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Molecular and Functional Imaging of Cancer

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Abstract **Because of the remarkable ability of cancer cells to adapt and survive, finding effective treatments against cancer depends upon identifying and attacking targets and pathways critically important for the cancer cell. The tumor physiological microenvironment, interactions between cancer cells and stromal cells such as endothelial cells, fibroblasts and macrophages, the extracellular matrix, and a multitude of secreted factors and cytokines influence progression, aggressiveness and response of the disease to treatment. Multi-modality and multi-parametric molecular and functional imaging provide unprecedented opportunities for understanding cancer. Some of our recent advances in molecular and functional imaging of cancer are discussed.**

I. INTRODUCTION

Recent advances in the development of molecular targeted contrast agents have expanded the traditional strengths of MRI and MRS of characterizing functional tumor parameters such as pH, vascularization, metabolism and cell death to include visualization of molecular pathways. Using combined MR and optical imaging of human breast and prostate cancer xenografts engineered to express green fluorescent protein (GFP) or red fluorescent protein (RFP) under hypoxia, we have obtained useful insights into the dynamics between the tumor ECM, vascularization, extracellular pH, interstitial fluid transport, and metabolism. MRI and MRSI were used to obtain co-localized maps of vascular volume, permeability, interstitial fluid transport, total choline and lactate/lipid, while optical imaging was used to obtain co-localized maps of hypoxia and ECM fiber distribution. These insights can be exploited to find effective treatment strategies. One common metabolic feature identified by MRS in most cancers is the elevation of total choline. This elevation of choline compounds provides a unique target to exploit for therapy; such targeting can be imaged noninvasively with MRS. We are developing molecular and molecular imaging based approaches to target choline metabolism, specifically choline kinase activity, which is the first step in choline phospholipids biosynthesis. The approaches that are being developed in our program include targeting choline kinase using siRNA delivered using lentiviral vectors, and performing image-guided targeting of choline kinase using siRNA in combination with a prodrug enzyme cytosine deaminase using a multi-modal imaging platform. These advances can be used to target specific pathways, microenvironments, and cell types within tumors.

Recent developments in the use of multi-nuclear magnetic resonance spectroscopy (MRS) as well as multi-modality approaches in animal models of cancer are discussed. The tumor physiological microenvironment, interactions between cancer cells and stromal cells such as endothelial cells, fibroblasts and macrophages, the extracellular matrix, and a multitude of secreted factors and cytokines influence progression, aggressiveness and response of the disease to treatment. The hypoxic environment of solid tumors has proven to be a major obstacle for successful radiation and chemotherapy of cancer for several decades. Because of the remarkable ability of cancer cells to adapt and survive, finding effective treatments against cancer depends upon identifying and attacking targets and pathways critically important for the cancer cell. Multi-modality and multi-parametric molecular and functional imaging provide unprecedented opportunities for imaging cancer in animal models and imaging interactions between cancer cells and stromal cells. Some of the applications of MRS, the nuclei commonly studied, and the information that can be obtained are summarized in Table 1.

From this table it is apparent that noninvasive MRS methods have wide-ranging pre-clinical and clinical applications in cancer that can be applied from bench to beside. The past decade has seen major advances in sequence design, the development of novel reporter probes, and technological advances that have significantly increased the uses of MRS in molecular-functional imaging applications in oncology. Some of these recent applications of ^{1}H, ^{13}C, ^{31}P and ^{19}F MRS in preclinical models of cancer will be reviewed. New developments such as hyperpolarization of spins to increase the sensitivity of detection of the MR signal of ^{13}C-labeled substrates will be discussed. Advantages and limitations of the spectroscopic techniques and challenges for the future will be outlined.

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Table 1: Nuclei commonly studied and some of the applications.

Nucleus	γ[MHz/T]	Application
^{1}H	42.58	• Total choline • Lactate • Lipid • Extracellular pH (pHe) • Treatment efficacy • Detection of metastasis • pO2
^{13}C	10.71	• Labeled substrate utilization to evaluate drug pharmacokinetics and metabolic pathways
^{19}F	40.08	• Drug pharmacokinetics • pHe • pO2 • Enzyme activity • Labeled substrate utilization
^{31}P	17.25	• Energy metabolism (ATP, PCr, Pi) • Intracellular pH (pHi) • Phospholipid metabolism

II. Molecular Targeting

The use of MR biomarkers such as total choline and perfusion are already being explored clinically for characterizing tumors and following treatment response [1, 2]. The elevation of choline compounds presents a unique target to exploit for molecular targeting; such targeting can be imaged noninvasively with MRS [3, 4]. We are developing molecular targeted imaging based approaches to target choline metabolism, specifically choline kinase activity, which is the first step in choline phospholipid biosynthesis. New areas that are being developed in our program include targeting choline kinase using siRNA delivered using lentiviral vectors, and performing image-guided targeting of choline kinase using siRNA in combination with a prodrug enzyme cytosine deaminase using a multi-modal imaging platform.

In an example of multinuclear MR in combination with optical imaging example, visualization of the prodrug enzyme cytosine deaminase (bCD) and poly-L-lysine (PLL) functionalized with biotin, rhodamine, and Gd^{3+}-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) with MRI was used to time prodrug administration. Image-guided timing of 5-fluorocytosine prodrug administration to coincide with the maximum concentration of the enzyme in the tumor and the minimum concentration in normal tissue, minimized damage from the active drug 5-fluorouracil in normal tissue while maximizing damage to cancer cells [5]. The conversion of the prodrug 5-fluorocytosine to the active drug 5-fluorouracil was detected by ^{19}F MRS. We are currently extending this approach to include siRNA targeting of choline kinase that can be detected with ^{1}H MRS of total choline in addition to the conversion of 5-fluorocytosine to 5-fluorouracil, to achieve better cancer cell kill.

The interaction between cancer cells and the tumor microenvironment is providing new insights into the etiology and progression of cancer. For example, **o**xygen partial pressure (pO_2) in tumors can be imaged by both ^{1}H and ^{19}F MRI [6, 7]. Oxygen is sensed by administration of reporter molecules through changes in the spin-lattice relaxation rate. Using combined MR and optical imaging of human breast and prostate cancer xenografts engineered to express green fluorescent protein (GFP) or red fluorescent protein (RFP) under hypoxia, we have obtained useful insights into the dynamics between hypoxia and the tumor extracellular matrix (ECM), vascularization, extracellular pH, interstitial fluid transport, and metabolism [8, 9]. MRI and MRSI were used to obtain co-localized maps of vascular volume, permeability, interstitial fluid transport, total choline and lactate/lipid, while optical imaging was used to obtain co-localized maps of hypoxia and ECM fiber distribution. These insights can be exploited to find effective treatment strategies.

Tumor recurrence and metastasis continue to be the leading causes of morbidity and mortality from cancer and, despite the tremendous advances in cancer research and treatment, even in the twenty-first century cancer continues to evade cure and frequently control. The discovery of cancer initiating cells or cancer stem cells is generating tremendous excitement and is offering new paradigms for understanding and treating cancer recurrence and metastasis. These stem-like cancer cell populations can form tumors in immune suppressed mice from low cell number inoculums, are more drug resistant, more invasive, and more likely to metastasize [10]. Identifying biomarkers associated with cancer stem cells, and imaging and targeting permissive or preventive microenvironmental niches for cancer stem cells is another area that will have significant impact on cancer research and treatment.

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