MINI-SYMPOSIUM: CANCER METABOLISM IN BRAIN TUMORS

Magnetic Resonance (MR) Metabolic Imaging in Glioma

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Abstract

This review is focused on describing the use of magnetic resonance (MR) spectroscopy for metabolic imaging of brain tumors. We will first review the MR metabolic imaging findings generated from preclinical models, focusing primarily on *in vivo* studies, and will then describe the use of metabolic imaging in the clinical setting. We will address relatively well-established ^{1}H MRS approaches, as well as ^{31}P MRS, ^{13}C MRS and emerging hyperpolarized ^{13}C MRS methodologies, and will describe the use of metabolic imaging for understanding the basic biology of glioma as well as for improving the characterization and monitoring of brain tumors in the clinic.

INTRODUCTION

Gliomas represent approximately 40% of all diagnosed primary central nervous system tumors (cbtrus.org). They are comprised of glioblastomas (GBMs), oligodendrogliomas and astrocytomas. GBMs are classified as histologically grade IV, are the most common and aggressive type of glioma, and standard of care is maximal safe resection and combination radiation and temozolomide (TMZ) treatment (88). Oligodendrogliomas and astrocytomas can present as histologically low grade (grade II) or higher grade (grade III). They are typically surgically excised, but subsequent treatment depends on tumor type, grade and a variety of other factors (53, 64). However, all brain tumors are highly infiltrative and will almost always recur. In addition, lower grade tumors will usually transform into higher-grade (grade III and grade IV) lesions. In this context, noninvasive imaging methods play an essential role not only in the initial detection of tumors but also in the evaluation of invasive tumor margins, monitoring of recurrence, assessment of grade and determination of response to therapy.

Computed tomography (CT) (63) and positron emission tomography (PET) (46) have been used to some extent in neuro-oncology imaging. However, magnetic resonance imaging (MRI) remains by far the most commonly used imaging modality in the clinic (139). Imaging sequences include T2-weighted, T1-weighted pre- and post-injection of gadolinium-based contrast agents, fluid-attenuated inversion recovery (FLAIR), dynamic susceptibility contrast-enhanced perfusion and diffusion-weighted imaging. These methods provide anatomic, structural and functional information about the lesion, its microvasculature and surrounding parenchyma, and as such, can be used for diagnosis and grading of gliomas (139). However, several challenges remain, including delineation of the invading tumor margins, prediction of outcome, monitoring of drug delivery and early response to therapy, distinction between true tumor progression and pseudoprogression, detection of genetic events such as the IDH mutation, etc. (97).

A way of enhancing the MRI information and addressing some of these challenges is to also acquire magnetic resonance spectroscopy (MRS) data. MRS can non-invasively measure both steady-state metabolite levels and metabolic fluxes. This provides metabolic information that co-localizes with, and complements, the anatomic and functional information generated by MRI (97, 109). Here, we will first review the MR metabolic imaging findings generated from preclinical models, focusing primarily on *in vivo* studies, and will then describe the use of metabolic imaging in the clinical setting. We will address relatively well-established ^{1}H MRS approaches, as well as ^{31}P MRS, ^{13}C MRS and emerging hyperpolarized ^{13}C MRS methodologies, and will describe the use of metabolic imaging for understanding the basic biology of glioma as well as for improving the characterization and monitoring of brain tumors in the clinic.

METABOLIC IMAGING: AN OVERVIEW

Although MRI methods are based on probing the properties of water, the most abundant molecule in living tissues (~40M), MRS

methods assess the levels of a range of metabolites, provided that they contain an MR-detectable nucleus and that their tissue concentration is at least 0.1–1 mM (50). In the field of neuro-oncology, the most commonly used method is ^{1}H MRS, but ^{31}P and ^{13}C MRS have also been shown to provide a wealth of metabolically interesting information.

In vivo MR spectra can be acquired from a single localized volume of interest (VOI) or voxel (single-voxel MRS), or from multiple voxels (MR spectroscopic imaging or MRSI), with the goal of single-voxel MRS being to inform on a single region, whereas MRSI is capable of probing a wider area of adjacent voxels simultaneously and thus can depict regions of heterogeneous tumor, surrounding edema and normal brain parenchyma. From a methodological standpoint, these different approaches require slightly different hardware, sequences and post-processing, and we refer the reader to previous recent reviews (50, 62, 77, 112, 126, 159). Additionally, it is also possible to investigate the global metabolic profile of live cells or cell extracts from established glioma models using standard nuclear magnetic resonance (NMR) methods, and tumor biopsies obtained from animal models or patients can be investigated using high-resolution magic angle spinning spectroscopy (HRMAS) (33, 41, 66, 67, 151). Such approaches are valuable in providing complementary data to the *in vivo* findings.

Metabolic imaging using ^{1}H MRS

The proton nucleus is the most sensitive for MRS because of its high natural abundance (>99.9%) and its high gyromagnetic ratio. As virtually all biologically relevant molecules contain protons, ^{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 (31). In the field of neuro-oncology, the most prevalent metabolites in the ^{1}H spectrum are N-acetylasparate (NAA), total-choline-containing metabolites (Cho), lactate (Lac), mobile lipids (Lip), creatine (Cre), glutamate (Glu), glutamine (Gln; the glutamate and glutamine signals cannot always be resolved, and studies will then refer to their composite Glx peak), *myo*-inositol (mIns), glycine (Gly), glutathione (GSH) and 2-hydroxyglutarate (2-HG).

NAA is the largest signal in normal healthy brain. It is synthesized in neurons and is considered a neuronal marker. It is thus an indicator of neuronal health, function and density, and NAA levels are typically decreased in gliomas (79, 159).

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 (51).

Lactate is the end product of aerobic glycolysis. It is rarely observed in the normal brain but its production is enhanced in cancer as part of the Warburg effect (150).

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 (161).

The Cre signal is a composite of creatine and phosphocreatine (PCr), which are involved in energy metabolism via the creatine kinase reaction that produces adenosine-triphosphate (ATP) from adenosine-diphosphate (ADP). Cre levels vary within normal brain regions and in some cases with tumorigenesis (36, 68, 135).

The amino acid Glu is the most abundant amino acid in the brain and an essential neurotransmitter. It has been found to play a key role in glioma pathogenesis through multiple mechanisms, including antioxidant synthesis, seizure induction and tumor cell invasion into the parenchyma (32). Gln plays a critical role in a variety of biochemical functions, including protein synthesis and nitrogen homeostasis. In gliomas, glutaminolysis is often required for tumor growth as an anaplerotic source of carbon complementary to glucose metabolism (33, 132).

MIns is part of the inositol triphosphate second messenger system, and glial cells have been shown to contain significantly higher levels of mIns than neurons (10).

Gly is a small non-essential amino acid that can play multiple roles, especially as an inhibitory neurotransmitter as well as a building block in protein synthesis. In glioma, gly levels are often elevated and could be linked to increased survival under hypoxic conditions (73).

GSH is an antioxidant that prevents damage from reactive oxygen species and whose metabolism was found to play an important role in tumor proliferation, invasion and resistance to therapy in many tumors *ex vivo* (6, 44, 114, 124).

Finally, with the recent discovery of the isocitrate dehydrogenase (IDH) mutation, the most common mutation in oligodendroglioma and astrocytoma tumors (29, 122, 155), elevated levels of 2-HG, which is producted 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 overlaps 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 (31). Additionally, detection of 2-HG *in vivo* has required specific methodological approaches (25, 80, 125).

Metabolic imaging using ^{31}P MRS

The ^{31}P nucleus is 100% naturally abundant, but ^{31}P MRS is intrinsically less sensitive than ^{1}H MRS (~7% of ^{1}H MRS), requiring longer acquisition times and/or larger voxels. Nonetheless, it provides valuable biological information with regard to ^{31}P-containing metabolites that are involved in energy and phospholipid metabolism. In the field of neuro-oncology, the most prevalent metabolites studied using ^{31}P MRS are PCr, ATP, PC, phosphoethanolamine (PE), GPC and glycerophosphoethanolamine (GPE) (31). As mentioned earlier, PCr serves as a cellular energy reserve for rapid replenishment of cellular ATP levels. PC and PE are the precursors of phosphatidylcholine and phosphatidylethanolamine, respectively, whereas GPC and GPE are the respective breakdown products.

Metabolic imaging using ^{13}C MRS

In contrast to ^{1}H and ^{31}P MRS, which directly detect the steady-state levels of endogenous metabolites, the sensitivity of ^{13}C MRS is intrinsically limited by the low natural abundance of the ^{13}C nucleus (1.1%) and the low gyromagnetic ratio of [13]C. As a result, ^{13}C MRS is used in combination with the exogenous infusion of a ^{13}C-labeled substrate of interest (104). By enriching the ^{13}C pool of a chosen metabolite, it is then possible to specifically monitor its flux via different metabolic pathways, with little or no background signal. The most commonly used ^{13}C-labeled substrate for ^{13}C MRS is glucose (91), but other metabolites such lactate (137) or glycine (145) have also been investigated in preclinical models of glioma. Nonetheless, the relative sensitivity of ^{13}C MRS remains relatively poor (~2% of ^{1}H MRS), requiring relatively long acquisition times and/or voxel sizes (152). The recent development of dissolution dynamic nuclear polarization (DNP) has significantly transformed the field of ^{13}C MRS, resulting in a >10 000-fold signal enhancement of hyperpolarized (HP) metabolites that enables the detection of metabolism for HP precursors within seconds. To date, the most commonly investigated compound is HP [1-^{13}C] pyruvate, but other compounds are being investigated as well (11, 17, 76).

PRECLINICAL STUDIES

Metabolic imaging for characterization of glioma

Steady-state metabolite levels by ^{1}H and ^{31}P MRS

Preclinical models of lower-grade gliomas are rare, and most established GBM models generate well-circumscribed tumors. Furthermore, a recent ^{1}H MRS study comparing seven different GBM lines from rat, mouse and human showed that the metabolic profiles could be variable among glioma models and pointed to the need for careful selection of appropriate models (38). Such issues limit the number of *in vivo* preclinical studies that use metabolic imaging to characterize tumor grade, monitor tumor upgrade or assess extent of invasion. Nonetheless, a number of preclinical studies have used ^{1}H MRS to determine steady-state metabolite levels in GBM models in rodents (58, 101, 103, 128, 135, 141, 160, 161). Early studies reported a drop in NAA and Cre levels, and an increase in Cho and Gly levels in tumor compared with normal brain (58, 135). As discussed below, these findings are in line with observations in the clinical setting, with reduced NAA reflecting a reduction in the density of viable neurons and elevated Cho reflecting increased oncogenic signaling and cell proliferation characteristic of cancer. Several subsequent investigations focused on the detection of Lac as a measure of increased glycolysis in GBM models, consistent with the Warburg effect as well as with clinical findings (89, 128, 141, 160, 161). An associated acidification of the microenvironment could also be detected in GBM models using exogenous ^{1}H or ^{31}P MRS-detectable exogenous pH probes (48, 49, 128). Additionally, it was shown that elevated tumoral Lip levels are associated with cell death and increased necrosis within the tumor (161).

Recent studies have investigated genetically engineered models of mutant IDH1-expressing cells and tumors, patient-derived tumor biopsies and a handful of *in vivo* patient-derived mutant IDH1 models. Investigating engineered cells, we showed that ^{1}H MRS could detect the accumulation of 2-HG in mutant IDH1 cells, but not in wild-type cells (66). A similar study was performed *in vivo* and showed that 2-HG could be detected in engineered mutant IDH1 tumors in rodents (80). More importantly, an *ex vivo* study of human recurrent low-grade glioma biopsies also reported detection of 2-HG by ^{1}H MRS in mutant IDH1 tumors (40). In addition, 2-HG levels in patient biopsies correlated with other metabolite levels, such as Cho, but it is unclear whether these correlations reflect metabolic reprogramming or increased tumor cellularity (40). In genetically engineered models, we found that the presence of the IDH1 mutation is associated with a ^{1}H MRS-detectable drop in Glu, PC and Lac, and a trend toward increased GPC (66), consistent with metabolic reprogramming observed in other engineered models and detected using mass spectrometry (134). Elevated levels of GPC and reduced levels of PE were also observed using ^{31}P MRS in mutant IDH1 patient-derived rodent tumors when compared to wild-type IDH1 tumors, a finding that was further confirmed by *ex vivo* studies of human tumor biopsies (42). In another *in vivo* study, we probed the ^{1}H MRS profile of a mutant IDH1 patient-derived oligoastrocytoma model and observed low levels of Lac, consistent with reports from the clinic and in line with the recent discovery that LDH-A is silenced in mutant IDH1 tumors (21, 23). We also used MRS to understand the underlying reasons for a drop in Glu levels. We found that it was associated with a drop in pyruvate dehydrogenase flux (65, 66) and identified PDH as a possible therapeutic target for mutant IDH1 tumors.

Metabolism using ^{13}C MRS

Early ^{13}C MRS investigations were used to monitor glucose metabolism and Lac turnover during steady-state hyperglycemia with [1-^{13}C] glucose in C6 glioma-bearing rats (9, 138, 144). 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. Furthermore, Gln was the source of anaplerotic oxaloacetate production, whereas Gln derived-nitrogen was secreted from the cell via alanine and ammonia, collectively pointing to the essential role of Gln in enabling GBM cells to use glucose-derived carbon and TCA cycle intermediates as biosynthetic precursors (33). 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 (94).

Finally, 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 (95). 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.

Metabolism using hyperpolarized ^{13}C MRS

As mentioned earlier, the recent development of dissolution DNP has lead to a >10 000-fold signal enhancement for HP ^{13}C-labeled compounds (4), enabling the acquisition of high-resolution (~5 mm) ^{13}C MRSI metabolic data within a very short time frame (~10 s). Because HP ^{13}C MRS can rapidly detect Lac production in real-time by measuring the conversion of HP [1-^{13}C] pyruvate to HP [1-^{13}C] Lac, it is of particular interest for monitoring the Warburg effect in cancer. As a result, HP [1-^{13}C] pyruvate has been the most extensively used HP probe to date.

In the context of glioma, multiple preclinical studies using HP ^{13}C MRS have been successfully conducted. The first preclinical study reporting the use of HP ^{13}C MRS in gliomas *in vivo* demonstrated that, following injection of HP [1-^{13}C] pyruvate, high levels of HP [1-^{13}C] Lac could be detected in glial tumors in rats, whereas little Lac could be detected in normal contralateral brain (120). Furthermore, Lac levels varied among different GBM models, in line with the pathophysiological characteristics of the studied tumors; lower levels of lactate were generated in the less hypoxic U87MG tumor compared with the more hypoxic U251MG model. This pointed to the value of imaging HP lactate production not only as a method for detection of tumor but also as a possible approach for tumor characterization.

Other studies that have reported the use of HP ^{13}C MRS for improving the diagnosis and characterization of tumors have focused on mutant IDH1 glioma models. We have developed a new HP probe, [1-^{13}C] α-ketoglutarate, and have shown that it can be used to non-invasively detect the production of HP [1-^{13}C] 2-HG in engineered mutant IDH1 glioma tumors in rats, but not in their wild-type IDH1 counterparts (19). This approach could potentially be used as a complementary method to ^{1}H MRS detection of 2-HG for identification of mutant IDH1 tumors. In a second study, modulations in HP [1-^{13}C] Glu production in the presence of the IDH1 mutation were detected in the same model following intravenous injection of HP [1-^{13}C] α-ketoglutarate (18). Here, we showed that the conversion of α-ketoglutarate to Glu was reduced in mutant IDH1 tumors when compared to their wild-type IDH1 counterparts. This effect was mediated by a drop in the expression and activity of the enzymes branched-chain amino acid transaminase 1, aspartate transaminase and glutamate dehydrogenase, identifying previously unreported metabolic consequences of the IDH1 mutation. Finally, consistent with the above-mentioned LDH-A silencing that occurs in mutant IDH1 tumors (23) and low Lac levels observed in a mutant IDH1 oligoastrocytoma model and in patients, we determined that HP Lac produced from HP [1-^{13}C] pyruvate was comparable to that observed in normal brain. This identified an additional metabolic imaging marker for mutant IDH1 tumors (21).

Metabolic imaging of response to therapy

In addition to the diagnostic value of metabolic imaging, preclinical studies show that this type of imaging also has potential for early monitoring of therapeutic response in glioma.

Steady-state metabolite levels by ^{1}H and ^{31}P MRS

Response to multiple therapeutic approaches has been evaluated in preclinical *in vivo* models of GBM using ^{1}H MRS (47, 61, 75, 96, 153). Among these, treatment with the phosphatidylinositol-3-kinase (PI3K) irreversible inhibitor, PX-886, resulted in a decrease in the MRS-detectable Cho-to-NAA ratio, which was linked to increased autophagy post-treatment (75). Following treatment with antitumor glycosides, an increase in free choline and PC was reported in C6 glioma tumors in mice, suggesting that the mode of action of glycosides involves alterations in phospholipid metabolism resulting in cell death (47). X-ray irradiation as well as treatments with the nitrones PBN and OKN007, both induced a significant decrease in the level of lipids related to the modulation of extent of necrosis (61, 96).

^{31}P MRS was used to study the response of GBM to treatment with two anti-neoplastic agents, a nitrosourea and a DNA intercalator. Both resulted in a significant depletion of high-energy phosphates as detected by ^{31}P MRS, which suggested a high dependence on energy metabolism during chemotherapy (81). A more recent ^{31}P MRS study on glioma cells showed a drop in the ratio of PC to GPC following TMZ treatment (93).

Metabolism using hyperpolarized ^{13}C MRS

To date, HP ^{13}C MRS has been used to evaluate response to three clinically relevant treatments: radiation therapy (RT), chemotherapy and PI3K-targeted therapies.

Following whole brain irradiation, levels of HP [1-^{13}C] lactate produced from HP [1-^{13}C] pyruvate were significantly decreased in C6 glial tumors in rats, potentially identifying a translational biomarker of response to RT (30).

Investigations of the effect of PI3K pathway inhibition using HP ^{13}C MRS methods demonstrated that treatment induced a drop in HP [1-^{13}C] Lac production in cells when exposed to the PI3K inhibitor, LY294002 (148, 151). A similar finding was made when investigating subcutaneous GBM tumors in mice (151) and orthotopic tumors in rats following treatment with Everolimus (20). More importantly, in the latter case, decreases in Lac could be detected before any changes in tumor volume based on MR imaging.

Three other studies have investigated the potential of HP ^{13}C MRS to monitor response to TMZ, the standard-of-care chemotherapeutic for patients with GBM. The first study reported that, following TMZ treatment, the level of HP [1-^{13}C] Lac significantly decreased as early as 2 days post-treatment in orthotopic rat tumors (117). The authors thus concluded that HP [1-^{13}C] Lac could serve as an early surrogate marker of TMZ treatment in GBM. In a follow-up study, we further confirmed this effect and demonstrated that TMZ-induced DNA damage was linked to pyruvate metabolism through pChk1 and reduced expression of pyruvate kinase M2 (PKM2), thus uncovering the biochemical mechanisms involved in reduced HP Lac production following TMZ treatment

(121). In contrast, we recently showed that, in mutant IDH1 glioma with silenced LDH-A (23), HP [1-^{13}C] Lac levels are not modulated in response to TMZ treatment, precluding the use of this metabolic imaging approach to monitor response in mutant IDH1 tumors (21).

Finally, a recent study expanded on previous findings, and investigated the potential of HP ^{13}C MRS to monitor glioma response to a new generation dual PI3K/mTOR inhibitor XL765 alone or in combination with TMZ treatment (131). This study showed that the drop in HP Lac production is a better predictor of survival than tumor size in treated GBM, highlighting the potential of this approach to improve patient management early during therapy.

CLINICAL ^{1}H MR SPECTROSCOPY

Numerous studies have highlighted the potential benefits of using ^{1}H MRS to estimate metabolite levels in brain tumors in the clinic (110). 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 (112, 118). More recent advances in pulse sequence and spectral editing schemes have facilitated the detection of metabolites with shorter T2 relaxation times and lower signal-to-noise ratios such a Glu, Gln, Glx, mIns and 2-HG, expanding the investigation of potential metabolic processes for both characterizing the spatial extent of gliomas and assessing therapeutic response.

Metabolic imaging for tumor characterization

Long-echo metabolites

The first studies to identify metabolic differences between gliomas and normal brain tissue date back to the mid-1990s using a long TE (144 ms) ^{1}H MRS acquisition (45, 108, 113, 127, 147). 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 (39, 88, 100, 116), define the spatial extent of abnormal metabolism due to tumor beyond the contrast-enhancing lesion (35, 37, 57, 123), guide the selection of biopsied tissue samples to the most aggressive part of the tumor (16, 28, 156) and differentiate among tumor grades and types (8, 16, 70, 79). 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 (99). 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 (16, 26, 40, 70, 98). However, despite the benefits of ^{1}H MRSI in improving sensitivity to metabolically active tumor and differentiating gliomas from metastatic disease (12), 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 (149).

Other metabolites of interest that can add to the specificity of CNI *in vivo* are levels of Lac and Lip. Lesions with elevated Lac and Lip have been shown to have higher-grade histology with shorter progression-free and overall survival (27, 83). Increased Lac levels have been found in both grade III and grade IV gliomas, while Lip levels are significantly elevated in patients with GBM, with volumes of abnormal Lac plus Lip also distinguishing grade (16, 82, 83, 154). In GBMs, reduced Cre and increased Lac levels were found specifically in hypoxic regions with leaky vessels and elevated Cho (90). Within the oligodendrogliomas subtype, the combination of elevated Cho, Lip and Lac was shown to distinguish grade II and grade III gliomas (70), and in low-grade gliomas specifically, Lac and Lip levels have been reported as predictors for proliferation based on KI67 analysis from biopsied samples (55). These results all support the close relationship between increased tumor growth and hypoxia.

Short-echo metabolites

mIns and Gly On clinical scanners, the mIns peaks often overlap with the peak of Gly. Elevations in the sum of mIns and Gly relative to Cre from *in vivo* MRS data at 1.5 T have been observed in low-grade astrocytomas but were shown to decrease in higher-grade lesions, coinciding with higher levels of Lac (13, 14, 86). However, in the contralateral hemisphere of GBM tumors, increased concentrations of mIns were significantly increased relative to levels observed in control subjects and tended to be higher relative to levels in patients with low-grade glioma (72). Significant differences in mIns/Cre have also been reported between astrocytomas and oligodendrogliomas preoperatively (22). The increased spectral resolution available at 7 T facilitates the quantification of the Gly peak. Although significant increases in mIns /Cre, [mIns + Gly]/Cre and Gly/Cre ratios were found within the T2-hyperintensity lesion compared with normal-appearing white matter at 7 T in all grades of glial tumors, no differences were observed among grades (84). This variability among findings in the literature highlights the need for further investigation of both the roles of mIns and Gly in brain tumors.

Glu, Gln and GSH Short echo *in vivo* MRS has been implemented to resolve spin-coupled metabolites such as Glu, Gln and GSH without the need for sophisticated editing sequences (24, 84). As Glu is the main excitatory neurotransmitter in the brain and is converted to Gln by the re-absorption by neurons or reuptake by astrocytes to avoid Glu excitotoxicity, glioma cells that secrete Glu lead to an increase in extracellular Glu (143, 157). Although Gln concentrations in the contralateral brain tissue in patients with GBM were significantly elevated compared with the levels found in both low-grade gliomas and normal brain (84), Glu and Gln levels were found to be most useful in differentiating oligodendrogliomas from astrocytomas (22, 136). The level of Gln + Glu (Glx) has been shown to be significantly higher in low-grade oligodendrogliomas than in low-grade astrocytomas, the exception being reported in cases when Lac and Lip are also present (69, 136). Finally, the ratio of GSH/Cre has been found to be significantly elevated in the T2-hyperintensity lesion compared with normal appearing white matter in gliomas of all grades (84).

IDH status and 2-HG

As the presence of the IDH mutation in a tumor can be noninvasively detected by measuring 2-HG via MRS, differentiating 2-HG from neighboring metabolites, such as gamma amino butyric acid, Gln and Glu *in vivo* has become an important area of research (3, 24, 25, 41, 43, 71, 125). After initial analysis of brain tumor specimens to demonstrate the feasibility of using MRS to quantify 2-HG for the classification of IDH mutant tumors (71), Pope *et al* demonstrated detection of 2-HG by MRS in patients with glioma prior to resection, with measurement of 2-HG concentrations and other metabolites by liquid chromatography–mass spectroscopy and analysis of IDH1 status by DNA sequencing (125). They found that 2-HG levels measured *in vivo* using water-suppressed ^{1}H-MRS correlated with the measured amounts in the resected tumor specimens in tumors with IDH1 mutations. These tumors also had elevated Cho and decreased GSH levels. Similarly, analysis of *ex vivo* tissue samples by Elkhaled *et al* also demonstrated that the levels of 2-HG correlate with Cho, Lac and GSH, as well as with histopathologic tumor grade (40). In a separate study of patients with IDH1-mutated low-grade gliomas that utilized 2D ^{1}H MRSI, maps of 2-HG showed similar spatial distribution as that of Cho (25). Although it appears that MRS can provide a noninvasive measure of 2-HG *in vivo* in human gliomas, as mentioned earlier, further studies are needed to validate the relevance of other alterations in metabolites as prognostic biomarkers, in addition to the utility of 2-HG quantification in patient management.

Metabolic imaging for predicting outcome

For patients with newly diagnosed grade IV glioma, higher levels of Lac and Lip in the region with abnormal CNI have been found to be associated with worse overall survival, even when controlling for the volume of the contrast-enhancing lesion (27), suggesting that lesions that are both highly cellular and have regions of hypoxia and necrosis are more malignant. This was found to be true for MRSI parameters and volumes of abnormal CNI obtained at both pre-surgical and pre-RT examinations (27, 78, 115, 140), and was consistent with the previous findings that used recursive partitioning to select MRSI parameters of higher Cho-to-Cre, higher Cho-to-NAA, higher Lac plus Lip and lower Cr-to-NAA abnormalities as being at high risk for poor survival (82). Using the entire spectrum together, other groups have also been able to demonstrate the prognostic value of ^{1}H MRSI for high-grade gliomas by stratifying groups based on median survival (92). Finally, using short echo MRS and multivariate analyses, ratios of Lip/Cr and mI/Cre of the solid tumor region before surgery were found to be associated with both RT response and time to progression in patients with high-grade gliomas who were subsequently treated by radiation alone after surgery (129).

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 (59, 60). With the more recent ability to detect 2-HG accumulation by ^{1}H-MRS *in vivo*,

Natsumeda *et al* found that 2-HG accumulation detected by 3 T MRS not only correlated well with IDH status, but also with survival in World Health Organization (WHO) grade II and grade III gliomas (107).

Metabolic imaging for assessing response to therapy

Pseudoprogression and distinguishing treatment effect from recurrent tumor

Pseudoprogression, or an increase in the contrast-enhancing lesion that occurs after RT without any associated worsening of clinical symptoms and disappears on subsequent scans without a change in therapy, is a concern for patient management in the clinic. Numerous studies have been performed using physiological (perfusion- and diffusion-weighted) imaging techniques to be able to distinguish pseudoprogression from true tumor progression. Although on its own, ^{1}H-MRS has had little success in being able to differentiate between the two more robustly than physiological imaging (2), when used in conjunction with physiological MRI, the discriminatory accuracy of the combined methodologies has been reported as high as 96% when Cho/NAA, Cho/Cre, apparent diffusion coefficients and cerebral blood volume are included as predictors (36, 158).

In the recurrent tumor setting, mIns and Cho have also been shown to play a role in distinguishing recurrent tumor from treatment effect. Using *ex vivo* HRMAS of tissue samples from image-guided biopsy studies at 14 T, mIns/Cho was found to differentiate tumor from treatment-induced reactive astrocytosis in both newly diagnosed and recurrent GBM, as well as separate upgraded recurrent low-grade gliomas from those that remained low-grade at the time of recurrence (40, 142). *Ex vivo* analyses of tissue from human gliomas have also shown the potential roles of other metabolites, such as Glu, Gln and GSH, in differentiating between tumor and gliosis and/or associating with malignant transformation, but these markers have yet to be investigated in the clinical setting *in vivo* (40).

Predicting response to therapy

The information provided by MRSI 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 RT and gamma knife radiosurgery (15), and to assess the response to therapy (88).

Assessing response to RT The evolving pattern of spectral changes over the course of and following radiotherapy, in particular those associated with choline-containing compounds, appears to be prognostic of tumor response and outcome. 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 (1). 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. Overall, the more MRS-derived prognostic factors a patient had, the shorter their overall survival. More recently, patients with an increase in mean or median Cho/NAA values during the third week of RT were also found to have a significantly greater chance of early progression (106), and changes in normalized Cho at 2 months post-RT have been reported as being highly prognostic for both progression-free and overall survival (130). In recurrent gliomas treated with gamma knife radiosurgery, response within the gamma knife target was observed as a reduction of Cho levels and an increase in Lac + Lip levels, typically within 6 months of treatment (52). Again, increases in Cho were correlated with poor outcome, indicating tumor recurrence that typically preceded the presence of new contrast enhancement.

Assessing response to cytotoxic and anti-angiogenic chemotherapies Although most of the literature on monitoring response to cytotoxic and anti-angiogenic chemotherapies utilizes anatomical, perfusion-weighted and diffusion-weighted MRI, there have been a few key studies demonstrating the utility of serial ^{1}H-MRS in early response assessment.

In newly diagnosed GBM being treated with radiation and concurrent chemotherapy, more three-dimensional (3D) MRSI parameters that predicted 6-month progression-free survival and overall survival were found than for parameters derived from anatomic, diffusion or perfusion imaging (85). Specifically, decreases in NAA/Cho in the region with CNI > 3 at baseline and an increase of the CNI within elevated CNI regions (>2) at 2 months after RT were associated with shorter progression-free survival and patients with higher normalized Lac + Lip at either baseline or post-RT had significantly worse overall survival. Larger volumes of abnormal CNI after RT were also associated with worse progression-free and overall survival. In patients with recurrent GBM who received anti-angiogenic therapy in the form of bevacizumab at the time of tumor recurrence, NAA/Cho was associated with a positive therapeutic response as early as 2 weeks into treatment (133). Similar increases in NAA/Cho were found as early as 1-month post-treatment initiation of cediranib, highlighting the importance of MRS-derived parameters as early markers of response in the setting where traditional anatomical measures of tumor burden are no longer valid (74).

In low-grade gliomas, significant reductions in the mean Cho signal were initially observed after 1 year of TMZ administration compared with baseline and 3-month time points that paralleled a change in tumor volume (105). However, subsequent studies in this patient population over 14 months of follow-up showed that the ^{1}H MRS profile changed more widely and rapidly than tumor volume during the initial response and subsequent progression phases, representing an early prognostic factor of outcome that may be a promising, non-invasive tool for monitoring the clinical response to TMZ (56).

Clinical metabolic imaging with other nuclei

Non-proton nuclei MR techniques are in general less prevalent in the clinical setting because of the low concentration of these metabolites and their reduced sensitivity. Initial studies using ^{31}P MRS have shown elevated PC levels and reduced pH in more aggressive tumors (54, 102), while changes in the levels of PC, PE, GPC and GPE have been associated with response to therapy (34, 87). Another approach, [23]Na imaging, has also been applied in brain tumors, with increased [23]Na signal intensity, an indicator of neuronal integrity, observed in low-grade gliomas that was significantly correlated with NAA and Glu and inversely correlated with Cho from ^{1}H MRS (7). With the advent of higher field clinical and research scanners, the ability to detect non-proton nuclei has recently become more feasible. [23]Na imaging and MR-based cerebral metabolic rate of oxygen consumption mapping (^{17}O MRS) are two examples that have come to fruition in healthy controls because of ultra-high-field systems (5) and their initial application in patients with brain tumors is similarly promising (146).

The first demonstration of the feasibility of ^{13}C MRS in a brain tumor patient was reported in 2010 on a 3 T system by Wijnen *et al* (152). In this study, the glucose-derived [3-^{13}C] Lac signal appeared after ~20 min of [1-^{13}C] glucose infusion, but only in spectra from the tumor voxel. A 50% elevation in [1-^{13}C] glucose signals compared with normal brain was also observed, likely due to blood–brain barrier breakdown, resulting in more glucose in the extracellular space. Two years later, Maher *et al* infused uniformly labeled ^{13}C glucose prior to surgical resection of high-grade gliomas in order to subsequently analyze Lac production with MR spectroscopy (91). Besides Lac, ^{13}C-labeled Gln and Gly were also detected, consistent with the *ex vivo* and preclinical findings demonstrating functional TCA flux and channeling of glucose-derived metabolites to the biosynthesis of cellular building blocks (95).

The HP ^{13}C technology has recently been successfully applied in a phase I clinical trial, which evaluated the safety and feasibility of HP [1-^{13}C] pyruvate as an agent for noninvasively characterizing alterations in tumor metabolism for patients with prostate cancer (111). This important study not only confirmed the safety of the agent but also revealed elevated lactate-to-pyruvate ratios in regions of biopsy-proven cancer, confirming the clinical value of this method. Finally, in anticipation of an upcoming clinical trial in brain tumor patients aimed at detecting the presence of tumors and their response to therapy, a preclinical study was recently performed to monitor HP pyruvate delivery into the normal brain of a non-human primate. The study confirmed that ^{13}C MRS could be used to probe HP pyruvate metabolism in the brain and sets the scene for implementing this emerging metabolic imaging methodology in glioma patients (119).

SUMMARY AND CONCLUSIONS

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 metabolic imaging 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 HP 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.

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