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Novel Approaches to Imaging Tumor Metabolism

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Abstract

The field of metabolism research has made a dramatic resurgence in recent years, fueled by a newfound appreciation of the interactions between metabolites and phenotype. Metabolic substrates and their products can be biomarkers of a wide range of pathologies, including cancer, but our understanding of their *in vivo* interactions and pathways has been hindered by the robustness of non-invasive imaging approaches. The last 3 decades have been flushed with the development of new techniques for the study of metabolism *in vivo*. These methods include nuclear based, predominantly positron emission tomography (PET) and magnetic resonance imaging (MRI), many of which have been translated to the clinic. The purpose of this review is to introduce both long standing imaging strategies as well as novel approaches to the study of perturbed metabolic pathways in the setting of carcinogenesis. This will involve descriptions of nuclear probes labeled with ¹¹C and ¹⁸F as well ¹³C for study using hyperpolarized MRI. Highlighting both advantages and disadvantages of each approach, the aim of this summary is to provide the reader with a framework for interrogation of metabolic aberrations in their system of interest.

Keywords

MRI; PET; imaging probes; metabolic pathways

Elucidation of the defining features of carcinogenesis represents our ever-increasing understanding of the etiology of this disease. While cancers have been long known to display unique nutrient consumption characteristics, the recognition of abnormal metabolism as a widely-accepted ‘cancer’ phenotype has occurred relatively recently¹. This increasing interest in cancer metabolism has led to the desire to non-invasively measure metabolism in the whole organism using novel molecular imaging platforms.

Imaging Tumor Metabolism

Imaging tumor metabolism is a subset of molecular imaging where biological processes are visualized and quantified at cellular and subcellular levels within an intact organism. Specifically, the biological processes that generate energy (catabolism) or are involved in

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providing building blocks (anabolism) constitute the field of imaging tumor metabolism and will be the focus of this review. We will specifically discuss imaging methods available to visualize and quantify various catabolic and anabolic processes at a cellular level, limiting discussion to methods that have the potential to be applied non-invasively in a clinical setting. The two metabolic imaging modalities that will be most commonly encountered will be positron emission tomography (PET) and magnetic resonance imaging (MRI). The most widely used approaches and probes are illustrated in Figure 1 and expanded on structurally in Table 1.

Positron Emission Tomography (PET) Imaging

The physics of nuclear imaging are beyond the scope of this review, but a brief discussion of the parameters most relevant to imaging tumor metabolism will be provided. PET signals are derived from the ejection of a positron from the nucleus, which subsequently combines with an electron in tissues, resulting in the release of a pair of annihilation photons that can be detected by a ring of scintillation counters, enabling localization and quantification of the radioisotope^{2,3}.

PET has unparalleled sensitivity with no background signal making it suitable for measuring small differences in metabolism. The spatial resolution of the majority of clinical PET scanners is (6–8 mm³)⁴. This review focuses on ¹⁸F and ¹¹C with half-lives of 110 and 20 minutes, respectively. ¹¹C labeled compounds have a limited temporal resolution, requiring metabolic processes to occur relatively quickly. Most tracers described in this review are naturally occurring metabolites or analogs and substitution with ¹¹C results in less of a biological effect compared to substitution with ¹⁸F. Additionally, it is important to note that PET tracers are infused at low concentrations (fM), but it is not possible to differentiate between the tracer and any metabolic product. Moreover, nuclear imaging experiments cannot be repeated indefinitely due to radiation hazard limiting the ability to observe dynamic metabolic fluxes over an extended period of time. These advantages and disadvantages are important when considering the imaging approach chosen for the metabolic process to be interrogated.

Magnetic Resonance Spectroscopy and Imaging (MRS/MRI)

Nuclear magnetic resonance (NMR) is a phenomenon derived from the interaction of nuclear spins inside of a strong external magnetic field. Atomic nuclei possess an intrinsic property known as spin, dictated by the number of neutrons and protons in the nucleus. Nuclei that are particularly useful in NMR have spin 1/2, and include ¹H, ¹³C and ³¹P. The physics of NMR and MRI are well described elsewhere⁵, but it is important to introduce some basic features of the MRI signal. The MRI signal intensity is dependent on many factors including the concentration of the relevant nuclei in the region of interest, the gyromagnetic ratio of the nuclear spins and two rate constants that govern the time dependence of the relaxation of the magnetization signal to a perturbation: the spin-lattice or longitudinal relaxation time, T₁, and the spin-spin or transverse relaxation time, T₂. Chemical shift, derived from the difference in electronic shielding experienced by nuclei, enables differentiation of various chemical environments, enabling the identification of

molecules in a solution. This is conventionally called magnetic resonance spectroscopy (MRS) and has the distinct advantage of being able to identify specific compounds in a chemical reaction. MR spectra can be acquired with or without spatial localization. Spectral information obtained without spatial localization is a sum of all signals in the sensitive volume of the coil that detects the RF signal. Localized spectra can either be acquired from a single volume element (single voxel), or from multiple voxels (multi-voxel). Multivoxel MRS was originally introduced as chemical shift imaging (CSI) and is also known as MRS imaging (MRSI).

For imaging metabolism using MR-based methods, ^1H , ^{13}C and ^{31}P nuclei are most relevant and these will be evaluated critically in subsequent sections. The theoretical spatial resolution for MRI is high, but this is dependent on the nuclei of interest, though the sensitivity of traditional *in vivo* methods is low (high μM to mM). Repeated imaging using some MRSI methods is possible, as the imaging signal is non-radioactive and because repeated sampling does not destroy certain MR signals, dynamic information can be derived to probe metabolic fluxes.

Imaging Metabolism using PET

In work pioneered by Wolf, Fowler and colleagues at the Brookhaven National Laboratory, the synthesis of ^{18}F -labelled fluorodeoxyglucose (^{18}FDG) was first described in 1978⁶, followed administration of ^{18}FDG in man⁷. This section of the review introduces the most commonly used radioactive imaging agent in PET imaging, with emphasis on the different metabolic pathways that may be detected in cancers in a clinical setting.

Imaging Metabolism using ^{18}F

Imaging metabolism of tumors using ^{18}F , specifically ^{18}FDG remains the best example of the utility of detecting the catabolic and anabolic processes in tumors. Imaging using ^{18}FDG -PET remains by far the most popular technique in the clinic with estimates of up to millions of scans performed⁸.

Glycolytic Metabolism

The popularity of this radiotracer lies in the observation that most human tumors display significantly increased glucose uptake. Thus, ^{18}FDG serves as a surrogate for tumor glycolysis, as sodium independent, facilitative glucose transporters (GLUTs) take up both glucose and FDG ⁹. With over fourteen members of facilitative transporters described so far, each having differing affinities for hexoses, the most relevant members of the GLUTs for ^{18}FDG are thought to be GLUT1 and GLUT3¹⁰. GLUT1 is the most common glucose transporter in humans and is believed to be responsible for basal glucose uptake in all cells while GLUT3 expression is considered to be primarily localized to the brain, specifically in neurons, but also in the testes, placenta and the embryo¹¹. In the context of cancer, both GLUT1 and GLUT3 have been found to be overexpressed in a large variety of human cancers^{10,12}. Significantly, for these tumors, an association between GLUT1 with metastasis and/or poor prognosis has been reported¹³.

Once ^{18}F FDG crosses the plasma membrane *via* GLUTs, it is phosphorylated by the cytoplasmic enzyme, hexokinase (E.C. 2.7.1.1) to produce ^{18}F FDG-6-phosphate (^{18}F FDG6P). The fluorine atom at the C2 position prevents further oxidation of ^{18}F FDG6P in the cytosol, effectively trapping the imaging agent in the cell. ^{18}F FDG has been used successfully in cancer patients for many purposes and include tumor diagnosis, staging and restaging, evaluation of therapeutic efficacy as well as a prognostic indicator for chemo and radiotherapy of tumors^{14–16}. Nevertheless, ^{18}F FDG present some shortcomings as a radiotracer including false positives in non-cancerous tissues and regions of inflammation, proximity to excretory tissues and low avidity certain tumors^{14,16}. These shortcomings have been well documented and should be taken into consideration when designing imaging experiments to probe tumor metabolism.

Other sugars have been fluorinated with the hope of interrogating metabolism resulting from upregulated glycolysis, these include fluorinated mannose¹⁷ as well as positional labeling of fructose¹⁸. Fructose is transported predominantly *via* the GLUT5 transporter, can be phosphorylated both by hexokinase as well as fructokinase (predominantly in the liver) and has been shown to be a potential probe for studying breast cancer. Further investigation into other sugars and sugar analogues will be necessary to achieve higher specificity in these systems.

Nucleotide biosynthesis

Given the high rates of proliferation in cancer cells, it has been long postulated that imaging changes in nucleotide synthesis and metabolism may provide a means of study cancer biology *in vivo* and response to therapy. The utility of [^{18}F]flurothymidine (^{18}F FLT) as an imaging agent is based on the pyrimidine salvage pathway, where thymidine and its analogs are taken up, phosphorylated and used as nucleotides for DNA synthesis in proliferating cells. Uptake of the agent is thought to be facilitated by the equilibrative nucleoside transporters (ENTs) as well as passive diffusion¹⁹. ENT expression is ubiquitous and facilitates bi-directional transport of nucleosides, resulting in an overall lower signal-to-noise ratio in cancers arising from higher background signals compared to ^{18}F FDG. The ability of ^{18}F FLT to inform on proliferation stems from the metabolism of the compound once it has entered the cell. In proliferating cells, cytosolic thymidine kinase (TK-1, E.C. 2.7.2.21) phosphorylates ^{18}F FLT to ^{18}F FLT-monophosphate. Several studies have shown that phosphorylation of the tracer by TK-1 is the rate-limiting step and the resulting accumulation of imaging signal being proportional to the activity of TK-1, which is up-regulated in S-phase of the cell cycle^{20,21}.

Early clinical studies have shown a correlation of ^{18}F FLT to the histological marker Ki-67 in solid tumors^{22,23,24,25,26}. However, the reliability of using ^{18}F FLT as a surrogate for cell proliferation has been disputed because tri-phosphorylated ^{18}F FLT is not incorporated during DNA replication^{27,28}. Other agents for the study of nucleotide metabolism have been developed to overcome this limitation and possible interrogate other nucleotide metabolic/salvage pathways including [^{18}F]fluoro- β -d-arabinofuranosyl)cytosine (^{18}F FAC)²⁹ and 1-(2'-deoxy-2- ^{18}F -fluoro- β -l-arabinofuranosyl)-5-methylcytosine (^{18}F MAC)³⁰ for the study of deoxycytidine kinase (dCK).

Lipid membrane metabolism

Recent experiments have led to the observation that increased glucose consumption in cancer might be used to provide building blocks for increasing biomass with glycolytic intermediates being postulated to support synthesis of lipids³¹. Phosphatidylcholine (PtdCho), a component of choline metabolism, is incorporated into the characteristic phospholipid bilayer of cellular membranes, underpinning the intimate association between choline and lipid metabolism in normal cell function. Choline uptake into cancer cells is mediated by four different choline-transporting transmembrane systems. Subsets of each of these transporter classes have been shown to be overexpressed in a variety of human cancers and have been reviewed³².

Radiolabeled ¹⁸F-choline is rapidly taken up by tumors plateauing within 10–20 minutes of injection³³, but studies have shown that a significant proportion of intracellular tracer remains as non-metabolized choline³⁴. These observations suggest that choline transport is the major component regulating signal intensity in PET imaging, distinct from intracellular imaging agent trapping seen in typical PET agents. Transport of choline into the cell may inform on the rate of membrane synthesis, assuming transport of choline is rate limiting, but this remains to be proven. In the clinic, PET imaging with radiolabeled choline has been most useful in prostate cancer. This is due in part to the limited sensitivity ¹⁸FDG as a radiotracer for prostate carcinomas³⁵ as well as high ¹⁸FDG excretion through the bladder. The clinical utility of radiolabeled choline compounds (both ¹¹C and ¹⁸F) in prostate cancer has been reviewed and the use of choline as an imaging agent for restaging of patients with biochemical recurrence (increasing PSA serum levels) after definitive local therapy has displayed promising results³⁶.

Glutamine/Glutamate Metabolism

While the essential role of glutamine in cell culture has long been known³⁷, more recent experiments have started to uncover the genetic basis behind glutamine requirement in the cell. The stimulation of glutamine metabolism due to Myc activation resulted in mitochondrial glutaminolysis that is used to sustain cell viability and TCA cycle anapleurosis³⁸. From an imaging perspective, it has also been speculated that tumors that are not ¹⁸FDG-avid might instead be relying on glutaminolysis³⁹. Several isomers of glutamine have been synthesized for PET imaging and ¹⁸F-(2S,4R)-4-fluoroglutamine (¹⁸FGln) was reported to display good cellular uptake and retention. In follow up *in vivo* studies in a c-Myc upregulated xenograft model, ¹⁸FGln displayed selective tumor uptake with good retention. The first clinical trials using ¹⁸FGln are underway and in a first study, uptake of ¹⁸FGln is demonstrated *in vivo*, though it appears that like ¹⁸F-choline, ¹⁸FGln remains intact within the cell, incorporating into a large pool⁴⁰.

There is also great interest in studying the pool of glutamate and its use inside of the cell. Recently ¹⁸F glutamate and analogues of glutamate have been developed to interrogate this intracellular pool ((4S)-4-(3-¹⁸F-Fluoropropyl)-L-Glutamate or ¹⁸F-FSPG). Recent work has identified the xCT cysteine/glutamate antiporter as a mechanism for bringing glutamate into the cell and has been used for radioactive probes. This glutamate has been postulated to be related to the need for glutamate in synthesis of the antioxidant tripeptide glutathione^{41,42}.

Metabolic imaging using ^{18}F -based compounds is dominated by using ^{18}F FDG as the primary radiotracer with the compound being one of the only ^{18}F radioisotopes approved for use by the FDA. The suitably long half-life also means that imaging centers are not obliged to synthesize tracers on-site, further encouraging widespread use. It is worth noting however, that ^{18}F FLT and ^{18}F -choline (and its analogs) are able to reveal distinct biochemical processes within a tumor.

Imaging Metabolism using ^{11}C

While the vast majority of PET images are obtained using ^{18}F -based imaging agents, other radioisotopes have provided information on different aspects of cancer metabolism (Table 1). One such radiotracer is ^{11}C -acetate.

Fatty acid metabolism

The use of ^{11}C acetate as a tracer in cancer lies in part to cancers displaying a propensity for reactivating de novo fatty acid synthesis⁴³. Fatty acids are crucial to the synthesis of the lipid bilayer as well as for catabolic processes. While most cells derive fatty acids from circulating dietary lipids, cancer cells have been shown to obtain up to 93% of their triacylglycerol fatty acids from de novo fatty acid synthesis^{43,44}. Fatty acid synthesis occurs in the cytosol, with acetyl-coA functioning as the precursor. Acetyl-coA can be derived from either citrate in the mitochondria, or from acetate. ^{11}C -acetate uptake is thought to be facilitated by proton-linked monocarboxylate transporters (MCTs) part of the solute carrier (SLC) 16 family. There is a large variation in the type of MCT transporter expressed in different cancers and the exactly member/s of MCT that facilitate ^{11}C -acetate transport has yet to be elucidated^{45,46}.

In tissues with active oxidative phosphorylation acetate is converted to acetyl-CoA, facilitated by acetyl-coA synthase (E.C.6.2.1.1). In these tissues, the radiolabel from acetate is lost as $^{11}\text{CO}_2$. In contrast, ^{11}C -acetate has been observed to accumulate in a number of different cancers⁴⁷. Labeling experiments using ^{14}C -acetate have long been used to demonstrate incorporation of acetate into fatty acids⁴⁸. Fatty acid synthase (FAS, E.C. 2.3.1.85) catalyzes the first step in the formation of complex fatty acids from acetyl-coA, in an NADPH requiring reaction. Inhibition of FAS, as well as other fatty acid synthesis enzymes has been shown to decrease ^{11}C -acetate accumulation in mouse models⁴⁹. While these experiments lend support to the hypothesis that ^{11}C -acetate provides some information on tumor lipid metabolism, there is also evidence that suggest alternative mechanisms of retention, including metabolism of ^{11}C -acetate in the TCA cycle⁵⁰. Additionally, neither of the two pharmacological inhibitors of fatty acid synthesis described earlier completely abrogated ^{11}C -acetate retention in xenograft prostate models, indicating other pathways of radiolabel retention⁴⁹.

While the exact metabolic fate of ^{11}C -acetate in tumors remain controversial, this tracer has been used in many clinical trials especially for imaging the prostate. Several reviews have evaluated the utility of using ^{11}C -acetate in prostate cancer and are in agreement that the effectiveness of this radiotracer for the detection purposes might be limited^{14,51,52}. The use

of ^{11}C -acetate to image recurrent disease appears more promising in several clinical trials, but detectability seems to be correlated with serum PSA levels^{53,54}.

Amino acids and protein synthesis

The use of radiolabeled amino acids in oncology has been reported since the 1970s^{55,56}. 13 amino acid transport systems have since been elucidated including the sodium-dependent systems A, ASC, N, Gly, B⁰, B, B^{0,1}, and the sodium-independent systems L, y¹, b^{0,1}, and x^{c2}. These transport systems have overlapping substrate specificities and have been shown to be overexpressed in a variety of cancer cell lines⁵⁷.

For the sake of brevity, [^{11}C] methionine (MET) will be used as the main example to illustrate the principles of imaging amino acid metabolism with PET, primarily in the context of brain tumors. MET is taken up by the system L amino acid transporters, which has 4 members, LAT1-4. LAT1 overexpression has been observed in a variety of human tumors^{58,59} and was observed to be a significant prognostic factor^{60,61}. Both LAT1 and LAT2 are obligatory exchanging transporters, exporting an amino acid for every amino acid imported. By themselves, both these transporters are not able to change overall amino acid concentrations but are able to modify concentrations of individual amino acids in a cell⁵⁷. Once amino acids are transported across the membrane, they can be used for a variety of catabolic and/or anabolic processes, with MET predominantly incorporated into proteins⁶². However, it has also been argued that within the time frame of PET imaging using ^{11}C radioisotopes, amino acid transport would be the dominating factor in signal accumulation instead of metabolism inside the cell⁶³. This is supported by work using amino acid analogs that are not incorporated into protein showing similar uptake to natural amino acids^{64,65}.

The popularity of MET in the clinic is due to several factors including convenient and rapid radiochemical synthesis, as well as high yields without the need for purification steps⁶⁶. One challenge preventing widespread use of ^{11}C radioisotopes in general is the relatively short half-life, resulting in the need for an on-site cyclotron. The uncertain metabolic fate after intracellular uptake justifies further investigation.

Imaging Metabolism using Magnetic Resonance Spectroscopy (MRS)

MRS-based methods first developed without the need for delivery of an exogenous contrast agent, with imaging signals produced by tissues in a volume of interest. This represents an overall advantage over nuclear-based methods, where pharmacokinetics of the contrast agent needs to be taken into consideration, as well as excretion through different organs.

Additionally, MRS enables imaging of endogenous metabolites, without the concern of contrast agents interfering with normal physiology. Although newer MRS methods have incorporated delivery of contrast agents, the most commonly used clinical applications of MRS remain label-free. While MRS remains the most commonly used MR-based method to image tumors, there are recent reports using chemical exchange saturation transfer (CEST) to detect non-labeled glucose uptake in cells⁶⁷. Although CEST has the potential to be translated to the clinic, this review will not discuss saturation transfer but instead focus on the more widely used MRS methodologies.

³¹P MRS for Metabolism

Some of the earliest work on using MRS to study tumors was performed using ³¹P MRS. This spin ½ nuclei has an isotopic abundance of 100% and a relatively high gyromagnetic ratio. The convention of comparing the sensitivity of detection of any NMR-visible nuclei to ¹H, as protons are the most abundant nuclei in living systems, would see ³¹P MRS possess a 6.6% compared to 100% in proton MRS. Of the most studied resonances, the three phosphates corresponding to the nucleotide triphosphates (NTPs), predominantly ATP and phosphocreatine (PCr) have been the most widely studied. In the setting of cardiac metabolism especially these have been used to characterize the energy state of cells^{68,69}.

In terms of choline phospholipid metabolism, the main contribution to *in vivo* ³¹P spectra is the phosphomonoester and phosphodiester peaks. The phosphomonoester metabolites feature at resonances between 3.2 and 3.6 parts per million (ppm) and consist of small molecular weight phosphorylated intermediates, phosphocholine as well as sugar phosphates. However, the sugar phosphate peaks are often obscured by phosphoethanolamine (PEtn) and phosphocholine (PCho) peaks. The inability to resolve peaks demonstrate a shortcoming of ³¹P and other MRS techniques – molecules with similar chemical structures resonate at frequencies that may be indistinguishable *in vivo*. Additionally, sensitivity is another consideration as well, with MR-based imaging modalities estimated to be limited to the mM range⁴. Clinical trials using ³¹P MRS have nevertheless been performed in cancer patients. A recent clinical trial was performed at 7T and using an endo-rectal coil for the prostate in healthy volunteers achieved 4cm³ spatial resolution in 18 minutes⁷⁰. The significance of PCho in cancer has led to the preference of ¹H MRS to detect the total pool of choline clinically due to its higher sensitivity and this will be discussed below.

The popularity of ³¹P MRS in the clinic is on the wane. This is due to limited sensitivity of the technique, resulting in long scan times for patients. While many groups are attempting to investigate ³¹P MRS at high fields (7T), ¹H MRS is preferred for metabolic imaging of tumors in the clinic.

Tumor metabolism by ¹H MRS

¹H MRS or proton MRS is the most commonly used spectroscopic imaging modality in the clinic. This stems in part due to its high sensitivity. As a result, it is conventional to compare all other nuclei to ¹H as a standard for sensitivity where ¹H MRS is denoted to be the most sensitive at 100%. The other significant advantage is the possibility of current clinical hardware to perform MRS without further modifications as well as the ability to obtain anatomical information in the same experiment. The dominant peak in ¹H spectroscopy is the water peak, which can be up to 10,000 fold more intense than metabolites of interest. Early development work on *in vivo* imaging recognized this problem and water suppression via an application of an RF pulse was developed⁷¹. Today, ¹H MRS is widely available and this review will summarize the most common metabolites detectable.

In clinical imaging today, the most commonly interrogated metabolic resonance is the ¹H MRS peak at 3.2 ppm that is contributed by the methyl carbons of Cho, PCho, GPC, betaine

and taurine. These resonances are usually termed the total choline peak (tCho). The observation that cancers have dysregulated choline metabolism was made over two decades ago facilitated by both ^1H and ^{31}P MRS imaging⁷². PCho levels are frequently elevated in a number of tumors with a concomitant overexpression of the first enzyme involved in choline metabolism, choline kinase (ChoK, E.C. 2.7.1.32). ChoK phosphorylates choline that is taken up by cells through the transporters discussed earlier in the review. Both the expression and activity of ChoK have been identified to be upregulated in many cancers and are also correlated with tumor grade^{32,73}. Oncogenic signaling has been implicated in the control of ChoK expression and these include the Ras and PI3K/AKT pathways, as well as its co-expression with Src family kinases and EGFR⁷³. Quantification of the tCho region provides a robust and consistent marker for malignancy, with normal tissues displaying low levels of tCho while many tumors having high levels of these metabolites^{74,75}. The addition of MRS to standard radiological imaging protocols such as dynamic contrast enhanced (DCE) MRI resulted in significant increases of up to 91% in sensitivity, specificity and diagnostic accuracy in a variety of tumors^{76,77,78}. Additionally, because MRSI is quantitative, calculating ratios of different metabolites can further increase sensitivity and specificity of diagnosis. This has been utilized in the setting of citrate and choline quantification in order to differentiate benign prostatic hyperplasia (BPH) from prostate cancer^{79,80}.

While total choline has been one of the most studied MRS observable resonances, a number of other metabolites, indicative of changes in cancer metabolism have been both identified and quantified. Mutations in isocitrate dehydrogenase (IDH) results in the formation of 2-hydroxyglutarate (2HG) from isocitrate instead of α -ketoglutarate and is present in most grade 2 and 3 gliomas⁸¹. 2HG has been detected by several groups using ^1H MRS in patients with glioma^{82,83} and has been postulated as a biomarker for diagnosis and monitoring treatment⁸⁴. Increases in the lactate peak observed by ^1H MRS has also been associated with higher-grade gliomas as well as related to necrosis and hypoxia^{85,86}.

It is important to note that while the sensitivity of ^1H MRS is low in contrast to nuclear techniques, steady state concentrations of molecules are detected and are not confounded by metabolic transport. Advances in the future will likely include efforts to shorten scan time and to increase sensitivity and the ability to resolve overlapping spectra, allowing the quantification of even more metabolites from a single scan and their combination with *ex vivo* metabolomics methods^{87,88}.

Imaging Metabolism using ^{13}C MRS and Hyperpolarized ^{13}C MRS

Of the total carbon naturally occurring, 1.1% is present in the MR-active form of ^{13}C with a gyromagnetic ratio that is about a quarter of that of ^1H . As a result, the sensitivity of ^{13}C MRS is low, estimated to be 0.02% compared to 100% for ^1H . However, ^{13}C MRS has been performed in humans⁸⁹ with these experiments requiring the administration of ^{13}C -enriched compounds to supplement the low natural abundance of ^{13}C *in vivo*. These experiments were prohibitively expensive to perform in humans⁹⁰ and long scan times were also required for the generation of adequate signal, resulting in ^{13}C MRS being of limited routine use in the clinic.

Recent developments in hyperpolarization techniques have resulted in a dramatic increase in the sensitivity of MR signal detection. Hyperpolarization describes several techniques that have been used to enhance the polarization of nuclear spins, with dissolution dynamic nuclear polarization (dDNP)⁹¹ as the most widely used for studying metabolism. dDNP is based on polarizing nuclear spins in the solid state in the presence of unpaired electrons where an MR-visible isotope is mixed with an organic free radical and frozen to form a glass at low temperatures (1K). Rapid dissolution⁹² provides molecules with greatly enhanced signal to noise (> 10,000 fold), though it does not last indefinitely and they decay as a function of their T₁ relaxation time (typically on the order of 10–60 s). Since each molecule decays at a different rate, flux modeling of bolus infusions *in vivo* may become critical for the understanding of metabolic rates.

Many substrates have been hyperpolarized to date with varying lifetimes and uses⁹², though the best studied of these is [1-¹³C] pyruvate. Intravenous administration of hyperpolarized [1-¹³C] pyruvate allows the visualization of different metabolites in different tissues, including tumors. Pyruvate is rapidly taken up by cells, facilitated by MCTs, primarily MCT1⁹³. It is rapidly converted into multiple molecules including lactate (reduction via lactate dehydrogenase (LDH, E.C. 1.1.1.27), alanine (transamination, alanine transaminase, E.C. 2.6.1.2) and carbon dioxide (decarboxylation, pyruvate dehydrogenase, E.C. 1.2.4.1) indicative of the hyperpolarized nucleus presence in each molecule. In cancer models, the primary metabolic fate of hyperpolarized [1-¹³C] pyruvate is its reduction to [1-¹³C] lactate. This has been attributed to high levels of (LDH) and its cofactor, NADH. The observation that tumors preferentially utilize aerobic glycolysis has long been known⁹⁴ and it is likely that the production [1-¹³C] lactate is linked to this aberrant metabolic phenotype, where preservation of redox homeostasis leads to increased metabolism to lactate^{95,96}. Initial experiments with hyperpolarized pyruvate focused on tumor detection, with high increased lactate production seen in a number of models^{97,98} as well as a decrease post-radiation therapy⁹⁹ and a variety of targeted chemotherapies^{98,100–102}. Additionally, much work has been done in a number of cancer cell suspensions^{103,104} as well as tissues^{105,106}, potentially adding metabolic flux data to metabolomics, genomic and proteomic data that can be derived from *ex vivo* specimens of patients. Currently, hyperpolarized pyruvate is the only to be used in humans with the first safety study in biopsy proven prostate cancer patients demonstrating no dose-limiting toxicities or adverse effects, and areas of high [1-¹³C] lactate in tumor regions¹⁰⁷.

Although [1-¹³C] pyruvate has been the most studied, a number of other molecules have also informed on different aspects of cancer metabolism. Other carbohydrates have been studies including uniformly labeled glucose¹⁰⁸ and fructose¹⁰⁹. While interesting, in model systems they may be difficult to translate to the clinic due to their short relaxation times. Hyperpolarized [1-¹³C] dehydroascorbic acid (DHA) has been developed as a redox probe and utilized in preclinical models^{110,111}. DHA is the oxidized form of vitamin C and is taken into cells *via* GLUT 1,3, and 4 and has been postulated to inform on the redox capacity of cells and changes in reactive oxygen species¹¹². [5-¹³C] glutamine has been converted by cell suspensions to [5-¹³C] glutamate, in a reaction catalyzed by intramitochondrial glutaminase and has the potential to inform on rates of glutaminolysis¹¹³.

Hyperpolarized MRS is still a technology in its infancy, although it has shown great promise in terms of delivering information on enzyme kinetics that no other modality has been able to offer. The hardware requirements may be financially restraining as well as the need to inject relatively high amounts of ^{13}C -labeled probes. However, with a sustained interest in the field and cooperation of both industry, preclinical and clinical laboratories, hyperpolarized MRS will receive the attention it is needed to realize its full potential.

Conclusion

The field tumor metabolic imaging has grown immensely in recent years, scaled to our further understanding of changes in metabolism with oncogenesis. The progress though in this field has been hindered due to lack of robust imaging strategies to interrogate metabolism non-invasively. This review has introduced a number of long-standing and novel approaches to dissecting metabolic pathways *in vivo*. Many PET radiotracers have been discussed that have exquisite sensitivity for detecting accumulation in the setting of tumors. Future work though is required to determine whether novel probes are completely dominated by their uptake dynamics or informs on pathways downstream. Steady state measurements of metabolites using ^1H MRS and new techniques such as hyperpolarized MRS have the ability to address some of these issues by denoting the magnitude to which substrates are converted in living systems. It is most likely the complimentary nature of hyperpolarized MRS and PET will provide the most useful application of combined MR/PET machines in the research setting as well as the clinic. Through collaboration between imaging scientists, cancer biologists and synthetic chemists, these metabolic imaging strategies have the potential to greatly enhance our understanding of metabolism in a living system.

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Figure 1.

Schematic of metabolic pathways that are detectable *via* nuclear and MR-based imaging modalities. Transport enzymes are highlighted in red. Metabolites in blue are detectable by ^1H MRS. DHA = dehydroascorbic acid; ENT = equilibrative nucleotide transporter; FACBC = 1-amino-3-fluorine-cyclobutane-1-carboxylic acid; FDOPA = L-6-fluoro-3, 4-dihydroxyphenylalanine; FET = O-2-fluoroethyl-L-tyrosine; FGln = (2S,4R) 4-fluoroglutamine; FLT = 3-deoxy-3-fluorothymidine; FMT = L-3-alpha-methyltyrosine; FSPG = (4S)-4-3-fluoropropyl-L-glutamate; GLUT = glucose transporter; MCT = monocarboxylic acid transporter.

Table 1

A selection of contrast agents and metabolites in cancer that can be detected using PET and MR-based imaging methods

Compound Name	Chemical Structure	Nucleus	Metabolic Fate/Pathway	References
Alanine		^{13}C	Pyruvate <i>Transamination</i>	114
N-acetylaspartate		^1H	<i>Fatty acid/Lipid metabolism</i>	8, 115
Acetate		^{11}C , ^{13}C , ^{18}F	<i>Lipid/Fatty Acid metabolism</i>	47, 116, 117
Choline		^1H , ^{11}C , ^{18}F	Phosphocholine <i>Lipid metabolism</i>	36, 76
Citrate		^1H	<i>TCA cycle, Lipid metabolism</i>	79

Compound Name	Chemical Structure	Nucleus	Metabolic Fate/Pathway	References
Dehydroascorbate		^{13}C	Vitamin C <i>Redox metabolism</i>	110, 111
Fluorodeoxyglucose		^{18}F	^{18}F FDG-6-P <i>Glycolysis</i>	14, 16
Fluorothymidine		^{18}F	^{18}F FLT-phosphate <i>Nucleotide transport/ Nucleotide synthesis</i>	25
Glucose		^{13}C	Multiple sugar phosphates <i>Glycolysis Pentose Phosphate Pathway</i>	108, 118
Glutamine		^1H , ^{13}C , ^{18}F	Glutamate <i>Glutaminolysis Amino acid transport</i>	113, 119

Compound Name	Chemical Structure	Nucleus	Metabolic Fate/Pathway	References
Glutamate		^1H , ^{13}C , ^{18}F	α -ketoglutarate Amino acid transport Transamination	120, 121
2-Hydroxyglutarate		^1H	Isocitrate metabolism and Redox	83, 84
Lactate		^1H , ^{13}C	Pyruvate Aerobic glycolysis Pyruvate oxidation	85, 122
Methionine		^{11}C	Amino acid transport Protein incorporation	64
Pyruvate		^{13}C	Lactate Alanine ^{13}C Bicarbonate/ CO_2 Glycolysis, TCA cycle, Transamination	97, 98, 107