Weekend Educational Program at the ISMRM Meeting in Milan, Italy, 2014

Modeling Cancer in MR Studies: Cells and Animals

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Target Audience: Basic and translational researchers interested in experimental models to study cancer with multimodal MR techniques

Introduction

Hanahan and Weinberg (1) summarized the biology of tumor cells and categorized 8 distinct hallmarks and 2 enabling characteristics of cancer. The hallmarks are sustaining proliferative signaling, evading growth suppressors, enabling replicative immortality, inducing angiogenesis, resisting cell death, activating invasion and metastasis, avoiding immune destruction, and deregulating cellular energetics. The 2 characteristics are tumor-promoting inflammation, and genome instability and mutation (1). MR methods can be applied to investigate these hallmarks and characteristics either *in vitro* or *in vivo*.
This course will describe the different cell and animal models that can be used to investigate these with MRI, MRS and MRSI. The complementary role of *in vitro*, *ex vivo* and *in vivo* experiments will be detailed. Examples of typical MR application in cells and mice will be given, and limitations relative to each model will be discussed.
At the end of the course, the attendants should be familiar with the advantages and disadvantages of the different experimental models available to study cancer with MRI, MRS and MRSI, and should be able to decide which model would be the best to use for their specific study.

A. MRI and MRS of cancer cells

The invasive potential of cancer cells can be characterized *in vitro* by quantifying the penetration of cells into reconstituted basement membrane gel or other extracellular matrix (ECM) (2). An MR compatible invasion assay system can be used to dynamically track the invasion of cancer cells and simultaneously characterize oxygen tensions, physiological and metabolic parameters (**Figure 1**) (3, 4). Incorporation of perfluorotripropylamine (FTPA) doped alginate beads in the MR tube allows direct measurement of oxygen tensions in the sample. The assay can be used to derive quantitative indices of invasion of a Matrigel layer, and determine changes in the invasion index in response to different physiological conditions, or from the downregulation or over-expression of specific genes (**Figure 2**).
It is also possible to use the system to study the interaction between cancer cells and stromal cells, by adding a layer of stromal cells on top of the Matrigel layer and determine their effect on cancer cell invasion under different physiological conditions. This MR assay has been used to observe the effects of paracrine factors secreted by cancer cells on human vascular endothelial cell (HUVEC), and on lymphatic endothelial cell (LEC) motility (6, 7). This assay can be expanded to study cancer cell-endothelial cell interactions in response to single perturbations such as hypoxia, a chemokine, or a cytokine. By acquiring high-resolution three-dimensional MR images, further insights into the remodeling of basement membranes can be obtained.

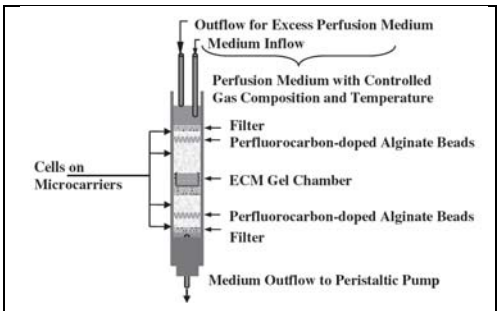

Figure 1: Schematic illustration of the MR-compatible cell perfusion assay used to derive an invasion index and to quantify choline and lipid metabolites (5).

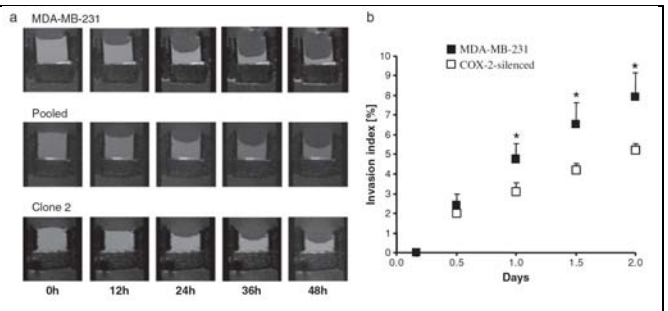

Figure 2. (a) Representative ^{1}H MR images showing reduced degradation of ECM by Pooled and Clone 2 COX-2-silenced MDA-MB-231 cells relative to parental MDA-MB-231 cells. (b) Quantitative time-dependent invasion indices obtained from diffusion-weighted intracellular water profiles of MDA-MB-231 cells and COX-2-silenced cells (5).

Glucose, lipids, and energy metabolism is profoundly altered in cancer cells compared to normal cells. MRS can be applied to access a detailed metabolic overview in the cells under investigation. ^{31}P, ^{13}C and ^{1}H MR spectra can be acquired in perfused cells to study metabolism, intracellular pH (pHi), extracellular pH (pHe), or glycolysis. The system maintains viable cells for up to four days and can also be used to study the effects of gene deletion, surexpression, or therapeutic agents without the complexities associated to *in vivo* systems (**Figure 3**).

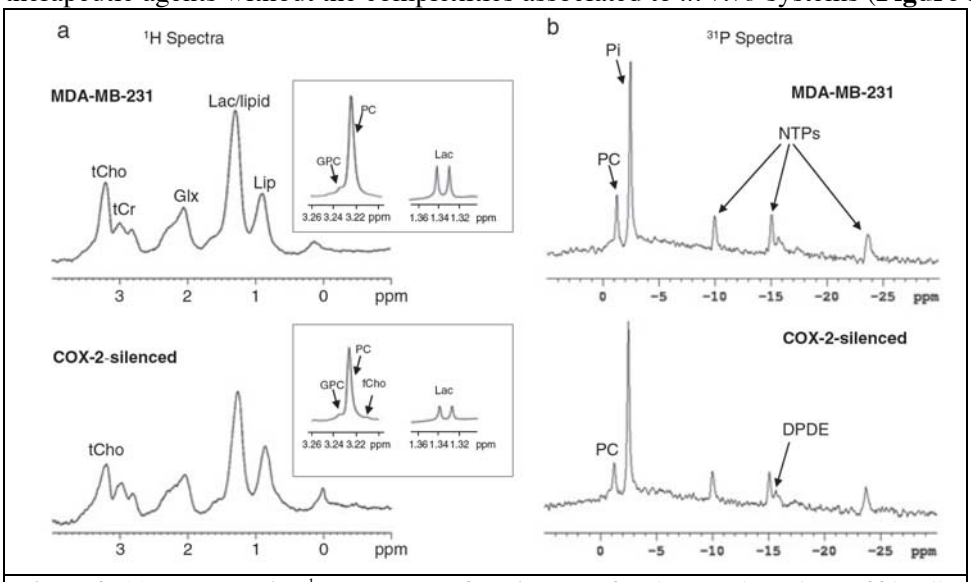

Figure 3: (a) Representative ^{1}H MR spectra from intact perfused parental MDA-MB-231 cells (top) and Pooled cyclooxygenase-2 (COX-2)-silenced cells (bottom) at 24 h. The insets in the corresponding top and bottom panels display representative high-resolution ^{1}H MR spectra from the water-soluble fraction of cell extracts, and confirm a decrease in phosphocholine (PC) and lactate (Lac) with COX-2 silencing. (b) ^{31}P MR spectra acquired from intact parental MDA-MB-231 cells (top) and Pooled COX-2-silenced cells (bottom) at 24 h, showing the decrease in PC with COX-2 silencing. DPDE, diphosphodiester; Glx, glutamine + glutamate; GPC, glycerophosphocholine; NTPs, nucleoside triphosphates; Pi, inorganic phosphate; tCho, total choline; tCr, total creatine (5).

To keep the cells alive in the MR spectrometer, they have to be kept at physiological conditions either in a cell suspension/pellet, or in a perfusion or bioreactor system. Real-time experiments to investigate time courses of metabolic changes can only be performed in live cells. Metabolites can be also extracted from the cells to provide a high spectral resolution. MRS of extracted cells requires the chemical extraction of biomolecules based on their solubility in different solvents. The higher spectral resolution gives access to a higher number of metabolites, and the technique has been applied to study metabolic pathways in detail, or to investigate effects of treatment, of gene overexpression or silencing, or of physiological changes (8, 9).

B. MRI, MRSI and MRS of mouse models of cancer

1. Preclinical mouse models of cancer

Preclinical model	Advantages	Disadvantages
Spontaneous carcinoma	- Immunocompetent host - Evaluation tumor-host interaction with an intact immune system	- Low incidence rate - Tumor development slow and variable - Tumor burden assessment can be difficult
Syngeneic models	- Immunocompetent host - Evaluation of tumor-host interaction - Possible orthotopic inoculation of cancer cells creating a model that mimics human disease progression and metastatic distribution	- Systems obtained from *in vitro* transformation of mouse surface epithelial cells, they might not be representative of the typical genetic transformation in cancer
Xenografts models	- Easy to use for therapeutic assays of cytotoxic and targeted agents - Tumor assessment depends on the site of implantation, it can more complicated if orthotopic - Realistic heterogeneity of tumor cells	- Immunocompromised mice, providing a less realistic tumor microenvironment - Lack of a competent immune response - The stroma is murine, the tumor is human
	- From cells, generally good take, inexpensive, time required relatively short	- Show more homogeneous, undifferentiated histology
	- From fresh tumor samples, morphological and molecular properties reminiscent of the original human tumor retained	- Not always high tumor takes, more expensive based on labor, time required is longer (several weeks)
Transgenic models (genetically engineered mice)	- Immunocompetent host - Assessment of specific genetic alterations on tumor progression - Investigation of the role of oncogenes, tumor suppressor genes - Can provide proof of principle for novel targeted therapy	- Variation in genetic background, random nature in the genetic insertion process, limited number of genes targeted (not reflective of the complex heterogeneity of human tumor cells) - Expensive for labor and housing - Very long time required before validation - Tumor burden assessment can be difficult

2. *In vivo* studies

In vivo MR studies are necessary to explore the tumor microenvironment. Multi-nuclear *in vivo* MR techniques have been widely used in preclinical studies of cancer. With their versatility, they can provide information on the tumor microenvironment, tumor metabolism, pH, hypoxia, vascularization, treatment efficacy, or apoptosis. Examples of some of these applications are described here.

During an *in vivo* MR experiment, the animal scanned has to be stably anesthetized, using either gas anesthetic, such as isoflurane, or injectable anesthetic, such as mixture of ketamine and acepromazine or xylazine. Body temperature, heart and breathing rates can be followed during the acquisition using specific equipment compatible with high magnetic fields. The body temperature needs to be maintained by using, for example, a blanket circulating with warm water.

MRI can provide functional characterization of tumor vasculature in tumor models with blood oxygen level dependent (BOLD) or dynamic contrast enhanced (DCE)-MRI techniques (10, 11). The BOLD technique uses intrinsic contrast arising from deoxyhemoglobin circulating in the blood and the inherent paramagnetism of deoxyhemoglobin that can be observed on T_2*- or T_2-weighted MR images (12). DCE-MRI is based on the use of MR contrast agents to characterize tumor vasculature, that are detected indirectly by their effects on water proton T_1, T_2, or T_2* relaxation time constants (13). Low molecular weight gadolinium-based agents can be used to derive tracer kinetic parameters such as K^{trans} (min^{-1}), the volume transfer constant between the

blood plasma and the extravascular extracellular space (EES), k^{ep} (min^{-1}), the rate constant between the EES and blood plasma, and ve (%), the volume of the EES per unit volume of tissue (13). For preclinical studies, a wide variety of contrast agents, macromolecular as well as targeted, are available for characterizing tumor vasculature. MRI of the macromolecular intravascular contrast agent albumin-GdDTPA has been used to characterize tumor vascular volume and permeability surface area product (PSP) (14). It can also be used to explore the tumor macromolecular transport by measuring how the contrast agent is cleared from the tumor interstitial space (15). The importance of the tumor growth location is critical in the vascularization of the tumor, and several studies have shown the importance of an orthotopic implantation *versus* a subcutaneous implantation (16). MRI can be used to visualize angiogenesis *in vivo* by using nanoparticles loaded with either gadolinium or iron oxide particles that are targeted to $\alpha_v\beta_3$ integrin (17, 18). Studies to date indicate that more aggressive tumors are characterized by increased vascular volume and permeability (19).

Hypoxia has been closely associated with tumor aggressiveness, invasion, and resistance to radiation and chemotherapy (20, 21). Because of its importance in cancer progression and treatment, the development of noninvasive methods to detect hypoxia is of major importance in cancer discovery and treatment (22). Nitroimidazole compounds have been developed to detect hypoxia *in vivo*, they are reduced by intracellular reductases in an oxygen-dependent manner, and accumulate in viable hypoxic cells by binding covalently to the thiol groups of intracellular proteins (23). Tumor oxygen tensions can be measured *in vivo* using ^{19}F and ^{1}H MRS to detect nitroimidazole-based compounds (24, 25). The spatial distribution of the probe can be visualized with ^{19}F MR spectroscopic images overlaid with anatomic images (22). Hypoxia in tumors can also be detected with ^{19}F MRS of hexafluorobenzene (HFB), a reporter molecule with an oxygen dependent T_1 relaxation rate (24). A ^{1}H MRS-detectable analog of HFB, hexamethyldisiloxane (HMDSO), is also available for *in vivo* quantitative pO_2 measurements (25).

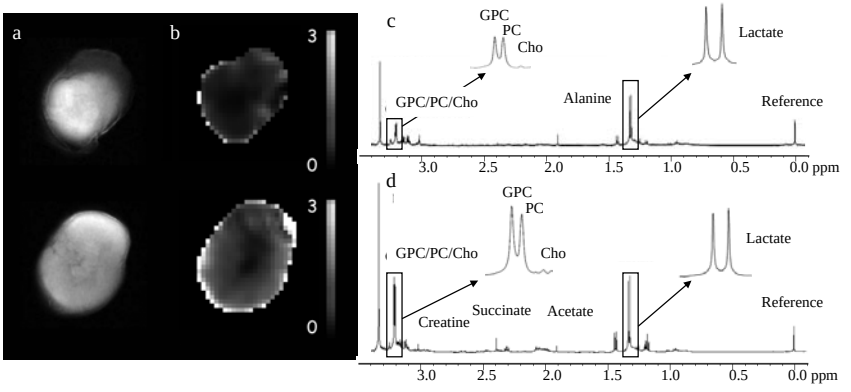

Figure 4: Representative (a) T_1-weighted images, and (b) tCho maps of a non-cachectic MAC13 (upper panel) and a cachectic MAC16 (lower panel) tumors. Representative ^{1}H MR high-resolution spectra of water-soluble (c) MAC13 and (d) MAC16 tumor extracts. Cho (free choline), PC (phosphocholine), GPC (glycerophosphocholine). Modified from (35).

Tumor extracellular pH (pHe) is usually acidic, whereas the intracellular pH (pHi) is neutral-to-alkaline (26). High glycolytic activity even in the presence of oxygen, termed the 'Warburg Effect', together with poor blood flow and hypoxia contribute to this acidic pHe. Tumor pH plays an important role in tumor progression, metastasis and treatment efficacy (27), and neutralization of the acidic tumor microenvironment has been shown to reduce metastasis (28). Tumor pH can be measured *in vivo* from the pH-dependent chemical shift of the phosphate inorganic signal in ^{31}P MR spectra. Since inorganic phosphate is mostly intracellular, it reports on pHi (29). pHe can be measured from the pH-dependent chemical shift of extracellular ^{31}P MR detectable probes such as 3-aminopropylphosphonate (3-APP) (30). In preclinical studies, ^{1}H MRS imaging has been used to obtain pHe maps of tumors by determining the chemical shift of extracellular compounds such as

(imidazol-1-yl)3-ethyoxycarbonylpropionic acid (IEPA), or 2-(imidazol-1-yl)succinic acid (ISUCA), (31, 32). Another MR technique that can be applied to measure pHe *in vivo* consists of using hyperpolarized ^{13}C-labeled bicarbonate (33).

Tumor metabolism can be studied *in vivo* or in excised tumor tissue following extraction with similar approaches as for extracted cells. *In vivo* studies in tumors allow for time course studies as they are noninvasive, and provide spatially resolved metabolic information in the case of performing MRSI (**Figures 4a-b**). However, the spectral resolution of *in vivo* MR spectra is quite limited as compared to the much higher spectral resolution achieved in MR spectra of tumor extracts (**Figures 4c-d**). Cell and tumor tissue extracts provide a higher spectral resolution as compared to perfused cells, and *in vivo* MRS and MRSI. By applying different chemical extraction procedures, the spatial information of the extracted tissue or cells is typically lost during the extraction procedure, which is not the case in MRSI studies *in vivo*, in which spatially resolved MR spectra can be obtained. Metabolic pathways, such as glycolysis, can be assessed using MRS. One of the characteristic features of cancers is aerobic glycolysis. ^{1}H MRS can be used to detect lactate, and ^{13}C MRS can be used to follow the utilization of labeled glucose through metabolic products such as glutamine or lactate, they can be applied to explore tumor glycolysis in preclinical models (34).

Treatment efficacy can be assessed on perfused cells (36), or on cell extracts (8), however, the method presents several limitations, especially related to the delivery of the treatment and to the role of the ECM and the stromal cells. MRI and MRS can be applied *in vivo* to assess treatment efficacy. The effect can be explored not only by following non-invasively the tumor growth overtime, but also by measuring other factors such as vascular parameters or total choline levels. Effects of anti-angiogenic agents can be detected with imaging (37, 38). MRS offers also the possibility to perform noninvasive assessment of the action of targeted treatments by detecting changes in metabolic biomarkers in preclinical models of cancer. Altered choline metabolism is often observed in cancers, and provides unique biomarkers to detect and characterize cancer *in vivo*, and to monitor response to treatment (39). The aberrant choline metabolism observed in most tumors results in the detection of high total choline (tCho) in ^{1}H MR spectra of tumors.

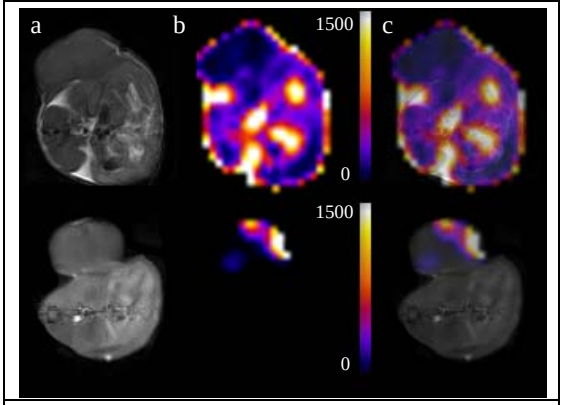

Figure 5: (a) Cross-sectional T_1-weighted images, (b) cross-sectional lactate+lipids maps, and (c) merged images from (a) and (b) of non-cachectic MAC13 (upper panel) and cachectic MAC16 (lower panel) tumor-bearing mice (35).

Thus, the tCho signal has been used as a spectroscopic marker of response to treatment in several preclinical studies (40). Studies showed that the differences in tCho levels could be detected before the tumor growth inhibition, suggesting that early changes in tCho levels may be more sensitive than changes in tumor volume to assess treatment efficacy (40). Several molecular targeted agents induce apoptosis, and therefore MRS methods have been evaluated for the ability to detect apoptosis following such treatments (41). It has been shown that lipids accumulate in tumor tissues as apoptotic death occurs (42-44), reinforcing the potential role of analyzing the lipid signal detected by ^{1}H MRS to detect tumor apoptosis *in vivo*. Whole body MR imaging allows the investigation of the impact of the tumor growth on normal tissue, as shown in **Figure 5**, where weight-loss inducing cachectic MAC16 tumors induced a loss of lipids in normal tissues, but not in the tumor itself (35).

To conclude, this lecture will provide an overview of the multiple MR applications on cell and

mouse models to investigate the tumor physiology and metabolism. It will emphasize the advantages and limitations of each experimental model. It will outline the use of MRI, MRS and MRSI in discovering and monitoring novel molecular therapies, and in translating them into the clinic.

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