Tumor Physiology

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This course will describe the distinct characteristics of tumor physiological phenotype and MRI-based approaches to assess tumor physiology. The main objectives of this lecture are to provide instructions/ updates on

(i) Physiological consequences of oncogenesis;
(ii) Application of MRI in visualizing tumor physiology;
(iii) Establishing physiological MRI end-points for targeted anti-cancer agents;
(iv) Introducing multimodal imaging approach for tumor physiology.

Physiological consequences of oncogenesis: It is well established that the host organ environment in which a tumor grows can greatly influence characteristics such as growth rate, vascularization, metastatic potential and inflammatory environment. The tumor "microenvironment" can be thought of as the biological, biochemical, genetic and immunologic characteristics regulated or deregulated by autocrine and paracrine effects between the host environment, the tumor, and the tumor vasculature [1-3]. Solid tumors are intrinsically heterogeneous in nature exhibiting an array of altered cellular receptors, enzymes, morphologies and growth properties. The main hallmarks of "cancer phenotype" are (i) tumor angiogenesis; (ii) tumor cellularity; and (iii) tumor inflamed microenvironment.

- Angiogenesis: As tumors grow in size (>1mm diameter), the ability of oxygen and nutrients to diffuse and reach all the cells in the mass becomes limited requiring the recruitment of new vasculature to the tumor. Poor tumor perfusion can result in microenvironmental conditions of hypoxia, acidic pH, and low glucose, which can stimulate the up-regulation of pro-angiogenic factors such as, VEGF, platelet-derived growth factor (PDGF), transforming growth factor α (TGF- α) and basic fibroblast growth factor (bFGF)[3, 4]. Angiogenesis is the process of the formation of new abnormal blood vessels for delivery of nutrients needed for tumor growth, invasion, and metastatic spread. Tumors do not grow beyond a size of 1-2 mm without initiating angiogenesis and producing new blood vessels. Tumor-induced blood vessels exhibit discontinuous basement membranes, and lack tight endothelial cell junctions making them highly permeable to macromolecules. Thus, microvasculature of tumors, in contrast to normal tissue, is structurally and functionally abnormal, tortuous and poorly organized, making the vessels "leaky".
- Cellularity: Cell density is an important tumor property. Tumor have highly elevated proliferation rate and decreased apoptosis making them a very dense tissue. It has been shown that tumor aggressiveness often correlates with cell density. As tumor cells are eliminated by therapeutic intervention (due to either apoptosis or necrosis) the cell density decreases[3].
- Inflammation: Inflamed tumor microenvironment is known to facilitate cell migration and metastases formation as well as to trigger highly permeable vasculature[5, 6]. Inflamed microenvironment is formed by recruiting tumor-associated macrophages from the blood stream[7]. It has been recently shown that pro-inflammatory stromal milieu with resulting intra-tumoral macrophage infiltration correlates with poor prognosis in breast cancer. Influx of TAMs is also

involved in postpartum mammary gland involution and correlates with increased risk of postpartum breast cancer[8, 9].

Application of MRI/MRS in visualizing tumor physiology and treatment response: There is a significant demand for developing of morphological and functional imaging as a noninvasive tool to characterize and evaluate tumor physiology with the ability for serial imaging. In the past, oncologic imaging of anatomical end-points and radiological assessment of tumor size and dimensions was considered the "gold standard" for clinical diagnosis. In the past decade, standard clinical practice predominantly used CT imaging for tumor detection and therapy planning. This was sometimes supported by other anatomic imaging techniques, e.g. T1- and T2-weighted MRI or ultrasound. Unfortunately, true extent of viable tumor is often obscured on standard imaging, meaning that edema, cysts, fibrosis, or necrosis cannot be distinguished from primary lesions. Currently, sophisticated physiological and metabolic imaging end-points are emerging which provide more specific information than just the margin of neoplasm. Using modern imaging technologies, various tumor phenotypic characteristics, such as cell density, angiogenesis, perfusion, apoptosis, glucose uptake or hypoxia, can be non-invasively evaluated[10]. MRI is, without doubt, the modality of choice for imaging tumor physiology since it can rely on either intrinsic contrast such as arterial spin labeling or blood oxygenation level-dependent (BOLD) contrast or can rely on exogenous contrast agents by intravenous injection of gadolinium chelates or iron oxide nanoparticles. As mentioned above, a wide spectrum of physiological changes happens during oncogenesis. This lecture will focus on describing MRI approaches to image physiological changes in tumor microenvironment related to three major hallmarks of cancer: (i) angiogenesis and tumor perfusion; (ii) tumor cellularity; and (iii) tumor inflammation.

- DCE-MRI for imaging angiogenesis: Dynamic contrast-enhanced (DCE)-MRI allows for the assessment of tumor vascular physiology. By kinetic modeling of T1-signal intensity changes (which result from the passage of a gadolinium chelate through the tumor vascular bed) physiologically based parameters, including volume transfer constant (K^{trans}), extravascular extracellular volume fraction (v_e), rate constant (k_{ep}), can be established to quantitatively assess tumor perfusion and permeability[11, 12]. DCE-MRI has most notably been used as a method for detecting disrupted tumor vascularization following treatment with targeted anti-vascular and anti-angiogenic compounds (mostly VEGF/ VEGFR2 inhibitors)[13, 14].

  Tumor blood flow can also be quantified by dynamic susceptibility contrast MRI. Another MR-based technique to evaluate changes in tumor oxygenation due to possible hypoxia employs the blood oxygenation level-dependent (BOLD) effect (no exogenous agent is required). BOLD MRI is based on the different magnetic properties of deoxyhemoglobin (paramagnetic) and oxyhemoglobin (diamagnetic). Deoxyhemoglobin increases the transverse magnetization decay rate ($R_2^* = 1/T_2^*$) resulting in signal loss in gradient-echo MRI. Thus, variations in hypoxia in the tumor lesion can be monitored quantitatively using variations in R_2^*; however, further validation studies are required.
- DWI for tumor cellularity: Diffusion-weighted (DW)-MRI another MR technique, which does not require an injection of exogenous contrast agent. Contrast, based on the diffusivity of water molecules in tissue, is afforded by DW-MRI. In DW-MRI, the motion of water molecules in the tissue is detected from the additional changes in their physical properties as the molecules diffuse through the cell

membrane. Because free diffusion of protons is inhibited by cell membranes, DW-MRI can sensitively detect deteriorations in cell membranes and, therefore, cellular injury. Most tumors show reduced water diffusion (hypodensity signal in DW-MRI) frequently attributed to increased cellularity in tumors. Tumor cellularity can then be quantitatively assessed by comparing apparent diffusion coefficients (ADCs) from malignant areas to the ADCs of normal tissues which has proven to increase tumor detection[15]. Tumor response to classic chemotherapeutics is characterized by increased ADC values[16].

- T2-MRI with iron oxide nanoparticles for tumor inflammation: The primary function of macrophages is phagocytosis, therefore they have a very high uptake of iron oxide. Super-paramagnetic iron oxide (SPIO) nanoparticles have a variety of applications for cellular MR imaging, since they are so-called T2-MRI contrast agents, which shorten T2-relaxation times of surrounding tissues and produce negative contrast in T2-MRI. As such, inflamed tumors which express high level of TAMs, can be potentially imaged using SPIO-enhanced T2-MRI[17]. This approach might also be used in the future to assess the efficacy of anti-inflammatory treatment (such as NSAIDs in breast cancer or the inhibitors of colony stimulated factors, CSF).

Introducing multimodal imaging approach for tumor physiology: MRI is, without doubt, the most desirable and widely used imaging modality for imaging of tumor physiology (an outstanding functional imaging potential supported by excellent spatial resolution and a high intrinsic soft-tissue contrast). The innovations in medical imaging also include computed tomography (CT), positron emission tomography (PET), single photon emission tomography (SPECT), ultrasonography (US) and, in the pre-clinical arena, optical imaging (Table 1).

Table 1: Advantages and disadvantages of single imaging modalities in clinical and pre-clinical cancer research.

Modality	Resolution [mm]		Specificity/ Sensitivity	Anatomic Potential	Functional Potential	Molecular Potential
	Clinical	*Research*				
MRI	5	0.1	high	excellent	outstanding	high
CT	5	0.05	high-moderate	excellent	moderate	low
SPECT	10-15	1.5-2	moderate-high	moderate	moderate	high-moderate
PET	10-15	1.5-2	high-excellent	moderate	excellent	high
Ultrasound	5-10	0.2-0.5	moderate	High-moderate	high	moderate-low
Optical	N/A	(0.5)	excellent	moderate-low	moderate	excellent

- CT: Similarly to DCE-MRI, quantitative contrast-enhanced (CE)-CT based on time-dependent contrast enhancement after injection of iodine-based contrast agents can be performed. Due to some technical limitation as well as potential toxicity of CT-contrast agents, CE-CT is less accepted than DCE-MRI, even though kinetic modeling on CE-CT yields similar results as from gadolinium kinetics.
- PET: In addition to the main FDG-PET protocols (FDG is a radioactive glucose analogue to measure metabolic activities in tumors, ^{18}F-fluorothymidine (FLT)-PET is used to assess tumor proliferation rates non-invasively. PET-based protocols with fluoromisonidazole (^{18}FMISO, which has a high “hypoxia-specific”

factor) and copper bis(thiosemicarbazones) derivatives (^{64}Cu-ATSM, which are sensitive to high levels of NADH in hypoxic tissues) are in advanced stages of clinical development and further development of oxygen-sensing imaging probe is feasible. One of the best approaches (although rather expensive and limited only to the institutions with on-site radiochemistry facilities) is quantitative $H_2[^{15}O]$ PET.

- Ultrasound: Doppler ultrasound is used for assessment of tumor blood flow. Novel micro-bubble contrast agents are used for ultrasound-based assessment of angiogenesis.
- Optical: While optical imaging is a great modality for molecular imaging, it had only limited application for imaging physiology (such as HIF-1α luciferase expressed cells for hypoxia imaging).

The future of functional imaging for tumor physiology and response for targeted treatment definitely depends on multi-parametric and multi-modality approaches.

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