a 500 MHz Bruker spectrometer (GPC, glycerophosphocholine). The tCho signal observed *in vivo* in **Figure 1** is the combination of the signals of free choline, PC and GPC.

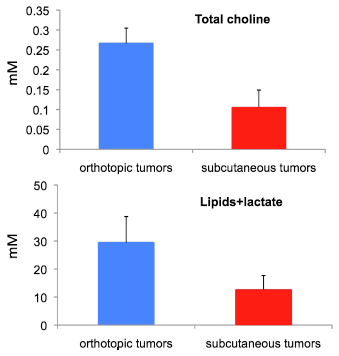

Figure 3: *In vivo* total choline and lipids+lactate quantifications in OVCAR3 orthotopic and subcutaneous tumors (n = 3 and n = 8 respectively, $P < 0.05$ for total choline)

Results and Discussion High-resolution ^{1}H MRS of OVCAR3 cell extracts revealed a high level of PC in those ovarian cancer cells (data not shown). T_1-weighted images and DWI images acquired from mice orthotopically implanted with OVCAR3 tumors are shown on **Figure 1** (top row); corresponding 2D CSI and a representative spectrum from a single voxel are shown in the bottom row. Orthotopic OVCAR3 tumors were characterized by a high level of tCho compared to subcutaneous tumors. High-resolution MRS analysis of tumor extracts revealed that the high level of tCho in the orthotopic tumors was due to a high level of PC, as shown in **Figure 2**. The orthotopic model presents several advantages compared to the subcutaneous one. It mimics human disease since we observed peritoneal invasion by the ovarian cancer cells, with ascites formation, and metastases present in the liver, on the diaphragm, and seeding into the abdominal cavity. Moreover, we observed significantly higher tCho in orthotopically-implanted tumors (**Figure 3**). Increased tCho and an accumulation of PC, associated with an increase of choline kinase (Chk) activity, have been observed in human ovarian cancer biopsies and ovarian carcinoma cell lines (1,4,5). Silencing Chk in human breast cancer cells has been shown to reduce growth, induce differentiation, and increase the effect of conventional chemotherapy (6,7). We intend to target ovarian cancer by developing liposomes loaded with gadolinium, as MR contrast agent, that will contain small interfering RNA (siRNA) to downregulate Chk. The orthotopic OVCAR3 xenograft model described here will be used in preclinical studies to evaluate this treatment strategy. Liposome delivery will be followed with MRI. Treatment efficacy can be assessed by following the tumor growth *in vivo* with MRI, and downregulation of Chk can be detected by a decrease of tCho signal in ^{1}H MRS images.

References: (1) Iorio *et al.*, Cancer Res 65: 9369 (2005), (2) Hamilton et al., Cancer Res 43: 5379 (1983), (3) Bolan *et al.*, MRM 50:1134 (2003), (4) Ferretti *et al.*, Br J Cancer 86:1180 (2002), (5) Iorio et *al.*, Cancer Res 70:2126 (2010), (6) Mori *et al.*, Cancer Res 67:11284 (2007), (7) Glunde *et al.*, Cancer Res 65:11034 (2005),

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