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Interrogating Metabolism in Brain Cancer

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1. MRS/NMR spectroscopy based metabolomics in brain tumor

Metabolomics is the “systematic study of the unique chemical fingerprints that specific cellular processes leave behind”, the study of their small-molecule metabolite profiles (1). Metabolomics in cancer research is gaining considerable importance recently. The applications of Nuclear Magnetic Resonance (NMR) or magnetic resonance spectroscopy (MRS) based metabolomics as applied to brain cancer will be discussed. In an NMR/MRS approach to the diagnosis of disease, it is necessary to develop classifiers that deal with the many spectral components of the entire spectra. A common method or classifier is the principal component analysis (PCA) that has been used widely in chemical and biological sciences. In brain tumors, diagnosing tumor type and grade non-invasively has been a clinical challenge. ^{1}H MRS/NMR based metabolomics has been explored to identify elevated metabolites in malignant tissue in contrast to normal brain. Interestingly, most of the ^{1}H MRS *in vivo* study to date has been done on the brain. This is primarily due to the reduced effects of motion and lipid contamination in the brain. The global metabolic profile of live cells or cell extracts from established glioma models are being investigated using standard nuclear magnetic resonance (NMR) methods, and tumor biopsies obtained from animal models or patients have been investigated using high resolution magic angle spinning

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spectroscopy (HRMAS) (2, 3). Such approaches are valuable in providing complementary data to the *in vivo* findings.

Detected metabolites using ^{1}H MRS/NMR

^{1}H MRS is extensively used to monitor the steady-state levels of major endogenous cellular metabolites. For a full review of *in vivo* ^{1}H MRS-detectable metabolites, see reference (4). In the field of neuro-oncology, the most prevalent metabolites in the ^{1}H MR spectrum are N-acetylasparate (NAA), total-choline containing metabolites (Cho), lactate (Lac), mobile lipids (Lip), creatine (Cre), glutamate (Glu), glutamine (Gln), glutamine and glutamate (Glx), glycine (Gly), glutathione (GSH) and 2-hydroxyglutarate (2-HG). NAA shows the largest signal in normal healthy brain and NAA level typically decreases in gliomas (5). The Cho signal is a composite of free choline, phosphocholine (PC) and glycerophosphocholine (GPC), which are the precursors and breakdown products of the main membrane phospholipid phosphatidylcholine. The intensity of this peak is associated with cell proliferation and cell signaling and is typically elevated in cancer (6). Lactate is the end product of aerobic glycolysis and enhanced in cancer as part of the Warburg effect (7). Lipids (long chain fatty acids), especially lipid droplets known as mobile lipids or triglycerides, are rarely observed in the normal brain, but are often increased in glial tumors and are associated with cell death and increased necrosis (8). The Cre signal is a composite of creatine and phosphocreatine (PCr), which are involved in energy metabolism via the creatine kinase reaction. Creatine levels vary within normal brain regions and in some cases with tumorigenesis (9). The amino acid Glu is the most abundant amino acid in the brain and an essential neurotransmitter. In gliomas, glutaminolysis is often required for tumor growth as an anaplerotic source of carbon complementary to glucose metabolism (2). Finally, with the recent discovery of the isocitrate dehydrogenase (IDH) mutation, the most common mutation in oligodendroglioma and astrocytoma tumors (10), elevated levels of 2-hydroxyglutarate (2-HG), which is produced from α-ketoglutarate by mutant IDH, serve as a clear metabolic indicator for the presence of the mutation within a tumor and can also be detected by ^{1}H MRS when the mutation is present.

From a technical perspective, it is important to note that the length of the echo time (TE) used in ^{1}H MRS sequences defines which metabolites can be detected. Using a short echo time (TE < 50 ms), most metabolites can be observed, but overlappings between resonances often hamper proper quantification; on the other hand, when using a long echo time (TE > 120 ms), only a few metabolites remain visible but their respective resonances can be readily identified and quantified (9).

Detected metabolites using ^{13}C MRS/NMR

Early ^{13}C MRS/NMR investigations were used to monitor glucose metabolism and lactate turnover during steady-state hyperglycemia with stable isotopically labeled, [1-^{13}C] glucose in C6 glioma-bearing rats (11). Labeling of glucose-derived [3-^{13}C] Lac, [4-^{13}C] Glu, [4-^{13}C] Gln and [1-^{13}C] glycogen could all be detected. More importantly, greater labeled Lac and reduced Glu and Gln production were observed when comparing tumor to normal contralateral brain, consistent with the Warburg effect and a reduction in flux into the tricarboxylic acid (TCA) cycle. ^{13}C MRS studies investigating GBM cells and a

combination of ^{13}C-labeled glucose and ^{13}C-labeled Gln have also shed light on the possible role of Gln in high-grade brain tumors. Conversion of Gln to Lac via glutaminolysis was found to be sufficient to produce NADPH required for fatty acid synthesis. More recently, studies of primary human GBM models in mice infused with ^{13}C-labeled glucose further demonstrated not only elevated glycolysis but also active glucose metabolism via the TCA cycle to Glu and Gln, confirming that flux via pyruvate dehydrogenase was not suppressed in GBM. However, this study showed limited glutaminolysis (12). Using ^{13}C MRS to probe the fate of ^{13}C-labeled acetate in orthotopic brain tumors, a recent investigation demonstrated that acetate is oxidized via the TCA cycle, together with glucose, to generate labeled Gln and Glu. This identifies an additional metabolite that could help meet the high biosynthetic and bioenergetic demands of GBM tumor growth (13). Future studies using additional ^{13}C-labeled substrates could be envisaged to shed further light on the metabolism of GBM and, as models are being developed, on the metabolism of lower-grade brain tumors.

Clinical ^{1}H MRS/NMR spectroscopy

Numerous studies have highlighted the potential benefits of using ^{1}H MRS to estimate metabolite levels in brain tumors in the clinic (14). When combined with similar spatial localization techniques that are used in generating anatomic MR images, this strategy can be used to produce maps of the variations in levels of choline containing compounds, Cre, NAA, Lac and Lip. With increased magnetic field strengths, improvements in scanner hardware and developments in software capabilities, the acquisition time for volumetric data is on the order of 5–10 minutes and the spatial resolution of the voxels obtained is typically 0.5–1 cm^3 (15). More recent advances in pulse sequence development and spectral editing schemes have facilitated the detection of metabolites with shorter T_2 relaxation times and lower signal-to-noise ratios such a Glu, Gln, Glx, and 2-HG, expanding the investigation of potential metabolic processes for both characterizing the spatial extent of gliomas and assessing therapeutic response.

For tumor characterization

The first studies to identify metabolic differences between gliomas and normal brain tissue date back to the mid-1990s and used a long TE (144 ms) 1H MRS acquisition (16, 17). Since then, numerous studies have shown that elevated levels of Cho and reduced levels of NAA together can distinguish regions of tumor from normal brain (18, 19), define the spatial extent of abnormal metabolism due to tumor beyond the contrast-enhancing lesion (20, 21) guide the selection of biopsied tissue samples to the most aggressive part of the tumor (22, 23,) and differentiate among tumor grades and types. The Cho to NAA index (CNI) is a metric that has been developed in the clinical setting to describe such changes and has been found to be more robust than ratios and absolute quantification (24). These *in vivo* results have been confirmed by correlating with both *ex vivo* histological characteristics from image-guided tissue samples and, more recently, ^{1}H HRMAS of tumor biopsies to show that regions with elevated Cho and reduced NAA relative to normal brain have a high probability of corresponding to tumor (22). However, despite the benefits of ^{1}H MRSI in improving sensitivity to metabolically active tumor and differentiating gliomas from metastatic disease

(25), other disease processes such as inflammation can also cause a reduction in neuronal function while increasing cellularity, so alone it is not specific to tumor (26).

For predicting outcome or response to therapy

In high-grade gliomas, higher Cho-to-Cre, higher Cho-to-NAA, higher Lac plus Lip and lower Cr-to-NAA abnormalities have been found to be associated with poor survival (27). In low-grade gliomas, far less data are available on the prognostic value of MRS in the clinic. In one study, normalized Cre was found to be a significant predictor for tumor progression and for malignant tumor transformation in grade II gliomas, while gliomas with decreased Cre appeared to have longer progression-free times and later malignant transformation than low-grade gliomas with regular or increased Cre values (28). The information provided by MRS data is complementary to anatomic images and may often be more valuable than the contrast-enhancing lesion in assessing therapeutic response. The spatial extent of the metabolic lesion can also be used to plan focal therapy, such as external beam radiation therapy (RT) and gamma knife radiosurgery (29), and to assess the response to therapy (30). Alexander *et al* initially showed that the mean tumor Cho/NAA ratio and normalized Cho decreased from baseline to after completion of external beam RT (31). In this study, patients who exhibited more than a 40% decrease in normalized Cho between mid- and post-radiotherapy studies were associated with shorter survival times and faster disease progression. The Lac/NAA ratio at the fourth week of RT and the change in normalized Cho/Cre between baseline and week 4 of RT were also predictive of the outcome, suggesting the possible benefit of adaptive, response-based radiation treatment.

Summary

Although a breadth of MRI methods can provide extensive anatomical and functional information about brain tumors, the current use of co-localized ^{1}H MRS/NMR metabolomics approaches provides valuable information that can help clinicians determine tumor margins, distinguish between progression and pseudoprogression, characterize tumor grade and IDH status, and predict response to therapy. Additionally, preclinical studies of brain tumor models continue to shed light on the complexities of glioma metabolism, leading to an improved understanding of cellular events that could be targetable for new therapeutic approaches. Furthermore, methods currently optimized in the preclinical setting and most notably the use of hyperpolarized agents, are poised to enter the clinic and will enhance the steady-state metabolic data with dynamic flux information that could further improve the detection of tumors and the early monitoring of therapeutic response. Collectively, the breadth of existing and emerging MRS methodologies available for metabolic imaging of brain tumors could significantly improve current paradigms on diagnosis, treatment and response assessment, advancing personalized patient care and quality of life.

2. Gas Chromatography/Mass Spectrometry based metabolomics of brain cancer

Besides NMR based metabolomics, metabolomics based on gas chromatography/mass spectrometry are increasingly applied in brain cancer research and at this point mostly

restricted to *ex vivo* samples. However, mass spectrometry imaging is emerging as a powerful tool for directly determining the distribution of proteins, peptides, lipids, neurotransmitters, metabolites and drugs *in situ* (32) and has enormous potential as a clinical tool for interrogating metabolism in brain cancer.

Gas chromatography/mass spectrometry -based metabolomic analysis of cerebrospinal fluid (CSF) from glioma patients

Nakamizo et al. (33) analyzed cerebrospinal fluid (CSF) from glioma patients using gas chromatography/mass spectrometry (GC/MS) in order to correlate metabolomic profiles with World Health Organization (WHO) tumor grades, tumor location, contrast in magnetic resonance (MR) images, and isocitrate dehydrogenase (IDH) mutations. Samples were separated into three groups: grade I and II glioma, grade III glioma, and glioblastoma (GBM). It was found that grades I and II and grade III samples did not differ in their metabolomic content. However in GBM samples, citric and isocitric acid were elevated compared to the other two groups, and lactic acid was higher relative to grade I and II samples. Succinic, fumaric, and malic acid were decreased in GBM samples relative to grade I and II and grade III gliomas. For samples that originated from tumors proximal to the ventricles, high grade tumors possessed elevated levels of citric and isocitric acid, and low grade tumors had elevated levels of lactic acid, compared to samples originating in tumors distal to the ventricles. In high grade tumors which displayed gadolinium enhancement in MR images, quantities of citric and isocitric acid were higher than those tumors which did not display this enhancement. Levels of citric, isocitric, and lactic acid were higher in samples with mutated IDH than those with wild-type IDH, while the combined signal from pyruvate and oxaloacetic acid was decreased in the mutated IDH samples. The correlation between overall survival (OS) and these metabolites was also investigated. It was found that in malignant glioma (grade III and IV), elevated levels of lactic acid were correlated to lower OS. While not significant, there was a trend between elevated levels of citric and lactic acid and lower OS in all gliomas.

Metabolomic patterns in glioblastoma and changes during radiotherapy

Wibom et al. (34) examined the metabolic profile of GBM patients pre- and post-radiotherapy. Extracellular fluid was extracted from the tumor and brain adjacent to tumor (BAT) via microdialysis before and during treatment, similar to the procedure performed in (35), and was analyzed with gas chromatography time-of-flight mass spectrometry (GC-TOF MS). Reference samples were also collected subcutaneously from the abdomen. Tumor tissue was found to express lower levels of glucose than BAT and the subcutaneous tissue. Several amino acids were found in larger quantities in the tumor relative to the BAT including the essential amino acids, L-threonine, allothreonine, L-tryptophan, L-arginine, L-lysine, and L-valine. Glycine and glutamate levels were also elevated in the tumor compared to the BAT. After radiotherapy was administered, glutamate and glutamine levels increased in both tumor and BAT regions. Ethanolamine appeared to decrease in the tumor but increase in the BAT in addition to glycerol. Although the metabolic profile varied amongst the patients, it was suggested that the trends in the tumor metabolome could be monitored individually to help assess the efficacy of radiotherapy.

Metabolomic screening glioma patients reveals diagnostic and prognostic information

Mörén et al.(36) conducted a study which compared the metabolic profiles of tumor samples and serum from different WHO grades of GBM and oligodendroglioma via GC-TOF MS. Correlations were determined between metabolite levels and survivability of the two diseases. Elevated levels of 2-hydroxyglutaric acid, 4-Aminobutyric acid (GABA), creatinine, glycerol-2-phosphate, glycerol-3-phosphate, ribitol and myo-inositol were found in tumor samples, and lysine and 2-oxoisocaproic acid in serum from oligodendroglioma patients, relative to samples from GBM patients. Compared to oligodendroglioma samples, levels of mannitol and phenylalanine in GBM tumor samples and cysteine from serum were increased. Survival groups were split into short survival (4 months) and long survival (3 years), where the survival is measured as the time after diagnosis. In GBM, elevated levels of glycerol-3-phoshate, myo-inositol, ribitol and fructose were associated with long survival. In oligodendroglioma levels of ribitol, myo-inositol and spermidine were higher for long survival patients while short survival patients expressed elevated levels of glycine and aminomalonic acid.

Metabolomics of human cerebrospinal fluid identifies signatures of malignant glioma

The first study to delineate the metabolic differences in CSF samples between malignant and non-malignant glioma patients was investigated by Locasale et al. (37) through the use of a liquid chromatography/tandem mass spectrometry (LC-MS/MS). Several metabolites that were significantly altered between the malignant and non-malignant samples were identified. A correlation between tumor size and metabolite levels was also determined. It was found that acetylcarnitine, acetoacetate, phenylpropiolic acid, and cholesteryl sulfate were positively correlated with tumor size while myo-inositol and cytidine were negatively correlated.

The metabolomic signature of malignant glioma reflects accelerated anabolic metabolism

The metabolic differences between various WHO grades of glioma tumors were analyzed by Chinnaiyan et al. (38) via ultrahigh performance liquid chromatography/tandem mass spectrometry (UHPLC/MS-MS2) and gas chromatography/mass spectrometry (GC/MS) platforms. It was found that there was a poor distinction of grade III tumors from either grade II or grade IV due to overlap in their metabolomic profiles. Amino acid metabolism was altered in grade IV tumors including increased levels of glutathione and tryptophan and a decrease in creatine levels. An analysis of lipid metabolism in grade IV tumors revealed higher levels of essential and medium chain fatty acids and metabolites associated with carnitine metabolism and lower levels of glycolipids, lysoplipids, and sterols. While phosphoenolpyruvate (PEP) and 3-phosphoglycerate (3-PG) levels were elevated in grade IV tumors, there was an overall decrease in carbohydrate metabolism. Nucleotide metabolism, particularly pyrimidine catabolism, was found to increase in grade IV tumors. Random forest (RF) analysis was performed to determine which metabolites contributed the most to changes in global metabolism amongst the different grades of tumors. 2-HG was found to be the most significant biochemical which best delineates tumor grades. An investigation of glycolysis and the oxidative energy metabolism resulted in over a seven-fold increase in the glycolytic intermediates 3-PG and PEP in grade IV tumors compared to grade II.

Furthermore, the metabolites 6PG, R5P, serine, and glycine, which contribute to the glycolytic pathway, are significantly elevated in grade IV tumors. Interestingly, glutamine, which is found to be a major component of tumorigenesis, was not found to be associated with tumor grade.

3. Hyperpolarized Magnetic Resonance and metabolic imaging in brain cancer

MRI invaluably benefits many brain diseases patients due to its non-invasive investigation. ^{13}C MRS has been studied as another diagnostic modality in the brain tumor model of animals and in patients with brain tumor (39) because ^{13}C labeling compounds provide specific metabolic information with less background noise and it can provide dynamic metabolic analysis with ^{13}C flux analysis (40). However, the low inherent sensitivity compared with proton MRS must be compensated for in clinical applications. Recently, the novel rapid dissolution process of dynamic nuclear polarization (DNP) technique was developed to retain strongly polarized nuclear spins in the liquid state (41). This method can provide more than 10,000-fold signal enhancement enough to investigate the metabolic changes at the cellular level. Many ^{13}C labeled compounds are used for detecting the abnormality of metabolic fluxes or the accumulation of metabolites in human cancer (42) and specifically interrogating brain cancer such as succinate (43), α-ketoglutarate(α-KG) (44,45) and pyruvate and ethyl pyruvate (46–48). Hyperpolarized ^{13}C pyruvate and α-KG are now actively studied for brain cancer metabolism and the first clinical trial with hyperpolarized ^{13}C pyruvate in GBM patients are underway at the University of California, San Francisco. This same group at USCF did the pioneering first in human clinical study of metabolic imaging in prostate cancer employing hyperpolarized ^{13}C pyruvate (49).

[1-^{13}C] Pyruvate

A number of preclinical studies in brain tumors have shown that one can distinguish brain tumors from normal tissue by examining *in vivo* metabolism and assess early response to treatment of high-grade gliomas in animal models (46–47). Recently, hyperpolarized ^{13}C pyruvate kinetic data were acquired from a healthy cynomolgus monkey brain using optimized ^{13}C coils and pulse sequences and demonstrated the feasibility of using hyperpolarized ^{13}C pyruvate for assessing *in vivo* metabolism for brain tumor patients in the near future (48), like the first human clinical trial using ^{13}C metabolic imaging for prostate cancer (49). All these findings suggest that hyperpolarization will provide a promising tool for non-invasive cancer diagnosis and treatment assessment in a clinical setting. The novel hyperpolarized ^{13}C MRS is thus more promising for interrogating the cancer metabolism of a tumor in real-time and monitoring the efficacy and prognosis after therapy to help to tailor treatment for each individual patient.

[1-^{13}C] α-ketoglutarate

Mutations in isocitrate dehydrogenase (IDH) 1 and 2 present in 70% of low-grade gliomas and secondary high-grade gliomas in adults (10). These mutations are associated with the production of the oncometabolite, 2-hydroxyglutarate (2-HG) instead of α-KG in the brain

tumor, which contributes to the formation and malignant progression of gliomas (50). This accumulated 2-HG was detected using *in vivo* proton MRS acquisitions in patients on a 3 T MRI scanner and this noninvasive 2-HG detection shows its feasibility as a diagnostic and prognostic biomarker (51). Also, the injected hyperpolarized ^{13}C α-KG interrogated the accumulation of hyperpolarized 2HG (44) or the conversion to hyperpolarized ^{13}C glutamate, a potential biomarker for glioma (45).

Acetate metabolism in the brain

It has been known for some time that acetate is heavily utilized in the brain. Many studies were done ten to fifteen years ago showing this high utilization of acetate directly in the brain and its specific utilization as a glial fuel (52–54). Recently, this dependence on acetate as an energy source has been found not only in glial cells but in glioblastoma (GBM) and brain metastases (55). Most studies determine and compare the amount and rate of conversion of isotopically labeled acetate to labeled glucose using direct infusion techniques. In one such paper the kinetic parameters for 2-^{13}C acetate transport and conversion was determined by infusing the compound into rats at different rates (56). It was found that the labeled compound can be observed quickly in the brain but its conversion to other metabolites is the rate limiting step. With plasma concentrations around 2–3 mM the rate of acetate utilization was 0.5 μmol/g/min while its transport into the brain was calculated to be 0.96 μmol/g/min (56). In addition, the carbon-13 label enrichment of the C4 position of glutamine was 44% and the C4 position of glutamate was 14% with 2-^{13}C acetate infusion (56). Similar studies have been done in humans with plasma concentrations of 1 mM and show similar incorporation of the carbon-13 label of acetate into glutamine and glutamate in the brain (57–59).

The utilization of acetate in the glioblastoma and brain metastases was found using both *ex vivo* and *in vivo* nuclear magnetic resonance techniques after infusions of 1,2-^{13}C acetate in human orthotopic tumor mouse models of glioblastoma and brain metastases. They found the acetate-to-glucose ratio in the five GBM tumor models was around 5-fold higher than in normal control brain tissue. The rate of incorporation of acetate into the citric acid cycle was found to be directly correlated to the amount of acetyl-CoA synthetase enzyme 2 (ACSS2) (55). In humans, there are three acetyl-CoA synthetase enzymes – ACSS1, ACSS2, and ACSS3. Both ACSS1 and ACSS3 are found in the matrix of the mitochondria; however, ACSS2 is found mainly in the cytosol of the cell (60–61). In GBM, the amount of ACSS2 was found to be directly correlated to the grade of the tumor with high staining in (100% positive) in grade IV tumors compared to normal brain tissue (55). These recent findings have initiated new metabolic research in acetate and cancer progression (61).

Hyperpolarized 1-^{13}C Acetate

Acetate was one of the first compounds to be hyperpolarized using solid state dynamic nuclear polarization (DNP) (62, 63). Since its initial polarization, several improvements in formulation and use of other techniques have significantly improved the percentage of polarization and increased the amount (final concentration) of hyperpolarized acetate that can be generated (64–66). The compound has been polarized using both the pre-clinical DNP polarizer and the clinical polarizer (67). Our laboratory recently reported a method of

chemically converting hyperpolarized 1,2-^{13}C pyruvate to hyperpolarized 1-^{13}C acetate, ^{13}C-bicarbonate and ^{13}C carbon dioxide employing Chemical Reaction Induced Multi-molecular Polarization (CRIMP) (68). Even with these improvements in 1-^{13}C acetate polarization, significant progress needs to be made for its rate of conversion to glutamine, glutamate, acetyl-CoA, and citrate to be useful in diagnostic imaging in the time scale of polarization (less than 5 minutes). A thorough discussion and execution of utilizing hyperpolarized acetate to diagnosis metabolic changes in diabetic rats was recently published (67). One of the main mechanisms acetylCoA gets shuttled into the mitochondria is through its conversion into acetylcarnitine through (CAT-1, carnitineacetyltransferase) and then back to carnitine and acetyl-CoA in the mitochondria thorough CAT-2 (64). The Stodkilde-Jorgensen group could not detect a significant difference in the conversion rate of hyperpolarized acetate to acetylcarnitine in the time window of hyperpolarization and stated this is probably due to low rate of conversion of the enzyme (67). They observe similar results to the carbon-13 non-hyperpolarized infusion studies done in the brain; the transport of acetate into specific tissues is fast, however the rate of conversion is the rate limiting step and is significantly slower than hyperpolarized 1-^{13}C pyruvate. Another problem with 1-^{13}C acetate is that other metabolites that would incorporate the carbon-13 label such as citrate have similar chemical shifts to acetate. Using *in vivo* spectroscopy, it is thus difficult to separate the hyperpolarized resonance of hyperpolarized 1-^{13}C acetate from its metabolite 5-^{13}C citrate. Other compounds might be better suited to interrogate the citric acid cycle or fatty acid synthesis in the brain in the time scale of polarization (69–71).

A recent paper by Rolf Gruetter's laboratory illustrates another method of utilizing the hyperpolarization of 1-^{13}C acetate (72). His group generated a pulse sequence that transferred the polarized signal in 1,2-^{13}C acetate from the carbonyl carbon (C1) to the methyl carbon (C2) in the brain of a rat and from the C1 position to a proton on the methyl group of acetate. This method allows for a whole new group of metabolites and chemical resonances to be observed. This transfer sequence could allow for hyperpolarized acetate to become clinical metabolic imaging agent.

Brain cancer cell line work on hyperpolarization

Hyperpolarized MRS has been applied in brain tumor cell line models in efforts to potentially inform medical decision making in novel and clinically useful ways. Various studies have used this technique which could provide information on the diagnosis, treatment monitoring, and prognosis of brain tumors in a noninvasive manner.

In regards to diagnosing brain tumors, the differing signal levels provided by hyperpolarized ^{13}C-labeled pyruvate and ^{13}C-labeled lactate can be used to distinguish brain tumor and normal brain tissue. In one study, different glioblastoma cell lines (U251 and U87) were used to generate orthotopic xenografts in the rat brain. Levels of ^{13}C-labeled pyruvate and ^{13}C-labeled lactate were elevated in the tumor tissue compared to the normal brain tissue (46). Furthermore, the pattern of histopathology in the cell lines studied correlated with the amount of signal detected (46). The U251 glioblastoma cell line exhibits large areas of necrosis and hypoxia; in comparison, the U87 glioblastoma cell line has very little necrosis and hypoxia. The Signal to Noise ratio (SNR) of hyperpolarized ^{13}C -labeled

lactate, pyruvate, and total carbon was significantly greater in U87 tumors than in U251 tumors; this was thought to be due to the greater number of viable cells in the U87 line.

Compressed sensing has been used with hyperpolarized MRS to evaluate tumor tissues with heterogeneous metabolic profiles due to varying levels of necrosis and hypoxia. Compressed sensing allows for the reduction of acquisition time and higher spatial resolution than conventional imaging; this permits the characterization of tumors with a heterogeneous nature like GBM (47). Differentiation of tumor tissue that was hypoxic/necrotic, non-hypoxic/non-necrotic, or normal brain tissue in an orthotopic human glioblastoma xenograft model was possible using this technique. Specifically, highly necrotic and hypoxic tumor tissue had absent or low levels of ^{13}C-labeled pyruvate and ^{13}C-labeled lactate, whereas tumor tissue with minimal levels of necrosis and hypoxia had high levels of ^{13}C-labeled pyruvate and ^{13}C-labeled lactate (47). If used in the clinic, hyperpolarized pyruvate with MRS and compressed sensing could be particularly useful in noninvasively determining tumor tissue types in GBM.

In addition to characteristic signal levels of hyperpolarized ^{13}C-labeled metabolites in tumor versus normal brain tissue, the apparent rate constant (k_{PL}) for the conversion of pyruvate to lactate can also be used to distinguish cancer and normal tissue. Orthotopic xenografts of C6 glioma had larger lactate/pyruvate ratios as well as larger k_{PL} compared to normal tissue (73). Furthermore, k_{PL} may even be a more robust marker than the lactate/pyruvate ratio in distinguishing cancerous brain tissue. k_{PL} has significantly less variability than the lactate/pyruvate ratio when used to differentiate tumor tissue from normal brain tissue (73).

Hyperpolarized techniques could be applied to monitor treatment response in brain tumors; tumor response to radiation therapy and to different methods of chemotherapy, such as temozolomide, everolimus, and LY294002, have been studied with hyperpolarized ^{13}C-labeled metabolites as a metric for response. Temozolomide (TMZ) is an alkylating agent used to treat brain tumors and is the first-line agent in treating GBM. Everolimus and LY294002 are drugs that target the PI3K/AKT/mTOR pathway which regulates the cell cycle. Using conversion of hyperpolarized pyruvate to lactate as a metric, tumor response to radiation treatment by a C6 glioma orthotopic tumor model was examined. By 72 hours after irradiation, the ratio of hyperpolarized lactate in tumor-to-maximum pyruvate in blood vessels was decreased by 34% compared to untreated tumors (74).

Treatment with TMZ was found to decrease the hyperpolarized pyruvate/lactate ratio in an orthotopic rat model of human GBM (75). This response could be detected just 1 day after TMZ treatment, whereas imaging-based evidence of tumor volume shrinkage due to treatment did not occur until 5 to 7 days after treatment (75). These results were supported by a different study, which utilized a bioreactor to maintain GBM cells treated with TMZ during hyperpolarized imaging. TMZ-treated cells showed a decrease in conversion of hyperpolarized pyruvate to lactate compared to untreated cells (76). Additionally, the authors of the study found that treatment with TMZ correlated with a decrease in pyruvate kinase (PKM2), which is a glycolytic enzyme that indirectly controls pyruvate to lactate conversion (76).

In hyperpolarized studies of everolimus treatment in an orthotopic rate model of GBM, a significant drop in hyperpolarized lactate/pyruvate ratio compared to control was observed 7 days after treatment (77). At this same time point, conventional MRI was unable to detect a difference in tumor size between the treated and control group. It was not until 15 days after treatment that inhibition of tumor growth was appreciated on MRI (77). Bioreactor studies of everolimus and LY294002 treated glioblastoma cells support these results. Decreased hyperpolarized lactate levels were observed in cells treated with these drugs compared to untreated (78,79). In addition to decreased hyperpolarized lactate levels, decreased phosphocholine levels were found to correlate with treatment using these agents (78). This is logical because the PI3K/AKT/mTOR pathway controls both the synthesis of phosphocholine and lactate via a common transcription factor HIF-1-α (78).

The balance between glycolysis and oxidative phosphorylation can provide important information regarding treatment response. Because cancer cells derive most of their energy via glycolysis and normal cells via oxidative phosphorylation, the ability to detect if a tumor mainly utilizes one pathway over the other can indicate continued malignancy or positive response to treatment. One study was able to detect and quantify conversion of hyperpolarized ^{13}C-bicarbonate from ^{13}C-pyruvate *in vivo* for the first time (80). Because CO_2 is a byproduct of the flux from pyruvate to acetyl-CoA, bicarbonate can be used as a surrogate marker for mitochondrial metabolism, which is predominant in normal cells. The study found that lactate levels were significantly greater in glioma, and bicarbonate levels were significantly greater in normal brain tissue (80).

A bioreactor study of glioblastoma cells with mutated IDH1 showed elevated levels of hyperpolarized 2-HG compared to glioblastoma cells with wild-type IDH1 (78). IDH mutation was also found to correlate with decreased activity of branched chain amino acid transaminase (BCAT) (45). BCAT catalyzes the transamination of branched chain amino acids while converting α-KG to glutamate. Thus, in cells with IDH1 mutation, a decrease in BCAT activity is expected to correlate with decreased glutamate production. This was found to be the case in a bioreactor study of GBM cells with IDH1 mutation; hyperpolarized ^{13}C-glutamate production was decreased in mutant cells compared to wild-type (45). As biomarkers of metabolic imaging, hyperpolarized 2-HG and glutamate may potentially provide useful information in regards to detecting IDH1 mutation and treatment monitoring of brain tumors with IDH1 mutation.

Currently, the main tool for cancer diagnosis and monitoring is ^{18}Fluoro-2-deoxyglucose positron emission tomography (FDG-PET). In brain tumors however, FDG-PET shows increased signal in surrounding brain tissue due to the high uptake which masks the signal generated by the tumor itself (74). Furthermore, pseudoprogression is a misleading phenomenon in which there is contrast enhancement of the tumor after treatment with radiotherapy or chemotherapy despite positive tumor response to the treatment. Diagnosis and treatment monitoring with hyperpolarized metabolic techniques can help mitigate this confounding clinical problem.

4. Imaging Inflammation in brain

Broadly, it is known that the heterogeneous microenvironment of brain tumors includes both the tumor and a variety of altered stromal compounds. These altered stromal components include epithelial cells, infiltrative inflammatory cells, and adipose cells. The genesis of the milieu has not yet been determined, but it is clear that the net effect of local environment is mostly tumorigenic. Indeed, cells present at these sites do not behave as they would in their normal microenvironment. For example, tumor associated macrophages and neutrophils exhibit a graded expression pattern that is typical for a tumor environment. This expression pattern takes on an M2 or N2 like characteristic typical of the anti-inflammatory state of these cells. Initial tests in pre-clinical models have shown that when treated with compounds that restore these cells to their normal M1 or pro-inflammatory state that tumor progression halts, and indeed can show regression. In the case of the adaptive immune system there are an abundance of the regulatory T (Treg) cells that are again typically anti-inflammatory. By blocking the ability of the Treg cells to interact with and halt the adaptive immune system, functional “cures” have been effected in metastatic melanoma cases. Such response would have been unthinkable a decade ago. Currently there are clinical trials underway at a host of institutions to study these immune checkpoint blockade inhibitors in the context of GBM and neuroblastomas.

There is a deep need to understand how and when the immune system begins to interact with the tumor environment and to understand how the status of the various types and states of the inflammatory cells might predict response to various classes of immune checkpoint blockade. There are starting points to dissect out these contributions but each of the currently existing approaches has limitations. Broadly people often interpret the flair phenomenon as inflammation, but this observation can also occur due to increased vascular permeability in and around tumor sites. Indeed, when patients are treated with modern chemotherapy protocols 20–30% of patients yield pseudo progression, an increase in T2 bright areas due to “inflammatory” processes. Effectively categorizing new tumor growth vs. inflammation is critically important for managing patient care (81). Similarly phase weighted MRI can also be used to find inflammatory lesions, but this again has been primarily attributed to an increase in the leaky blood vessels and an increase in accumulation of heme from hemoglobin at the site of inflammation. Similar to ^{111}Indium oxine labeling of white blood cells for tracing inflammation by nuclear imaging, macrophages have been loaded *ex vivo* with superparamagentic iron oxide nanoparticles and then their localization to otherwise T2 bright regions can be determined. Because these are T2* agents the tumor and surrounding brain tissue must be T2 bright a priori. The normal brain is actually quite well suited for such an application, but as mentioned above an increase in vascular permeability can result in locally high concentrations of heme that also result in T2 darkening. In the context of spinal injury, diffusion tensor imaging can be useful for detecting demyelenation that is downstream of neuroinflammation and in traumatic brain injury (TBI) (82, 83). However, particularly in the case of heterogeneous GBM and infiltrating immune cells assigning changes in diffusion to a specific cell type or biochemical pathway will be challenging. Classic targeting of surface receptors using contrast agents has been limited by the sensitivity of MRI relative to the abundance of surface receptors present

on the cells and the requirement for breakdown of the blood brain barrier (84). Enzymatic amplification of the targeting signal might solve this problem. In pre-clinical models of inflammation, bis HT-DTPA-Gadolinium utilizes a polymerization strategy for determining myeloperoxidase (MPO) enzymatic activity *in vivo* (85). This has shown to be selective in the context of MPO −/− mice (86). However, because there is induction of free radical polymerization processes *in vivo*, this technique has not been widely adopted. Others have utilized MRS for studying changes in NAA, choline and creatine levels which correlate to changes in the neural tissue. However, it is not clear whether the subsequent increase NAA has been proven to be cell type specific through either cell depletion studies or knockout mice (87). As such there is still significant room for pathway or cell type specific molecular probes for studying inflammation in the context of tumor initiation, growth, and treatment *in vivo*. Indeed, the need for enzymatic amplification and improvement in SNR suggests that hyperpolarized MRI may be able to address several of the current limitations for imaging inflammation in the context of tumors in near future.

5. Chemical Exchange Saturation Transfer (CEST) imaging in brain cancer

Chemical exchange saturation transfer (CEST) imaging offers enhanced indirect detection of exchangeable protons species, which can be endogenous such as hydroxyl, amide and amine protons in peptides or exogenously introduced such as liposomes. There are several classification schemes of CEST techniques that are currently in use: 1) classification based on exchange type: protons, molecules or compartments; 2) diamagnetic CEST (diaCEST) or paramagnetic CEST (paraCEST) (88). We will use the latter due to its prevalence in the literature. The CEST mechanism relies on the selective RF saturation of exchangeable proton species that resonate at a different frequency relative to the bulk water (~4.75 ppm). Thus the bulk water signal becomes attenuated to some extent after solute protons (μM to mM range) exchange with that of the bulk (~110 M). Provided that the RF saturation is long enough (seconds range) and the solute exchange rates are sufficiently fast (ms residence time), significant saturation will eventually be visible on the bulk water signal (S_{sat}). Normalizing the original proton spectrum without saturation (S_0) with S_{sat} (S_{sat}/S_0) leads to the CEST spectrum or Z-spectrum. The inverted peaks in the Z-spectrum then correspond to the selectively saturated proton species and the bulk water peak is redefined to 0 ppm by convention. The asymmetric Magnetization Transfer Ratio (MTRasym) plot is subsequently obtained by subtracting the left half of the Z-spectrum (> 0 ppm) from the right half in order to remove the bulk water signal for better visual representation (88). For the diaCEST effect, the saturation frequency ranges from 0–7 ppm, while for paraCEST agents, the offset frequency range can be over 100 ppm, which offers a much cleaner saturation excitation profile.

The CEST signal is a function of CEST agent concentration, pH, temperature, magnetic parameters (relaxation rate, magnetic field strength) and imaging parameters (repetition time, RF irradiation amplitude and power as well as imaging sequence). For the CEST effect to be efficiently observed, a relatively slow to intermediate exchange rate on the MR time scale is required. If the exchange rate is too slow, the saturated protons could have relaxed back to equilibrium by the time it affects to the bulk water pool; if the exchange rate is too fast, not enough saturation on the solute proton pool will have been accumulated. The CEST

effect also scales with B_0 due to better spectrum separation, so more accurate selective saturation and a higher B_0 leads to longer $T_{1\ water}$, resulting in longer storage of saturation in the bulk water pool. However, the specific absorption rate (SAR) increases quadratically with B_0, which places a constraint on sequence design and applications. Please refer to (88,89) for a comprehensive review of optimization and quantification techniques involved in CEST imaging. Here we will focus on the potential applications of CEST to image brain cancer.

GluCEST

Glutamate is a major excitatory neurotransmitter in the brain, which is restricted to the synaptic and perisynaptic space of glutamateric synapses under normal physiological conditions. Glutamate released by malignant gliomas has been shown to induce seizure and promote excitotoxicity, which help the invasion of tumor cells into normal tissues (90,91) CEST imaging of glutamate (GluCEST) can provide non-invasive monitoring of glutamate level in vivo, which can potentially be used to monitor disease progression and assess treatment efficacy.

GluCEST relies on the exchange of protons in the amine group with the bulk water. The resonance frequency of the amine protons is pH-dependent and centered around 3 ppm relative to the bulk water resonance frequency (92). In phantom studies at 7T and at pH 7, it has been demonstrated that under physiological concentrations, the majority of the CEST effect with saturation at 3 ppm (z-spectrum scale) came from Glutamate (~ 70–75% of total CEST contrast) while other metabolites contribute relatively little (GABA ~12 %, <6% from creatine and minimal from other metabolites). *In vivo* experiment with middle cerebral artery occlusion (MCAO) induced stroke models showed much higher GluCEST contrast compared to the normal contralateral side. GluCEST imaging offers a resolution of 0.27 mm × 0.27 mm × 2 mm for animal model and 1.9 mm × 1.9 mm × 2 mm for human subjects with an imaging time of around 16s per slice (1 average), which is much better compared to conventional chemical shift imaging techniques in terms of spatial and temporal resolution. The total scan time is around 12 mins, including the acquisition of B0 and B1 maps needed for corrections (92, 93).

GlucoCEST

Tumors tend to exhibit high glucose uptake, which forms the basis of FDG-PET. Non-invasive CEST imaging of unlabeled glucose (GlucoCEST) offers an alternative to assess glucose uptake *in vivo* without the use of ionizing radiation (94). GlucoCEST rests upon selective saturation of the hydroxyl protons of glucose at 1.2, 2.1 and 2.9 ppm. In two colorectal cancer models (LS174T and SW1222), GlucoCEST images taken 60 mins after glucose infusion (1.1 mmol/kg, i.p.) shows significant correlation with FDG-PET images ($r^2 = 0.70$, $P < 0.01$), offering a possible non-radioactive alternative and better spatial resolution (1 × 1 × 3 mm) to probe intratumoral heterogeneity.

CrEST

Creatine kinase reaction plays a vital role in energetics by regulating ATP reservoir ($Cr + ATP \rightleftharpoons ADP + PCr + H^+$). Reduced total creatine (tCr) concentration has been shown to be

a predictor for gliomas progression (95–97). With traditional MRS techniques, it's possible to detect the total creatine (Cr + PCr) but not the individual components. CrEST (creatine CEST) imaging provides a way to assess the free creatine level *in vivo* with much improved spatial and temporal resolution (98). But quantification using only asymmetry analysis would be problematic due to contributions from semi-solid magnetization transfer (MT) and aliphatic nuclear Overhauser effect (NOE). So the entire Z-spectrums for each pixel must be acquired for Lorentzian spectral fitting (5 components: free water, bound water, CrEST ~2 ppm, MT and CEST), which could lead to greatly increased scan time (~30 mins for the brain). After fitting, the integral of creatine component in the brain tumor region was shown to decrease with disease progression in an intracranial cancer model, consistent with previous studies. And interestingly, the tumor margin as defined by the creatine map is sometimes larger than those obtained from traditional MRI contrast such as proton density image, which may indicate that tumor could have negative impacts on the energetics of surrounding tissues (99).

APT

CEST imaging of amide protons is based on saturation at 3.5 ppm from the water peak. Termed amide proton transfer (APT), it's assumed to originate from the exchangeable amide protons of mobile tissue proteins and peptides. In tumors, APT contrast increases as the tumor progresses presumably due to increased cellular amide proton content compared to normal tissues while intracellular pH stays relatively constant (88,99–102). In ischemic models, however, the APT contrast is lower compared to the normal tissues, which could be attributed to lower amide proton exchange rate resulting from decreased intracellular pH (103,104).

paraCEST

ParaCEST agents are mainly metal ion complexes, which offers protons exchanging slow enough for detection (e.g.: lanthanide groups, iron and nickel). Aside from having large chemical shift away from the bulk water pool, paraCEST agents can be designed to target specific biological processes and improve detection sensitivity by polymerization to increase the number of exchange sites per mole. They have been used to measure tissue pH, temperature, enzymatic activity as well as specific metabolite levels. But since paraCEST agents are exogenously administered, tissue perfusion and clearance tend to complicate the quantification of CEST contrast. One example of paraCEST agent for *in vivo* glioma detection is based on dendrimer labeled with Europium CEST agents and fluorescent labels for dual modality imaging ([DyLight 680]-Eu-G5PAMAM) (105). Meser et al. has shown the paraCEST agent could be detected in the tumor region after around 10 mins, primary due to impaired blood-brain barrier in glioma models (105).

SPION

Superparamagnetic iron oxide nanoparticles (SPION) act as T2 shortening agents, providing negative contrasts in MRI. They are commercially available and have been used extensively for cell labeling after modifications (106,107). Recently, SPION has been conjugated with recombinant human epidermal growth factor (EGF) for *in vivo* detection of brain malignancies in C6 glioma models which overexpress EGFR (108). After 24 hrs after i.v.

injection (0.3 mg/kg), SPION-EGF showed much better accumulation inside the tumor region compared to bare SPIONs; reflected laser scanning revealed internalization of SPION-EGF inside the glioma cell cytoplasm (108). It has also been shown that radiation sensitizes the glioma model for better uptake of SPIONs conjugated with antibodies targeting membrane heat shock protein HSP 70 (109). These early pre-clinical studies were promising applications of SPIONs but toxicity issues need to be further addressed for *in vivo* applications in human.

6. Hyperpolarized Xenon imaging in brain

Hyperpolarized ^{129}Xenon (Xe) (110) has been developed for biomedical MR imaging over the last twenty-five years, with most emphasis on void-space and pulmonary imaging (111, 112). Because inhaled (or injected) xenon travels readily to the brain (and acts as an anesthetic (113), it may also function as a novel contrast agent for brain imaging. Indeed, non-polarized xenon gas has been used for enhancing computed tomography (CT) scans of the brain for decades (114). In recent years, limited MRI studies have already successfully shown hyperpolarized ^{129}Xe gas dissolved in the brain tissue. Below is a brief overview of the current state of clinical and pre-clinical work applying hyperpolarized ^{129}Xe to brain MRS/MRI.

^{129}Xe has many physical and magnetic properties that make it well-suited for physiological imaging. This inert gas (spin=1/2) is non-radioactive, has no biological background signal, and dissolves readily into blood, tissue, and fat (115). In fact, xenon's differential solubility (due to a slight hydrophobicity) may be developed as a form of contrast to distinguish areas of the brain that are rich in water vs. lipids (110). Importantly, ^{129}Xe possesses an incredibly sensitive chemical shift range (δ>7,500 ppm), which makes it well-suited to function as a spectroscopic chemical sensor (116). Indeed, differences in chemical shift have been shown to distinguish ^{129}Xe inside Cryptophane cages that are linked to different biological targets (117). Furthermore, ^{129}Xe MR spectra are exceedingly simple to analyze, and typically only consist of a handful of sharp, well-resolved NMR peaks that correspond to different chemical and magnetic environments. The relaxation rate (T_1) of ^{129}Xe can also be used to differentiate contact with different tissue types (118), as well as oxygenated vs. deoxygenated blood (119).

Because of its relatively low gyromagnetic ratio (γ_{Xe}/γ_H ~−0.28) and natural abundance (~26%) compared to ^{1}H, the signal from ^{129}Xe at thermal equilibrium is insufficient for conventional MRI detection. ^{129}Xe gas can be hyperpolarized through spin-exchange optical pumping (SEOP) (120)—a two-step process where angular momentum from resonant, circularly polarized laser light is transferred to the electronic spins of an alkali metal vapor, then subsequently exchanged with the ^{129}Xe nuclei via gas-phase collisions. This results in a build-up of ^{129}Xe nuclear spin polarization, which creates MR signal enhancement values of 4–5 orders of magnitude compared to thermal equilibrium—allowing ^{129}Xe MRS/MRI for biomedical studies. This enhanced signal decays over a time constant (T_1) that is dependent on the immediate environment of the gas, and can range from a few seconds to several hours. SEOP takes place in a dedicated 'hyperpolarizer' (121) that will dispense the polarized gas into a Tedlar bag for administration to the patient; due to recent improvements

in technology and methodology, ^{129}Xe nuclear spin polarization values of over 90% have been reported (122).

Hyperpolarized ^{129}Xe can be administered to patients through either inhalation or intravenous injection, and it has been shown that both methods lead to comparable ^{129}Xe concentrations in the brain (123). Due to the decreased invasiveness of the procedure, gas inhalation is typically the method used for *in vivo* studies. In this case, the patient will inhale as much of the gas as possible (ca. 0.5 L) along with atmospheric air or O_2, followed by a breath hold (15–60 s). The xenon travels from the lungs to the brain relatively quickly (ca. 5 s) (124). For pre-clinical studies, the animal is usually intubated and an MR-compatible respirator controls the administration of the gas. For intravenous injections, the hyperpolarized ^{129}Xe is typically dissolved in saline or an emulsion (such as perflurbon or Intralipid) prior to injection. As with all hyperpolarized contrast agents, one major main constraint is that the non-renewable enhanced signal decays with time. When held in pristine laboratory conditions, hyperpolarized ^{129}Xe will last for hours (125). However, when administered *in vivo*, the enhanced signal is depleted over the course of about 1 minute due to interactions with its environment. Indeed, the hyperpolarized T_1 of ^{129}Xe in the blood is only ~5 s, due to the abundance of paramagnetic hemoglobin (119). Additionally, the very act of acquiring a signal further depletes the magnetization of the contrast agent in a non-renewable fashion. To combat this, many MR acquisitions take advantage of a ‘fast low-angle shot’ (FLASH) sequence to mitigate signal losses caused by rf pulsing. The silver lining to this time constraint is that the effective clearance rate of the ^{129}Xe MR signal can be quite fast to allow for multiple studies in a single patient session.

Since the initial demonstration of hyperpolarized ^{129}Xe MRI in a rat brain (126) and ^{129}Xe MRS in a human brain (124) in 1997, the field has grown steadily. ^{129}Xe brain MRS typically displays a handful of discernable peaks that can be attributed to ^{129}Xe in the gas phase, dissolved in blood, and dissolved in different brain tissues (i.e., gray and white matter) (118; 127). It has been shown that xenon has an increased proclivity for gray matter compared to white matter (with different T_1 rates), and perfusion between brain compartments can also be tracked (118). A range of different administration methods, including inhalation and intravenous injection, have been compared and numerically modelled to show that inhalation is the preferred method of supplying the brain with hyperpolarized ^{129}Xe gas (118). A differential distribution of ^{129}Xe signal in the cerebral cortex in rats was found following a pain stimulus (and corroborated with ^{1}H MRI), which is early work in the development of ^{129}Xe for functional MRI (128). Indeed, ^{129}Xe may be further developed for functional MRI through an increase in concentration (due to increased blood flow to regions of brain activity) and longer ^{129}Xe T_1 values in oxygenated blood (vs. deoxygenated blood) (110)—acting as a proxy for BOLD (Blood Oxygen Level Dependent) contrast imaging. It has also been shown that a perfusion deficit, such as one caused by a stroke, can be detected with changes in ^{129}Xe signal. Because ^{129}Xe is a safe, inert, and inhalable contrast agent, it may prove to be a safer MR contrast agent compared to injectable gadolinium-based tracers (128).

Moving forward, several advances in hyperpolarized ^{129}Xe brain MRS/MRI are on the horizon. Most predominantly will be targeted imaging using functionalized biomarkers in

the form of a Cryptophane complex. As ^{129}Xe diffuses through the cage-shaped molecule, its chemical shift is changed—which allows it to be used as a sensitive chemical sensor that can differentiate whether the cage molecule is attached to a biomarker (117). However, because the amount of ^{129}Xe associated with these targeted Cryptophane cages at a given time is small, the resulting signal is often quite weak. One way around this is through a technique called 'HyperCEST' (Hyperpolarized Chemical Exchange Saturation Transfer), which saturates the "^{129}Xe in Cryptophane" resonance while monitoring the increased depletion of the "^{129}Xe gas resonance" as the xenon transfers between the targeted cage complex and the spin reservoir (129). This method can be used to indirectly detect the presence of low concentrations of transferrable species that are spectroscopically discernable, and has been recently demonstrated to show targeting of the human brain microvascular endothelial cells that comprise the blood-brain barrier (130). Another potential improvement to detection sensitivity would be through the implementation of a remote detection protocol, where the step of rf-encoding of nuclear spins is physically separated from the signal detection. This can be used to improve sensitivity through optimizing the detection filling factor; under this scenario, ^{129}Xe in the entire brain would be encoded at once, but then detected using a small surface coil placed over the jugular vein to monitor the ^{129}Xe as it exits the brain (131).

Summary

This article reviews the existing and emerging techniques of interrogating metabolism in brain cancer from well-established proton MR spectroscopy to the promising hyperpolarized metabolic imaging, GlucoCEST, HyperCEST and imaging inflammation. Many of these techniques are at an early stage of development and clinical trials employing these techniques are in progress in brain cancer patients to establish the clinical efficacy of these techniques. Nonetheless, it is likely that *in vivo* metabolomics and metabolic imaging is the next frontier in brain cancer diagnosis and assaying therapeutic efficacy; with the combined knowledge of genomics and proteomics a complete understanding of tumorigenesis in brain might be achieved.

References

1. Bennett A. Growing pains for metabolomics. The Scientist. 2005; 19:25–28.
2. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci U S A. 2007; 104:19345–19350. [PubMed: 18032601]
3. Elkhaled A, Jalbert LE, Phillips JJ, Yoshihara HA, Parvataneni R, Srinivasan R, et al. Magnetic resonance of 2-hydroxyglutarate in IDH1-mutated low-grade gliomas. Sci Transl Med. 2012; 4:116ra5.
4. De Graaf, RA. In vivo NMR spectroscopy: principles and techniques. 2. John Wiley & Sons: Chichester; West Sussex, England; Hoboken, NJ: 2007.
5. Zhu H, Barker PB. MR spectroscopy and spectroscopic imaging of the brain. Methods Mol Biol. 2011; 711:203–226. [PubMed: 21279603]
6. Glunde K, Bhujwalla ZM, Ronen SM. Choline metabolism in malignant transformation. Nat Rev Cancer. 2011; 11:835–848. [PubMed: 22089420]
7. Warburg O. On the origin of cancer cells. Science. 1956; 123:309–314. [PubMed: 13298683]

8. Zoula S, Herigault G, Ziegler A, Farion R, Decorps M, Remy C. Correlation between the occurrence of 1H-MRS lipid signal, necrosis and lipid droplets during C6 rat glioma development. NMR Biomed. 2003; 16:199–212. [PubMed: 14558118]
9. Remy C, Arus C, Ziegler A, Lai ES, Moreno A, Le Fur Y, Decorps M. In vivo, ex vivo, and in vitro one- and two-dimensional nuclear magnetic resonance spectroscopy of an intracerebral glioma in rat brain: assignment of resonances. J Neurochem. 1994; 62:166–179. [PubMed: 8263516]
10. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009; 462:739–744. [PubMed: 19935646]
11. Bouzier AK, Quesson B, Valeins H, Canioni P, Merle M. [1-(13)C]glucose metabolism in the tumoral and nontumoral cerebral tissue of a glioma-bearing rat. J Neurochem. 1999; 72:2445–2455. [PubMed: 10349854]
12. Marin-Valencia I, Yang C, Mashimo T, Cho S, Baek H, Yang XL, et al. Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain in vivo. Cell Metab. 2012; 15:827–837. [PubMed: 22682223]
13. Mashimo T, Pichumani K, Vemireddy V, Hatanpaa KJ, Singh DK, Sirasanagandla S, et al. Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. Cell. 2014; 159:1603–1614. [PubMed: 25525878]
14. Nelson SJ. Assessment of therapeutic response and treatment planning for brain tumors using metabolic and physiological MRI. NMR Biomed. 2011; 24:734–749. [PubMed: 21538632]
15. Nelson SJ, Ozhinsky E, Li Y, Park I, Crane J. Strategies for rapid in vivo 1H and hyperpolarized 13C MR spectroscopic imaging. J Magn Reson. 2013; 229:187–197. [PubMed: 23453759]
16. Fulham MJ, Bizzi A, Dietz MJ, Shih HH, Raman R, Sobering GS, et al. Mapping of brain tumor metabolites with proton MR spectroscopic imaging: clinical relevance. Radiology. 1992; 185:675–686. [PubMed: 1438744]
17. Negendank WG, Sauter R, Brown TR, Evelhoch JL, Falini A, Gotsis ED, et al. Proton magnetic resonance spectroscopy in patients with glial tumors: a multicenter study. J Neurosurg. 1996; 84:449–458. [PubMed: 8609557]
18. Dowling C, Bollen AW, Noworolski SM, McDermott MW, Barbaro NM, Day MR, et al. Preoperative proton MR spectroscopic imaging of brain tumors: correlation with histopathologic analysis of resection specimens. AJNR Am J Neuroradiol. 2001; 22:604–612. [PubMed: 11290466]
19. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007; 114:97–109. [PubMed: 17618441]
20. Di Costanzo A, Scarabino T, Trojsi F, Giannatempo GM, Popolizio T, Catapano D, et al. Multiparametric 3T MR approach to the assessment of cerebral gliomas: tumor extent and malignancy. Neuroradiology. 2006; 48:622–631. [PubMed: 16752135]
21. Di Costanzo A, Trojsi F, Giannatempo GM, Vuolo L, Popolizio T, Catapano D, et al. Spectroscopic, diffusion and perfusion magnetic resonance imaging at 3.0 Tesla in the delineation of glioblastomas: preliminary results. J Exp Clin Cancer Res. 2006; 25:383–390. [PubMed: 17167979]
22. Chang SM, Nelson S, Vandenberg S, Cha S, Prados M, Butowski N, et al. Integration of preoperative anatomic and metabolic physiologic imaging of newly diagnosed glioma. J Neurooncol. 2009; 92:401–415. [PubMed: 19357966]
23. Croteau D, Scarpace L, Hearshen D, Gutierrez J, Fisher JL, Rock JP, Mikkelsen T. Correlation between magnetic resonance spectroscopy imaging and image-guided biopsies: semiquantitative and qualitative histopathological analyses of patients with untreated glioma. Neurosurgery. 2001; 49:823–829. [PubMed: 11564242]
24. McKnight TR, Noworolski SM, Vigneron DB, Nelson SJ. An automated technique for the quantitative assessment of 3D-MRSI data from patients with glioma. J Magn Reson Imaging. 2001; 13:167–177. [PubMed: 11169821]
25. Caivano R, Lotumolo A, Rabasco P, Zandolino A, D'Antuono F, Villonio A, et al. 3 Tesla magnetic resonance spectroscopy: cerebral gliomas vs. metastatic brain tumors. Our experience and review of the literature. Int J Neurosci. 2013; 123:537–543. [PubMed: 23390934]

26. Venkatesh SK, Gupta RK, Pal L, Husain N, Husain M. Spectroscopic increase in choline signal is a nonspecific marker for differentiation of infective/inflammatory from neoplastic lesions of the brain. J Magn Reson Imaging. 2001; 14:8–15. [PubMed: 11436208]
27. Li X, Jin H, Lu Y, Oh J, Chang S, Nelson SJ. Identification of MRI and 1H MRSI parameters that may predict survival for patients with malignant gliomas. NMR Biomed. 2004; 17:10–20. [PubMed: 15011246]
28. Hattingen E, Delic O, Franz K, Pilatus U, Raab P, Lanfermann H, Gerlach R. 1)H MRSI and progression-free survival in patients with WHO grades II and III gliomas. Neurol Res. 2010; 32:593–602. [PubMed: 19726017]
29. Chan AA, Lau A, Pirzkall A, Chang SM, Verhey LJ, Larson D, et al. Proton magnetic resonance spectroscopy imaging in the evaluation of patients undergoing gamma knife surgery for Grade IV glioma. J Neurosurg. 2004; 101:467–475. [PubMed: 15352605]
30. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007; 114:97–109. [PubMed: 17618441]
31. Alexander A, Murtha A, Abdulkarim B, Mehta V, Wheatley M, Murray B, et al. Prognostic significance of serial magnetic resonance spectroscopies over the course of radiation therapy for patients with malignant glioma. Clin Invest Med. 2006; 29:301–311. [PubMed: 17144440]
32. Shariatgorji M, Svenningsson Per, Andrén PE. Mass Spectrometry Imaging, an Emerging Technology in Neuropsychopharmacology. Neuropsychopharmacology Reviews. 2014; 39:34–49. [PubMed: 23966069]
33. Nakamizo S, Sasayama T, Shinohara M, Irino Y, Nishiumi S, Nishihara M, et al. GC/MS-based metabolomics analysis of cerebrospinal fluid (CSF) from glioma patients. J Neurooncol. 2013; 113:65–74. [PubMed: 23456655]
34. Wibom C, Surowiec I, Moren L, Bergstrom P, Johansson M, Antti H, et al. Metabolomic patterns in glioblastoma and changes during radiotherapy: a clinical microdialysis study. J Proteome Res. 2010; 9:2909–2919. [PubMed: 20302353]
35. Tabatabaei P, Bergstrom P, Henriksson R, Bergenheim AT. Glucose metabolites, glutamate and glycerol in malignant glioma tumors during radiotherapy. J Neurooncol. 2008; 90:35–39. [PubMed: 18563297]
36. Moren L, Bergenheim AT, Ghasimi S, Brannstrom T, Johansson M, Antti H. Metabolomic screening of tumor tissue and serum in glioma patients reveals diagnostic and prognostic information. Metabolites. 2015; 5:502–520. [PubMed: 26389964]
37. Locasale JW, Melman T, Song S, Yang X, Swanson KD, Cantley LC. Metabolomics of human cerebrospinal fluid identifies signatures of malignant glioma. Mol Cell Proteomics. 2012; 11:M111.014688.
38. Chinnaiyan P, Kensicki E, Bloom G, Prabhu A, Sarcar B, Kahali S, et al. The metabolomic signature of malignant glioma reflect accerlated anabolic metabolism. Cancer Res. 2012; 72:5878–5888. [PubMed: 23026133]
39. Ross B, Lin A, Harris K, Bhattacharya P, Schweinsburg B. Clinical experience with 13C MRS in vivo. NMR Biomed. 2003; 16:358–369. [PubMed: 14679500]
40. Maher EA, Marin-Valencia I, Bachoo RM, Mashimo T, Raisanen J, Hatanpaa KJ, et al. Metabolism of [U-13C] glucose in human brain tumors in vivo. NMR Biomed. 2012; 25:1234–1244. [PubMed: 22419606]
41. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hannson L, Lerche MH, et al. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. Proc Natl Acad Sci U S A. 2003; 100:10158–10163. [PubMed: 12930897]
42. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, et al. Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research. Neoplasia. 2011; 13:81–97. [PubMed: 21403835]
43. Bhattacharya P, Chekmenev EY, Perman WH, Harris KC, Lin AP, Norton VA, et al. Towards hyperpolarized 13C-succinate imaging of brain cancer. J Magn Reson. 2009; 186:150–155.

44. Chaumeil MM, Larson PE, Yoshihara HA, Danforth OM, Vigneron DB, Nelson SJ, et al. Non-invasive in vivo assessment of IDH1 mutational status in glioma. Nat Commun. 2013; 4:2429. [PubMed: 24019001]
45. Chaumeil MM, Larson PE, Woods SM, Cai L, Eriksson P, Robinson AE, et al. Hyperpolarized [1-13C] glutamate: a metabolic imaging biomarker of IDH1 mutational status in glioma. Cancer Res. 2014; 74:4247–4257. [PubMed: 24876103]
46. Park I, Larson PEZ, Zierhut ML, Hu S, Bok R, Ozawa T, et al. Hyperpolarized 13C magnetic resonance metabolic imaging: application to brain tumors. Neuro Oncol. 2010; 12:133–144. [PubMed: 20150380]
47. Park I, Hu S, Bok R, Ozawa T, Ito M, Mukherjee J, et al. Evaluation of heterogeneous metabolic profile in an orthotopic human glioblastoma xenograft model using compressed sensing hyperpolarized 3D 13C magnetic resonance spectroscopic imaging. Magn Reson Med. 2013; 70:33–39. [PubMed: 22851374]
48. Park I, Larson PE, Tropp JL, Carvajal L, Reed G, Bok R, et al. Dynamic hyperpolarized carbon-13 MR metabolic imaging of nonhuman primate brain. Magn Reson Med. 2014; 71:19–25. [PubMed: 24346964]
49. Nelson SJ, Kurhanewicz J, Vigneron DB, Larson PEZ, Harzstark AL, Ferrone M, et al. Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [1-13C]Pyruvate. Sci Transl Med. 2014; 5:198ra108.
50. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009; 360:765–773. [PubMed: 19228619]
51. Choi C, Ganji SK, DeBerardinis RJ, Hatanpaa KJ, Rakheja D, Kovacs Z, et al. 2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated patients with gliomas. Nat Med. 2012; 18:624–629. [PubMed: 22281806]
52. Zielke HR, Zielke CL, Baab PJ. Direct measurement of oxidative metabolism in the living brain by microdialysis: a review. J Neurochem. 2009; 109(Suppl 1):24–29. [PubMed: 19393005]
53. Waniewski RA, Martin DL. Preferential utilization of acetate by astrocytes is attributable to transport. J Neurosci. 1998; 18:5225–5233. [PubMed: 9651205]
54. Wyss MT, Magistretti PJ, Buck A, Weber B. Labeled acetate as a marker of astrocytic metabolism. J Cereb Blood Flow Metab. 2011; 31:1668–1674. [PubMed: 21654698]
55. Mashimo T, Pichumani K, Vemireddy V, Hatanpaa KJ, Singh DK, Sirasanagandla S, et al. Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. Cell. 2014; 159:1603–1614. [PubMed: 25525878]
56. Deelchand DK, Shestov AA, Koski DM, Ugerbil K, Henry PG. Acetate transport and utilization in the rat brain. J Neurochem. 2009; 109:46–54. [PubMed: 19393008]
57. Bluml S, Moreno-Torres A, Shic F, Nguy CH, Ross BD. Tricarboxylic acid cycle of glia in the in vivo human brain. NMR Biomed. 2002; 15:1–5. [PubMed: 11840547]
58. Sailasuta N, Harris K, Tran T, Ross B. Minimally invasive biomarker confirms glial activation present in Alzheimer's disease: a preliminary study. Neuropsychiatr Dis Treat. 2011; 7:495–499. [PubMed: 21931491]
59. Sailasuta N, Tran TT, Harris KC, Ross BD. Swift Acetate Glial Assay (SAGA): an accelerated human 13C MRS brain exam for clinical diagnostic use. J Magn Reson. 2010; 207:352–355. [PubMed: 20934362]
60. Comerford SA, Huang Z, Du X, Wang Y, Cai L, Witkiewski AK, et al. Acetate dependence of tumors. Cell. 2014; 159:1591–1602. [PubMed: 25525877]
61. Lyssiotis CA, Cantley LC. Acetate fuels the cancer engine. Cell. 2014; 159(7):1492–1494. [PubMed: 25525870]
62. Brindle K. Watching tumours gasp and die with MRI: the promise of hyperpolarised 13C MR spectroscopic imaging. Br J Radiol. 2012; 85:697–708. [PubMed: 22496072]
63. Harada M, Kubo H, Abe T, Maezawa H, Otsuka H, et al. Selection of endogenous 13C substrates for observation of intracellular metabolism using the dynamic nuclear polarization technique. Jpn J Radiol. 2010; 28:173–179. [PubMed: 20182855]
64. Bastiaansen JA, Cheng T, Mishkovsky M, Duarte JMN, Comment A, Gruetter R. In vivo enzymatic activity of acetylCoA synthetase in skeletal muscle revealed by (13)C turnover from

hyperpolarized [1-(13)C]acetate to [1-(13)C]acetylcarnitine. Biochim Biophys Acta. 2013; 1830:4171–4178. [PubMed: 23545238]

65. Flori A, Liserani M, Bowen S, Ardenkjaer-Larsen JH, Menichetti L. Dissolution dynamic nuclear polarization of non-self-glassing agents: spectroscopy and relaxation of hyperpolarized [1-(13)c]acetate. J Phys Chem A. 2015; 119:1885–1893. [PubMed: 25686013]
66. Vuichoud B, Milani J, Bornet A, Melzi R, Jannin S, Bodenhausen G. Hyperpolarization of deuterated metabolites via remote cross-polarization and dissolution dynamic nuclear polarization. J Phys Chem B. 2014; 118:1411–1415. [PubMed: 24397585]
67. Koellisch U, Laustsen C, Norlinger TS, Ostergaard JA, Flyvbjerg A, Gringeri CV, et al. Investigation of metabolic changes in STZ-induced diabetic rats with hyperpolarized [1-13C]acetate. Physiol Rep. 2015; 3:1–9.
68. Lee Y, Zacharias NM, Piwnica-Worms D, Bhattacharya PK. Chemical Reaction-Induced Multi-molecular Polarization (CRIMP). Chem Commun (Camb). 2014; 50(86):13030–13033. [PubMed: 25224323]
69. Bhattacharya P, Chekmenev EY, Perman WH, Harris KC, Lin AP, Norton VA. Towards hyperpolarized (13)C-succinate imaging of brain cancer. J Magn Reson. 2007; 186:150–155. [PubMed: 17303454]
70. Ball DR, Rowlands B, Dodd MS, Le Page L, Ball V, Carr CA, et al. Hyperpolarized butyrate: a metabolic probe of short chain fatty acid metabolism in the heart. Magn Reson Med. 2014; 71:1663–1669. [PubMed: 23798473]
71. Ross BD, Bhattacharya P, Wagner S, Tran T, Sailasuta N. Hyperpolarized MR imaging: neurologic applications of hyperpolarized metabolism. AJNR Am J Neuroradiol. 2010; 31:24–33. [PubMed: 19875468]
72. Mishkovsky M, Cheng T, Comment A, Gruetter R. Localized in vivo hyperpolarization transfer sequences. Magn Reson Med. 2012; 68:349–352. [PubMed: 22190079]
73. Park J, Josan S, Jang T, Merchant M, Yen Y, Hurd R, et al. Metabolite kinetics in C6 rat glioma model using magnetic resonance spectroscopic imaging of hyperpolarized [1- 13 C]pyruvate. Magn Reson Med. 2012; 68:1886–1893. [PubMed: 22334279]
74. Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris HD, et al. Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized [1-13C]pyruvate and 13C magnetic resonance spectroscopic imaging. Magn Reson Med. 2011; 65:557–563. [PubMed: 21264939]
75. Park I, Bok R, Ozawa T, Phillips JJ, James CD, Vigneron DB, et al. Detection of early response to temozolomide treatment in brain tumors using hyperpolarized 13C MR metabolic imaging. J Magn Reson Imaging. 2011; 33:1284–1290. [PubMed: 21590996]
76. Park I, Mukherjee J, Ito M, Chaumeil MM, Jalbert LE, Gaensler K, et al. Changes in Pyruvate Metabolism Detected by Magnetic Resonance Imaging Are Linked to DNA Damage and Serve as a Sensor of Temozolomide Response in Glioblastoma Cells. Cancer Res. 2014; 74:7115–7124. [PubMed: 25320009]
77. Chaumeil MM, Ozawa T, Park I, Scott K, James CD, Nelson SJ, et al. Hyperpolarized 13C MR spectroscopic imaging can be used to monitor Everolimus treatment in vivo in an orthotopic rodent model of glioblastoma. NeuroImage. 2012; 59:193–201. [PubMed: 21807103]
78. Ronen SM, Izquierdo-Garcia JL, Chaumeil MM, Cai LM, Phillips JJ, Pieper RO. Metabolic Imaging Biomarkers For Mutant Idh1 Gliomas. Neuro Oncol. 2014; 16(Suppl 3):Iii12.
79. Venkatesh HS, Chaumeil MM, Ward CS, Haas-Kogan DA, James CD, Ronen SM. Reduced phosphocholine and hyperpolarized lactate provide magnetic resonance biomarkers of PI3K/Akt/mTOR inhibition in glioblastoma. Neuro-Oncol. 2011; 14:315–325. [PubMed: 22156546]
80. Park JM, Recht LD, Josan S, Merchant M, Jang T, Yen Y, et al. Metabolic response of glioma to dichloroacetate measured in vivo by hyperpolarized 13C magnetic resonance spectroscopic imaging. Neuro Oncol. 2013; 15:433–441. [PubMed: 23328814]
81. Huang RY, Neagu MR, Reardon DA, Wen PY. Pitfalls in the neuroimaging of glioblastoma in the era of antiangiogenic and immuno/targeted therapy- detecting illusive disease, defining response. Front Neurol. 2015; 6:33. [PubMed: 25755649]

82. Zakszewski E, Schmit B, Kurpad S, Budde MD. Diffusion imaging in the rat cervical spinal cord. J Vis Exp. 2015; 98:52390.
83. Budde MD, Shah A, McCrea M, Cullinan WE, Pintar FA, Stemper BD. Primary blast traumatic brain injury in the rat: relating diffusion tensor imaging and behavior. Front Neurol. 2013; 4:154. [PubMed: 24133481]
84. Serres S, O'Brien ER, Sibson NR. Imaging angiogenesis, inflammation, and metastasis in the tumor microenvironment with magnetic resonance imaging. Adv Exp Med Biol. 2014; 772:263–283. [PubMed: 24272363]
85. Rodriguez E, Nilges M, Weissleder R, Chen JW. Activatable magnetic Resonance imaging agents for myeloperoxidase sensing: mechanism of activation, stability, and toxicity. J Am Chem Soc. 2010; 132:168–177. [PubMed: 19968300]
86. Chen JW, Breckwoldt MO, Aikawa E, Chiang G, Weissleder R. Myeloperoxidase targeted imaging of active inflammatory lesions in murine experimental autoimmune encephalomyelitis. Brain. 2014; 131:1123–1133.
87. Stadler KL, Ober CP, Feeney DA, Jessen CR. Multivoxel proton magnetic Resonance spectroscopy of inflammatory and neoplastic lesions of the canine brain at 3.0 T. Am J Vet Res. 2014; 75:982–989. [PubMed: 25350088]
88. Van Zijl PC, Yadav NN. Chemical exchange saturation transfer (CEST): what is in a name and what isn't? Magn Reson Med. 2011; 65:927–948. [PubMed: 21337419]
89. Kim J, Wu Y, Guo Y, Zheng H, Sun PZ. A review of optimization and quantification techniques for chemical exchange saturation transfer MRI toward sensitive in vivo imaging. Contrast Media Mol Imaging. 2015; 10:163–178. [PubMed: 25641791]
90. Sontheimer H. A role for glutamate in growth and invasion of primary brain tumors. J Neurochem. 2008; 105:287–295. [PubMed: 18284616]
91. de Groot J, Sontheimer H. Glutamate and the biology of gliomas. Glia. 2011; 59:1181–1189. [PubMed: 21192095]
92. Cai K, Haris M, Singh A, Kogan F, Greenberg JH, Hariharan H, et al. Magnetic resonance imaging of glutamate. Nat Med. 2012; 18:302–306. [PubMed: 22270722]
93. Crescenzi R, DeBrosse C, Nanga RP, Reddy S, Haris M, Hariharan H, et al. In vivo measurement of glutamate loss is associated with synapse loss in a mouse model of tauopathy. NeuroImage. 2014; 101:185–192. [PubMed: 25003815]
94. Walker-Samuel S, Ramasawmy R, Torrealdea F, Rega M, Rajkumar V, Johnson SP, et al. In vivo imaging of glucose uptake and metabolism in tumors. Nat Med. 19:1067–1072.
95. Hattingen E, Raab P, Franz K, Lanfermann H, Setzer M, Gerlach R, et al. Prognostic value of choline and creatine in WHO grade II gliomas. Neuroradiology. 2008; 50:759–767. [PubMed: 18523762]
96. Usenius JP, Vainio P, Hernesniemi J, Kauppinen RA. Choline-containing compounds in human astrocytomas studied by 1H NMR spectroscopy in vivo and in vitro. J Neurochem. 1994; 63:1538–1543. [PubMed: 7931308]
97. Chang L, McBride D, Miller BL, Cornford M, Booth RA, Buchthal SD, et al. Localized in vivo 1H magnetic resonance spectroscopy and in vitro analyses of heterogeneous brain tumors. J Neuroimaging. 1995; 5:157–163. [PubMed: 7626823]
98. Haris M, Singh A, Cai K, Kogan F, McGarvey J, DeBrosse C. A technique for in vivo mapping of myocardial creatine kinase metabolism. Nat Med. 2014; 20:209–214. [PubMed: 24412924]
99. Cai K, Singh A, Poptani H, Li W, Yang S, Lu Y, et al. CEST signal at 2 ppm (CEST@2ppm) from Z-spectral fitting correlates with creatine distribution in brain tumor. NMR in Biomedicine. 2015; 28:1–8. [PubMed: 25295758]
100. Zhou J, Lai B, Wilson DA, Laterra J, van Ziji PC. Amide proton transfer (APT) contrast for imaging of brain tumors. Magn Reson Med. 2003; 50:1120–1126. [PubMed: 14648559]
101. Gillies R, et al. pH imaging. A review of pH measurement methods and applications in cancers. IEEE Eng Med Bio Mag. 2004; 23:57–64.
102. Sagiyama K, et al. In vivo chemical exchange saturation transfer imaging allows early detection of a therapeutic response in glioblastoma. Proc Natl Acad Sci U S A. 2014; 111:4542–4547. [PubMed: 24616497]

103. Ling W, et al. Chemical exchange saturation transfer by intermolecular double-quantum coherence. J Mag Res. 2008; 194:29–32.
104. Yan G, et al. Changes in Extracellular and Intracellular Ph in Ischemic Rabbit Papillary-Muscle. Cir Res. 1992; 71:460–470.
105. Meser A, et al. A nano-sized PARACEST-fluorescence imaging contrast agent facilitates and validates in vivoCEST MRI detection of glioma. Nanomedicine. 2012; 7:1827–1837. [PubMed: 22891866]
106. Li L, et al. Superparamagnetic iron oxide nanoparticles as MRI contrast agents for non-invasive stem cell labeling and tracking. Theranostics. 2013; 3:595–615. [PubMed: 23946825]
107. Chen CCV, et al. Simple SPION incubation as an efficient intracellular labeling method for tracking neural progenitor cells using MRI. PLoS ONE. 2013; 8:e56125. [PubMed: 23468856]
108. Shevtsov MA, et al. Superparamagnetic iron oxide nanoparticles conjugated with epidermal growth factor (SPION-EGF) for targeting brain tumors. Intl J Nanomed. 2014; 9:273–287.
109. Shevtsov MA, et al. Ionizing radiation improves glioma-specific targeting of superparamagnetic iron oxide nanoparticles conjugated with cmHsp70.1 monoclonal antibodies (SPION–cmHsp70.1). Nanoscale. 2015; doi: 10.1039/C5NR06521F
110. Goodson BM. Nuclear Magnetic Resonance of Laser-Polarized Noble Gases in Molecules, Materials, and Organisms. J Mag Res. 2002; 155:157–216.
111. Albert MS, Gates GD, Driehuys B, Happer W, Saam B, Springer CS Jr, et al. Biological Magnetic Resonance Imaging Using Laser-Polarized ^{129}Xe. Nature. 1994; 370:199–201. [PubMed: 8028666]
112. Mugler JP III, Altes TA, Ruset IC, Dregely IM, Mata JF, Miller GW, Ketel, et al. Simultaneous Magnetic Resonance Imaging of Ventilation Distribution and Gas Uptake in the Human Lung Using Hyperpolarized Xenon-129. Proc Natl Acad Sci U S A. 2010; 107:21707–21712. [PubMed: 21098267]
113. Franks NP, Dickinson R, de Sousa SLM, Hall AC, Lieb WR. How Does Xenon Produce Anaesthesia? Nature. 1998; 396:324. [PubMed: 9845069]
114. Drayer BP, Wolfson SK, Reinmuth OM, Dujovny M, Boehnke M, Cook EE. Xenon Enhanced CT for Analysis of Cerebral Integrity, Perfusion, and Blood Flow. Stroke. 1978; 9(2):123–130. [PubMed: 417427]
115. Cherubini A, Bifone A. Hyperpolarized Xenon in Biology. Prog Nuc Mag Res Spec. 2003; 42:1–30.
116. PeitraiB T, Gaede HC. Optically Polarized ^{129}Xe in NMR Spectroscopy. Adv Mater. 1995; 7:826–838.
117. Spence MM, Rubin SM, Dimitrov IE, Ruiz EJ, Wemmer DE, Pines A, et al. Functionalized Xenon as a Biosensor. Proc Natl Acad Sci U S A. 2001; 98:10654–10657. [PubMed: 11535830]
118. Kilian W, Seifert F, Rinneberg H. Dynamic NMR Spectroscopy of Hyperpolarized ^{129}Xe in Human Brain Analyzed by an Uptake Model. Mag Res Med. 2004; 51:843–847.
119. Wolber J, Cherubini A, Dzik-Jurasz ASK, Leach MO, Bifone A. Spin-Lattice Relaxation of Laser-Polarized Xenon in Human Blood. Proc Natl Acad Sci U S A. 1999; 96:3664–3669. [PubMed: 10097094]
120. Walker TG, Happer W. Spin-Exchange Optical Pumping of Noble-Gas Nuclei. Rev Mod Phys. 1997; 69:629–642.
121. Ruset IC, Ketel S, Hersman FW. Optical Pumping System Design for Large Production of Hyperpolarized ^{129}Xe. Phys Rev Lett. 2006; 96:053002. [PubMed: 16486926]
122. Nikolaou P, Coffey AM, Walkup LL, Gust BM, Whiting N, Newton H, et al. Near-Unity Nuclear Polarization with an Open-Source ^{129}Xe Hyperpolarizer for NMR and MRI. Proc Natl Acad Sci U S A. 2013; 110:14150–14155. [PubMed: 23946420]
123. Lavini C, Payne GS, Leach MO, Bifone A. Intravenous Delivery of Hyperpolarized ^{129}Xe: A Compartmental Model. NMR in Biomed. 2000; 13:238–244.
124. Mugler JP III, Driehuys B, Brookeman JR, Cates GD, Berr SS, Bryant RC, et al. MR Imaging and Spectroscopy Using Hyperpolarized ^{129}Xe Gas: Preliminary Human Results. Mag Res Med. 1997; 37:809–815.

125. Anger BC, Schrank G, Schoeck A, Butler KA, Solum MS, Pugmire RJ, et al. Gas-Phase Spin Relaxation of ^{129}Xe. Phys Rev A. 2008; 78:043406.

126. Swanson SD, Rosen MS, Agranoff BW, Coulter KP, Welsh RC, Chupp TE. Brain MRI with Laser-Polarized ^{129}Xe. Mag Res Med. 1997; 38:695–698.

127. Mazzanti M, Walvick RP, Zhou X, Sun Y, Shah N, Mansour J, et al. Distribution of Hyperpolarized Xenon in the Brain Following Sensory Stimulation: Preliminary MRI Findings. PLoS One. 2011; 6:e21607. [PubMed: 21789173]

128. Zhou X, Sun Y, Mazzanti M, Henninger N, Mansour J, Fisher M, et al. MRI of Stroke Using Hyperpolarized ^{129}Xe. NMR in Biomed. 2010; 24:170–175.

129. Witte C, Martos V, Rose HM, Reinke S, Kippel S, Schroder L. Live-Cell MRI with Xenon Hyper-CEST Biosensors Targeted to Metabolically-Labeled Cell-Surface Glycans. Angew Chem Inter. 2015; 54:2806–2810.

130. Schnurr M, Sydow K, Rose HM, Dathe M, Schroder L. Brain Endothelial Cell Targeting Via a Peptide-Functionalized Liposomal Carrier for Xenon Hyper-CEST MRI. Adv Healthcare Mater. 2015; 4:40–45.

131. Harel E, Schroder L, Xu S. Novel Detection Schemes of Nuclear Magnetic Resonance and Magnetic Resonance Imaging: Applications from Analytical Chemistry to Molecular Sensors. An Rev Anal Chem. 2008; 1:133–163.

Key Points

- Many of existing and emerging techniques of interrogating metabolism in brain cancer are at an early stage of development. and clinical trials.
- Clinical trials that employ these techniques are in progress in brain cancer patients to establish the clinical efficacy of these techniques.
- It is likely that *in vivo* metabolomics and metabolic imaging is the next frontier in brain cancer diagnosis and assaying therapeutic efficacy; with the combined knowledge of genomics and proteomics a complete understanding of tumorigenesis in brain might be achieved.