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Journal
Brain Pathology, 25(6)

ISSN
1015-6305

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Publication Date
2015-11-01

DOI
10.1111/bpa.12310

Peer reviewed

Brain Pathology ISSN 1015-6305

MINI-SYMPOSIUM: CANCER METABOLISM IN BRAIN TUMORS

Magnetic Resonance (MR) Metabolic Imaging in Glioma

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Keywords
brain tumors, glioma, magnetic resonance imaging, metabolic imaging.

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Received 20 August 2015 Accepted 25 August 2015

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doi:10.1111/bpa.12310

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.

REFERENCES

1. Alexander A, Murtha A, Abdulkarim B, Mehta V, Wheatley M, Murray B *et al* (2006) Prognostic significance of serial magnetic resonance spectroscopies over the course of radiation therapy for patients with malignant glioma. *Clin Invest Med* **29**: 301–311.
2. Alimenti A, Delavelle J, Lazeyras F, Yilmaz H, Dietrich PY, de Tribolet N, Lovblad KO (2007) Monovoxel 1H magnetic resonance spectroscopy in the progression of gliomas. *Eur Neurol* **58**:198–209.
3. Andronesi OC, Kim GS, Gerstner E, Batchelor T, Tzika AA, Fantin VR *et al* (2012) Detection of 2-hydroxyglutarate in IDH-mutated glioma patients by in vivo spectral-editing and 2D correlation magnetic resonance spectroscopy. *Sci Transl Med* **4**:116ra4.
4. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH *et al* (2003) Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR. *Proc Natl Acad Sci U S A* **100**:10158–10163.
5. Atkinson IC, Thulborn KR (2010) Feasibility of mapping the tissue mass corrected bioscale of cerebral metabolic rate of oxygen consumption using 17-oxygen and 23-sodium MR imaging in a human brain at 9.4 T. *Neuroimage* **51**:723–733.
6. Balendiran GK, Dabur R, Fraser D (2004) The role of glutathione in cancer. *Cell Biochem Funct* **22**:343–352.
7. Bartha R, Megyesi JF, Watling CJ (2008) Low-grade glioma: correlation of short echo time 1H-MR spectroscopy with 23Na MR imaging. *AJNR Am J Neuroradiol* **29**:464–470.
8. Bian W, Khayal IS, Lupo JM, McGue C, Vandenberg S, Lamborn KR *et al* (2009) Multiparametric characterization of grade 2 glioma subtypes using magnetic resonance spectroscopic, perfusion, and diffusion imaging. *Transl Oncol* **2**:271–280.
9. Bouzier AK, Quesson B, Valeins H, Canioni P, Merle M (1999) [1-(13)C]glucose metabolism in the tumoral and nontumoral cerebral tissue of a glioma-bearing rat. *J Neurochem* **72**:2445–2455.
10. Brand A, Richter-Landsberg C, Leibfritz D (1993) Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci* **15**:289–298.
11. Brindle KM, Bohndiek SE, Gallagher FA, Kettunen MI (2011) Tumor imaging using hyperpolarized 13C magnetic resonance spectroscopy. *Magn Reson Med* **66**:505–519.
12. Caivano R, Lotumolo A, Rabasco P, Zandolino A, D'Antuono F, Villonio A *et al* (2013) 3 Tesla magnetic resonance spectroscopy: cerebral gliomas vs. metastatic brain tumors. Our experience and review of the literature. *Int J Neurosci* **123**:537–543.
13. Candiota AP, Majos C, Julia-Sape M, Cabanas M, Acebes JJ, Moreno-Torres A *et al* (2011) Non-invasive grading of astrocytic tumours from the relative contents of myo-inositol and glycine measured by in vivo MRS. *JBR-BTR* **94**:319–329.
14. Castillo M, Smith JK, Kwock L (2000) Correlation of myo-inositol levels and grading of cerebral astrocytomas. *AJNR Am J Neuroradiol* **21**:1645–1649.
15. Chan AA, Lau A, Pirzkall A, Chang SM, Verhey LJ, Larson D *et al* (2004) Proton magnetic resonance spectroscopy imaging in the evaluation of patients undergoing gamma knife surgery for Grade IV glioma. *J Neurosurg* **101**:467–475.
16. Chang SM, Nelson S, Vandenberg S, Cha S, Prados M, Butowski N *et al* (2009) Integration of preoperative anatomic and metabolic physiologic imaging of newly diagnosed glioma. *J Neurooncol* **92**:401–415.
17. Chaumeil MM, Najac C, Ronen SM (2015) Studies of Metabolism Using (13)C MRS of Hyperpolarized Probes. *Methods Enzymol* **561**:1–71. doi: 10.1016/bs.mie.2015.04.001; [Epub 2015 Jun 19]; PMID: 26358901.
18. Chaumeil MM, Larson PE, Woods SM, Cai L, Eriksson P, Robinson AE *et al* (2014) Hyperpolarized [1–13C] glutamate: a metabolic imaging biomarker of IDH1 mutational status in glioma. *Cancer Res* **74**:4247–4257.
19. Chaumeil MM, Larson PE, Yoshihara HA, Danforth OM, Vigneron DB, Nelson SJ *et al* (2013) Non-invasive in vivo assessment of IDH1 mutational status in glioma. *Nat Commu* **4**:2429.
20. Chaumeil MM, Ozawa T, Park I, Scott K, James CD, Nelson SJ, Ronen SM (2012) Hyperpolarized 13C MR spectroscopic imaging can be used to monitor Everolimus treatment in vivo in an orthotopic rodent model of glioblastoma. *Neuroimage* **59**: 193–201.
21. Chaumeil MM, Radoul M, Eriksson P, Blough MD, Chesnelong C, Pieper RO *et al* (2015) The tumor exception that proves the rule: Hyperpolarized 13C MRS cannot be used to detect the presence of mutant IDH1 glioma or their responses to Temozolomide therapy. Proceedings of the conference of the International Societyt for Magnetic Resonance in Medicine, Abstract #1122, Toronto, ON.
22. Chawla S, Oleaga L, Wang S, Krejza J, Wolf RL, Woo JH *et al* (2010) Role of proton magnetic resonance spectroscopy in differentiating oligodendrogliomas from astrocytomas. *J Neuroimaging* **20**:3–8.
23. Chesnelong C, Chaumeil MM, Blough MD, Al-Najjar M, Stechishin OD, Chan JA *et al* (2014) Lactate dehydrogenase A silencing in IDH mutant gliomas. *Neuro-Oncol* **16**:686–695.
24. Choi C, Ganji S, Hulsey K, Madan A, Kovacs Z, Dimitrov I *et al* (2013) A comparative study of short- and long-TE (1)H MRS at 3 T for in vivo detection of 2-hydroxyglutarate in brain tumors. *NMR Biomed* **26**:1242–1250.
25. Choi C, Ganji SK, DeBerardinis RJ, Hatanpaa KJ, Rakheja D, Kovacs Z *et al* (2012) 2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated patients with gliomas. *Nat Med* **18**:624–629.
26. Constantin A, Elkhaled A, Jalbert L, Srinivasan R, Cha S, Chang SM *et al* (2012) Identifying malignant transformations in recurrent low grade gliomas using high resolution magic angle spinning spectroscopy. *Artif Intell Med* **55**:61–70.
27. Crawford FW, Khayal IS, McGue C, Saraswathy S, Pirzkall A, Cha S *et al* (2009) Relationship of pre-surgery metabolic and physiological MR imaging parameters to survival for patients with untreated GBM. *J Neurooncol* **91**:337–351.
28. Croteau D, Scarpace L, Hearshen D, Gutierrez J, Fisher JL, Rock JP, Mikkelsen T (2001) Correlation between magnetic resonance spectroscopy imaging and image-guided biopsies: semiquantitative and qualitative histopathological analyses of patients with untreated glioma. *Neurosurgery* **49**:823–829.
29. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM *et al* (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462**:739–744.
30. Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris HD *et al* (2011) Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized [1–13C]pyruvate and

13C magnetic resonance spectroscopic imaging. *Magn Reson Med* **65**:557–563.
31. De Graaf RA (2007) *In vivo NMR spectroscopy: principles and techniques*, 2nd edn. John Wiley & Sons: Chichester, West Sussex, England; Hoboken, NJ.
32. de Groot J, Sontheimer H (2011) Glutamate and the biology of gliomas. *Glia* **59**:1181–1189.
33. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB (2007) 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* **104**:19345–19350.
34. Dewhirst MW, Poulson JM, Yu D, Sanders L, Lora-Michiels M, Vujaskovic Z *et al* (2005) Relation between pO2, 31P magnetic resonance spectroscopy parameters and treatment outcome in patients with high-grade soft tissue sarcomas treated with thermoradiotherapy. *Int J Radiat Oncol Biol Phys* **61**:480–491.
35. Di. Costanzo A, Scarabino T, Trojsi F, Giannatempo GM, Popolizio T, Catapano D *et al* (2006) Multiparametric 3T MR approach to the assessment of cerebral gliomas: tumor extent and malignancy. *Neuroradiology* **48**:622–631.
36. Di. Costanzo A, Scarabino T, Trojsi F, Popolizio T, Bonavita S, de Cristofaro M *et al* (2014) Recurrent glioblastoma multiforme versus radiation injury: a multiparametric 3-T MR approach. *Radiol Med (Torino)* **119**:616–624.
37. Di. Costanzo A, Trojsi F, Giannatempo GM, Vuolo L, Popolizio T, Catapano D *et al* (2006) Spectroscopic, diffusion and perfusion magnetic resonance imaging at 3.0 Tesla in the delineation of glioblastomas: preliminary results. *J Exp Clin Cancer Res* **25**:383–390.
38. Doblas S, He T, Saunders D, Hoyle J, Smith N, Pye Q *et al* (2012) In vivo characterization of several rodent glioma models by 1H MRS. *NMR Biomed* **25**:685–694.
39. Dowling C, Bollen AW, Noworolski SM, McDermott MW, Barbaro NM, Day MR *et al* (2001) Preoperative proton MR spectroscopic imaging of brain tumors: correlation with histopathologic analysis of resection specimens. *AJNR Am J Neuroradiol* **22**:604–612.
40. Elkhaled A, Jalbert L, Constantin A, Yoshihara HA, Phillips JJ, Molinaro AM *et al* (2014) Characterization of metabolites in infiltrating gliomas using ex vivo (1)H high-resolution magic angle spinning spectroscopy. *NMR Biomed* **27**:578–593.
41. Elkhaled A, Jalbert LE, Phillips JJ, Yoshihara HA, Parvataneni R, Srinivasan R *et al* (2012) Magnetic resonance of 2-hydroxyglutarate in IDH1-mutated low-grade gliomas. *Sci Transl Med* **4**:116ra5.
42. Esmaeili M, Hamans BC, Navis AC, van Horssen R, Bathen TF, Gribbestad IS *et al* (2014) IDH1 R132H mutation generates a distinct phospholipid metabolite profile in glioma. *Cancer Res* **74**:4898–4907.
43. Esmaeili M, Vettukattil R, Bathen TF (2013) 2-hydroxyglutarate as a magnetic resonance biomarker for glioma subtyping. *Transl Oncol* **6**:92–98.
44. Estrela JM, Ortega A, Obrador E (2006) Glutathione in cancer biology and therapy. *Crit Rev Clin Lab Sci* **43**:143–181.
45. Fulham MJ, Bizzi A, Dietz MJ, Shih HH, Raman R, Sobering GS *et al* (1992) Mapping of brain tumor metabolites with proton MR spectroscopic imaging: clinical relevance. *Radiology* **185**: 675–686.
46. Galldiks N, Langen KJ, Pope WB (2015) From the clinician's point of view—What is the status quo of positron emission tomography in patients with brain tumors? *Neuro-Oncol*.
47. Garcia-Alvarez I, Garrido L, Romero-Ramirez L, Nieto-Sampedro M, Fernandez-Mayoralas A, Campos-Olivas R (2013) The effect of antitumor glycosides on glioma cells and tissues as studied by proton HR-MAS NMR spectroscopy. *PLoS ONE* **8**:e78391.
48. Garcia-Martin ML, Herigault G, Remy C, Farion R, Ballesteros P, Coles JA *et al* (2001) Mapping extracellular pH in rat brain gliomas in vivo by 1H magnetic resonance spectroscopic imaging: comparison with maps of metabolites. *Cancer Res* **61**:6524–6531.
49. Gillies RJ, Liu Z, Bhujwalla Z (1994) 31P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. *Am J Physiol* **267** (1 Pt 1):C195–C203.
50. Gillies RJ, Morse DL (2005) In vivo magnetic resonance spectroscopy in cancer. *Annu Rev Biomed Eng* **7**:287–326.
51. Glunde K, Bhujwalla ZM, Ronen SM (2011) Choline metabolism in malignant transformation. *Nat Rev Cancer* **11**:835–848.
52. Graves EE, Nelson SJ, Vigneron DB, Verhey L, McDermott M, Larson D *et al* (2001) Serial proton MR spectroscopic imaging of recurrent malignant gliomas after gamma knife radiosurgery. *AJNR Am J Neuroradiol* **22**:613–624.
53. Grier JT, Batchelor T (2006) Low-grade gliomas in adults. *Oncologist* **11**:681–693.
54. Griffiths JR, Cady E, Edwards RH, McCready VR, Wilkie DR, Wiltshaw E (1983) 31P-NMR studies of a human tumour in situ. *Lancet* **1**:1435–1436.
55. Guillevin R, Menuel C, Duffau H, Kujas M, Capelle L, Aubert A *et al* (2008) Proton magnetic resonance spectroscopy predicts proliferative activity in diffuse low-grade gliomas. *J Neurooncol* **87**:181–187.
56. Guillevin R, Menuel C, Taillibert S, Capelle L, Costalat R, Abud L *et al* (2011) Predicting the outcome of grade II glioma treated with temozolomide using proton magnetic resonance spectroscopy. *Br J Cancer* **104**:1854–1861.
57. Guo J, Yao C, Chen H, Zhuang D, Tang W, Ren G *et al* (2012) The relationship between Cho/NAA and glioma metabolism: implementation for margin delineation of cerebral gliomas. *Acta Neurochir (Wien)* **154**:1361–1370, discussion 70.
58. Gyngell ML, Hoehn-Berlage M, Kloiber O, Michaelis T, Ernestus RI, Horstermann D, Frahm J (1992) Localized proton NMR spectroscopy of experimental gliomas in rat brain in vivo. *NMR Biomed* **5**:335–340.
59. Hattingen E, Delic O, Franz K, Pilatus U, Raab P, Lanfermann H, Gerlach R (2010) 1)H MRSI and progression-free survival in patients with WHO grades II and III gliomas. *Neurol Res* **32**:593–602.
60. Hattingen E, Raab P, Franz K, Lanfermann H, Setzer M, Gerlach R *et al* (2008) Prognostic value of choline and creatine in WHO grade II gliomas. *Neuroradiology* **50**:759–767.
61. He T, Doblas S, Saunders D, Casteel R, Lerner M, Ritchey JW *et al* (2011) Effects of PBN and OKN007 in rodent glioma models assessed by 1H MR spectroscopy. *Free Radic Biol Med* **51**:490–502.
62. Hoyer C, Gass N, Weber-Fahr W, Sartorius A (2014) Advantages and challenges of small animal magnetic resonance imaging as a translational tool. *Neuropsychobiology* **69**:187–201.
63. Huang AP, Tsai JC, Kuo LT, Lee CW, Lai HS, Tsai LK *et al* (2014) Clinical application of perfusion computed tomography in neurosurgery. *J Neurosurg* **120**:473–488.
64. Huse JT, Wallace M, Aldape KD, Berger MS, Bettegowda C, Brat DJ *et al* (2014) Where are we now? And where are we going? A report from the Accelerate Brain Cancer Cure (ABC2) low-grade glioma research workshop. *Neuro-Oncol* **16**:173–178.
65. Izquierdo-Garcia JL, Cai LM, Chaumeil MM, Eriksson P, Robinson AE, Pieper RO *et al* (2014) Glioma cells with the IDH1 mutation modulate metabolic fractional flux through pyruvate carboxylase. *PLoS ONE* **9**:e108289.

66. Izquierdo-Garcia JL, Viswanath P, Eriksson P, Cai L, Radoul M, Chaumeil MM *et al* (2015) IDH1 Mutation Induces Reprogramming of Pyruvate Metabolism. *Cancer Res* **75**:2999–3009.
67. Izquierdo-Garcia JL, Viswanath P, Eriksson P, Chaumeil MM, Pieper RO, Phillips JJ, Ronen SM (2015) Metabolic Reprogramming in Mutant IDH1 Glioma Cells. *PLoS ONE* **10**:e0118781.
68. Jacobs MA, Horska A, van Zijl PC, Barker PB (2001) Quantitative proton MR spectroscopic imaging of normal human cerebellum and brain stem. *Magn Reson Med* **46**:699–705.
69. Jaskolski DJ, Fortuniak J, Majos A, Gajewicz W, Papierz W, Liberski PP *et al* (2013) Magnetic resonance spectroscopy in intracranial tumours of glial origin. *Neurol Neurochir Pol* **47**:438–449.
70. Jenkinson MD, Smith TS, Joyce K, Fildes D, du Plessis DG, Warnke PC, Walker C (2005) MRS of oligodendroglial tumors: correlation with histopathology and genetic subtypes. *Neurology* **64**:2085–2089.
71. Kalinina J, Carroll A, Wang L, Yu Q, Mancheno DE, Wu S *et al* (2012) Detection of ″oncometabolite″ 2-hydroxyglutarate by magnetic resonance analysis as a biomarker of IDH1/2 mutations in glioma. *J Mol Med* **90**:1161–1171.
72. Kallenberg K, Bock HC, Helms G, Jung K, Wrede A, Buhk JH *et al* (2009) Untreated glioblastoma multiforme: increased myo-inositol and glutamine levels in the contralateral cerebral hemisphere at proton MR spectroscopy. *Radiology* **253**:805–812.
73. Kim D, Fiske BP, Birsoy K, Freinkman E, Kami K, Possemato RL *et al* (2015) SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. *Nature* **520**:363–367.
74. Kim H, Catana C, Ratai EM, Andronesi OC, Jennings DL, Batchelor TT *et al* (2011) Serial magnetic resonance spectroscopy reveals a direct metabolic effect of cediranib in glioblastoma. *Cancer Res* **71**:3745–3752.
75. Koul D, Shen R, Kim YW, Kondo Y, Lu Y, Bankson J *et al* (2010) Cellular and in vivo activity of a novel PI3K inhibitor, PX-866, against human glioblastoma. *Neuro-Oncol* **12**:559–569.
76. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH *et al* (2011) Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research. *Neoplasia* **13**:81–97.
77. Kurhanewicz J, Vigneron DB, Nelson SJ (2000) Three-dimensional magnetic resonance spectroscopic imaging of brain and prostate cancer. *Neoplasia* **2**:166–189.
78. Kuznetsov YE, Caramanos Z, Antel SB, Preul MC, Leblanc R, Villemure JG *et al* (2003) Proton magnetic resonance spectroscopic imaging can predict length of survival in patients with supratentorial gliomas. *Neurosurgery* **53**:565–574, discussion 74–6.
79. Law M, Yang S, Wang H, Babb JS, Johnson G, Cha S *et al* (2003) Glioma grading: sensitivity, specificity, and predictive values of perfusion MR imaging and proton MR spectroscopic imaging compared with conventional MR imaging. *AJNR Am J Neuroradiol* **24**:1989–1998.
80. Lazovic J, Soto H, Piccioni D, Lou JR, Li S, Mirsadraei L *et al* (2012) Detection of 2-hydroxyglutaric acid in vivo by proton magnetic resonance spectroscopy in U87 glioma cells overexpressing isocitrate dehydrogenase-1 mutation. *Neuro-Oncol* **14**:1465–1472.
81. Le. Moyec L, Naruse S, Higuchi T, Hirakawa K, Watari H, de Certaines JD, Roques BP (1988) In vivo 31P MRS in new antineoplastic agents evaluation on experimental tumor models. *Cancer Biochem Biophys* **10**:31–45.
82. Li X, Jin H, Lu Y, Oh J, Chang S, Nelson SJ (2004) Identification of MRI and 1H MRSI parameters that may predict survival for patients with malignant gliomas. *NMR Biomed* **17**: 10–20.
83. Li X, Vigneron DB, Cha S, Graves EE, Crawford F, Chang SM, Nelson SJ (2005) Relationship of MR-derived lactate, mobile lipids, and relative blood volume for gliomas in vivo. *AJNR Am J Neuroradiol* **26**:760–769.
84. Li Y, Larson P, Chen AP, Lupo JM, Ozhinsky E, Kelley D *et al* (2015) Short-echo three-dimensional H-1 MR spectroscopic imaging of patients with glioma at 7 Tesla for characterization of differences in metabolite levels. *J Magn Reson Imaging* **41**:1332–1341.
85. Li Y, Lupo JM, Parvataneni R, Lamborn KR, Cha S, Chang SM, Nelson SJ (2013) Survival analysis in patients with newly diagnosed glioblastoma using pre- and postradiotherapy MR spectroscopic imaging. *Neuro-Oncol* **15**:607–617.
86. Londono A, Castillo M, Armao D, Kwock L, Suzuki K (2003) Unusual MR spectroscopic imaging pattern of an astrocytoma: lack of elevated choline and high myo-inositol and glycine levels. *AJNR Am J Neuroradiol* **24**:942–945.
87. Lora-Michiels M, Yu D, Sanders L, Poulson JM, Azuma C, Case B *et al* (2006) Extracellular pH and P-31 magnetic resonance spectroscopic variables are related to outcome in canine soft tissue sarcomas treated with thermoradiotherapy. *Clin Cancer Res* **12**:5733–5740.
88. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A *et al* (2007) The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* **114**:97–109.
89. Luo Y, Rydzewski J, de Graaf RA, Gruetter R, Garwood M, Schleich T (1999) In vivo observation of lactate methyl proton magnetization transfer in rat C6 glioma. *Magn Reson Med* **41**:676–685.
90. Lupo JM, Cha S, Chang SM, Nelson SJ (2007) Analysis of metabolic indices in regions of abnormal perfusion in patients with high-grade glioma. *AJNR Am J Neuroradiol* **28**:1455–1461.
91. Maher EA, Marin-Valencia I, Bachoo RM, Mashimo T, Raisanen J, Hatanpaa KJ *et al* (2012) Metabolism of [U-13 C]glucose in human brain tumors in vivo. *NMR Biomed* **25**:1234–1244.
92. Majos C, Bruna J, Julia-Sape M, Cos M, Camins A, Gil M *et al* (2011) Proton MR spectroscopy provides relevant prognostic information in high-grade astrocytomas. *AJNR Am J Neuroradiol* **32**:74–80.
93. Mancuso A, Zhu A, Beardsley NJ, Glickson JD, Wehrli S, Pickup S (2005) Artificial tumor model suitable for monitoring 31P and 13C NMR spectroscopic changes during chemotherapy-induced apoptosis in human glioma cells. *Magn Reson Med* **54**: 67–78.
94. Marin-Valencia I, Yang C, Mashimo T, Cho S, Baek H, Yang XL *et al* (2012) Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain in vivo. *Cell Metab* **15**:827–837.
95. Mashimo T, Pichumani K, Vemireddy V, Hatanpaa KJ, Singh DK, Sirasanagandla S *et al* (2014) Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. *Cell* **159**:1603–1614.
96. Matulewicz L, Cichon A, Sokol M, Przybyszewski W, Glowala-Kosinska M, Gibas M (2013) High resolution proton nuclear magnetic resonance (1H NMR) spectroscopy of surviving C6 glioma cells after X-ray irradiation. *Folia Neuropathol* **51**:33–43.
97. McKnight TR (2004) Proton magnetic resonance spectroscopic evaluation of brain tumor metabolism. *Semin Oncol* **31**:605–617.
98. McKnight TR, Lamborn KR, Love TD, Berger MS, Chang S, Dillon WP *et al* (2007) Correlation of magnetic resonance spectroscopic and growth characteristics within Grades II and III gliomas. *J Neurosurg* **106**:660–666.

99. McKnight TR, Noworolski SM, Vigneron DB, Nelson SJ (2001) An automated technique for the quantitative assessment of 3D-MRSI data from patients with glioma. *J Magn Reson Imaging* **13**:167–177.
100. McKnight TR, von dem Bussche MH, Vigneron DB, Lu Y, Berger MS, McDermott MW *et al* (2002) Histopathological validation of a three-dimensional magnetic resonance spectroscopy index as a predictor of tumor presence. *J Neurosurg* **97**:794–802.
101. Mirbahai L, Wilson M, Shaw CS, McConville C, Malcomson RD, Griffin JL *et al* (2011) 1H magnetic resonance spectroscopy metabolites as biomarkers for cell cycle arrest and cell death in rat glioma cells. *Inter J Biochem Cell Biol* **43**:990–1001.
102. Miyamachi K, Abe H, Miyasaka K (1990) [Phosphorus-31 MR spectroscopy of brain tumors]. *No Shinkei Geka* **18**:533–537.
103. Mlynarik V, Cudalbu C, Clement V, Marino D, Radovanovic I, Gruetter R (2012) In vivo metabolic profiling of glioma-initiating cells using proton magnetic resonance spectroscopy at 14.1 Tesla. *NMR Biomed* **25**:506–513.
104. Morris P, Bachelard H (2003) Reflections on the application of 13C-MRS to research on brain metabolism. *NMR Biomed* **16**:303–312.
105. Murphy PS, Viviers L, Abson C, Rowland IJ, Brada M, Leach MO, Dzik-Jurasz AS (2004) Monitoring temozolomide treatment of low-grade glioma with proton magnetic resonance spectroscopy. *Br J Cancer* **90**:781–786.
106. Murugananandham M, Clerkin PP, Smith BJ, Anderson CM, Morris A, Capizzano AA *et al* (2014) 3-Dimensional magnetic resonance spectroscopic imaging at 3 Tesla for early response assessment of glioblastoma patients during external beam radiation therapy. *Int J Radiat Oncol Biol Phys* **90**:181–189.
107. Natsumeda M, Igarashi H, Nomura T, Ogura R, Tsukamoto Y, Kobayashi T *et al* (2014) Accumulation of 2-hydroxyglutarate in gliomas correlates with survival: a study by 3.0-tesla magnetic resonance spectroscopy. *Acta Neuropathol Commun* **2**:158.
108. Negendank WG, Sauter R, Brown TR, Evelhoch JL, Falini A, Gotsis ED *et al* (1996) Proton magnetic resonance spectroscopy in patients with glial tumors: a multicenter study. *J Neurosurg* **84**:449–458.
109. Nelson SJ (2003) Multivoxel magnetic resonance spectroscopy of brain tumors. *Mol Cancer Ther* **2**:497–507.
110. Nelson SJ (2011) Assessment of therapeutic response and treatment planning for brain tumors using metabolic and physiological MRI. *NMR Biomed* **24**:734–749.
111. Nelson SJ, Kurhanewicz J, Vigneron DB, Larson PE, Harzstark AL, Ferrone M *et al* (2013) Metabolic imaging of patients with prostate cancer using hyperpolarized [1-(1)(3)C]pyruvate. *Sci Transl Med* **5**:198ra08.
112. Nelson SJ, Ozhinsky E, Li Y, Park I, Crane J (2013) Strategies for rapid in vivo 1H and hyperpolarized 13C MR spectroscopic imaging. *J Magn Reson* **229**:187–197.
113. Nelson SJ, Vigneron DB, Dillon WP (1999) Serial evaluation of patients with brain tumors using volume MRI and 3D 1H MRSI. *NMR Biomed* **12**:123–138.
114. Ogunrinu TA, Sontheimer H (2010) Hypoxia increases the dependence of glioma cells on glutathione. *J Biol Chem* **285**:37716–37724.
115. Oh J, Henry RG, Pirzkall A, Lu Y, Li X, Catalaa I *et al* (2004) Survival analysis in patients with glioblastoma multiforme: predictive value of choline-to-N-acetylaspartate index, apparent diffusion coefficient, and relative cerebral blood volume. *J Magn Reson Imaging* **19**:546–554.
116. Ozturk-Isik E, Pirzkall A, Lamborn KR, Cha S, Chang SM, Nelson SJ (2012) Spatial characteristics of newly diagnosed grade 3 glioma assessed by magnetic resonance metabolic and diffusion tensor imaging. *Transl Oncol* **5**:10–18.
117. Park I, Bok R, Ozawa T, Phillips JJ, James CD, Vigneron DB *et al* (2011) Detection of early response to temozolomide treatment in brain tumors using hyperpolarized 13C MR metabolic imaging. *J Magn Reson Imaging* **33**:1284–1290.
118. Park I, Chen AP, Zierhut ML, Ozturk-Isik E, Vigneron DB, Nelson SJ (2011) Implementation of 3 T lactate-edited 3D 1H MR spectroscopic imaging with flyback echo-planar readout for gliomas patients. *Ann Biomed Eng* **39**:193–204.
119. Park I, Larson PE, Tropp JL, Carvajal L, Reed G, Bok R *et al* (2014) Dynamic hyperpolarized carbon-13 MR metabolic imaging of nonhuman primate brain. *Magn Reson Med* **71**:19–25.
120. Park I, Larson PE, Zierhut ML, Hu S, Bok R, Ozawa T *et al* (2010) Hyperpolarized 13C magnetic resonance metabolic imaging: application to brain tumors. *Neuro-Oncol* **12**: 133–144.
121. Park I, Mukherjee J, Ito M, Chaumeil MM, Jalbert LE, Gaensler K *et al* (2014) 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* **74**:7115–7124.
122. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P *et al* (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**:1807–1812.
123. Pirzkall A, McKnight TR, Graves EE, Carol MP, Sneed PK, Wara WW *et al* (2001) MR-spectroscopy guided target delineation for high-grade gliomas. *Int J Radiat Oncol Biol Phys* **50**:915–928.
124. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF (2003) The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol* **66**:1499–1503.
125. Pope WB, Prins RM, Albert Thomas M, Nagarajan R, Yen KE, Bittinger MA *et al* (2012) Non-invasive detection of 2-hydroxyglutarate and other metabolites in IDH1 mutant glioma patients using magnetic resonance spectroscopy. *J Neurooncol* **107**:197–205.
126. Posse S, Otazo R, Dager SR, Alger J (2013) MR spectroscopic imaging: principles and recent advances. *J Magn Reson Imaging* **37**:1301–1325.
127. Preul MC, Caramanos Z, Collins DL, Villemure JG, Leblanc R, Olivier A *et al* (1996) Accurate, noninvasive diagnosis of human brain tumors by using proton magnetic resonance spectroscopy. *Nat Med* **2**:323–325.
128. Provent P, Benito M, Hiba B, Farion R, Lopez-Larrubia P, Ballesteros P *et al* (2007) Serial in vivo spectroscopic nuclear magnetic resonance imaging of lactate and extracellular pH in rat gliomas shows redistribution of protons away from sites of glycolysis. *Cancer Res* **67**:7638–7645.
129. Qu JR, Jiang T, Dai JP, Li HL, Luo JP, Li SW *et al* (2012) Proton magnetic resonance spectroscopy predicts radiotherapy response and time-to-progression in high-grade gliomas after surgery. *Chin Med J* **125**:4334–4337.
130. Quon H, Brunet B, Alexander A, Murtha A, Abdulkarim B, Fulton D *et al* (2011) Changes in serial magnetic resonance spectroscopy predict outcome in high-grade glioma during and after postoperative radiotherapy. *Anticancer Res* **31**:3559–3565.
131. Radoul M, Chaumeil MM, Eriksson P, Ronen SM (2015) Hyperpolarized 13C MRSI is a better predictor of survival than tumor size in treated glioblastoma. Proceedings of the conference of the International Society for Magnetic Resonance in Medicine, Abstract #326, Toronto, ON.
132. Rajagopalan KN, DeBerardinis RJ (2011) Role of glutamine in cancer: therapeutic and imaging implications. *J Nucl Med* **52**:1005–1008.

133. Ratai EM, Zhang Z, Snyder BS, Boxerman JL, Safriel Y, McKinstry RC *et al* (2013) Magnetic resonance spectroscopy as an early indicator of response to anti-angiogenic therapy in patients with recurrent glioblastoma: RTOG 0625/ACRIN 6677. *Neuro-Oncol* **15**:936–944.
134. Reitman ZJ, Duncan CG, Poteet E, Winters A, Yan LJ, Gooden DM *et al* (2014) Cancer-associated isocitrate dehydrogenase 1 (IDH1) R132H mutation and d-2-hydroxyglutarate stimulate glutamine metabolism under hypoxia. *J Biol Chem* **289**:23318–23328.
135. Remy C, Arus C, Ziegler A, Lai ES, Moreno A, Le Fur Y, Decorps M (1994) 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* **62**:166–179.
136. Rijpkema M, Schuuring J, van der Meulen Y, van der Graaf M, Bernsen H, Boerman R *et al* (2003) Characterization of oligodendrogliomas using short echo time 1H MR spectroscopic imaging. *NMR Biomed* **16**:12–18.
137. Rodrigues TB, Lopez-Larrubia P, Cerdan S (2009) Redox dependence and compartmentation of [13C]pyruvate in the brain of deuterated rats bearing implanted C6 gliomas. *J Neurochem* **109** (Suppl. 1):237–245.
138. Ross BD, Higgins RJ, Boggan JE, Willis JA, Knittel B, Unger SW (1988) Carbohydrate metabolism of the rat C6 glioma. An in vivo 13C and in vitro 1H magnetic resonance spectroscopy study. *NMR Biomed* **1**:20–26.
139. Ryken TC, Aygun N, Morris J, Schweizer M, Nair R, Spracklen C *et al* (2014) The role of imaging in the management of progressive glioblastoma: a systematic review and evidence-based clinical practice guideline. *J Neurooncol* **118**:435–460.
140. Saraswathy S, Crawford FW, Lamborn KR, Pirzkall A, Chang S, Cha S, Nelson SJ (2009) Evaluation of MR markers that predict survival in patients with newly diagnosed GBM prior to adjuvant therapy. *J Neurooncol* **91**:69–81.
141. Schupp DG, Merkle H, Ellermann JM, Ke Y, Garwood M (1993) Localized detection of glioma glycolysis using edited 1H MRS. *Magn Reson Med* **30**:18–27.
142. Srinivasan R, Phillips JJ, Vandenberg SR, Polley MY, Bourne G, Au A *et al* (2010) Ex vivo MR spectroscopic measure differentiates tumor from treatment effects in GBM. *Neuro-Oncol* **12**:1152–1161.
143. Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M (2001) Glutamate release promotes growth of malignant gliomas. *Nat Med* **7**:1010–1015.
144. Terpstra M, Gruetter R, High WB, Mescher M, DelaBarre L, Merkle H, Garwood M (1998) Lactate turnover in rat glioma measured by in vivo nuclear magnetic resonance spectroscopy. *Cancer Res* **58**:5083–5088.
145. Thelwall PE, Yemin AY, Gillian TL, Simpson NE, Kasibhatla MS, Rabbani ZN *et al* (2005) Noninvasive in vivo detection of glutathione metabolism in tumors. *Cancer Res* **65**: 10149–10153.
146. Thulborn KR, Lu A, Atkinson IC, Damen F, Villano JL (2009) Quantitative sodium MR imaging and sodium bioscales for the management of brain tumors. *Neuroimaging Clin N Am* **19**:615–624.
147. Usenius JP, Kauppinen RA, Vainio PA, Hernesniemi JA, Vapalahti MP, Paljarvi LA, Soimakallio S (1994) Quantitative metabolite patterns of human brain tumors: detection by 1H NMR spectroscopy in vivo and in vitro. *J Comput Assist Tomogr* **18**:705–713.
148. Venkatesh HS, Chaumeil MM, Ward CS, Haas-Kogan DA, James CD, Ronen SM (2012) Reduced phosphocholine and hyperpolarized lactate provide magnetic resonance biomarkers of PI3K/Akt/mTOR inhibition in glioblastoma. *Neuro-Oncol* **14**:315–325.
149. Venkatesh SK, Gupta RK, Pal L, Husain N, Husain M (2001) Spectroscopic increase in choline signal is a nonspecific marker for differentiation of infective/inflammatory from neoplastic lesions of the brain. *J Magn Reson Imaging* **14**:8–15.
150. Warburg O (1956) On the origin of cancer cells. *Science* **123**:309–314.
151. Ward CS, Venkatesh HS, Chaumeil MM, Brandes AH, Vancriekinge M, Dafni H *et al* (2010) Noninvasive detection of target modulation following phosphatidylinositol 3-kinase inhibition using hyperpolarized 13C magnetic resonance spectroscopy. *Cancer Res* **70**:1296–1305.
152. Wijnen JP, Van der Graaf M, Scheenen TWJ, Klomp DWJ, de Galan BE, Idema AJS, Heerschap A (2010) In vivo 13C magnetic resonance spectroscopy of a human brain tumor after application of 13C-1-enriched glucose. *Magn Reson Imaging* **28**:690–697.
153. Xu Y, Li YH, Wang Y, Cui JL, Yin XB, He XW, Zhang YK (2014) 13C-engineered carbon quantum dots for in vivo magnetic resonance and fluorescence dual-response. *Analyst* **139**:5134–5139.
154. Yamasaki F, Takaba J, Ohtaki M, Abe N, Kajiwara Y, Saito T *et al* (2005) Detection and differentiation of lactate and lipids by single-voxel proton MR spectroscopy. *Neurosurg Rev* **28**:267–277.
155. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W *et al* (2009) IDH1 and IDH2 mutations in gliomas. *N Engl J Med* **360**:765–773.
156. Yao C, Lv S, Chen H, Tang W, Guo J, Zhuang D *et al* (2015) The clinical utility of multimodal MR image-guided needle biopsy in cerebral gliomas. *Int J Neurosci* [*Epub ahead of print*].
157. Ye ZC, Sontheimer H (1999) Glioma cells release excitotoxic concentrations of glutamate. *Cancer Res* **59**:4383–4391.
158. Zeng QS, Li CF, Liu H, Zhen JH, Feng DC (2007) Distinction between recurrent glioma and radiation injury using magnetic resonance spectroscopy in combination with diffusion-weighted imaging. *Int J Radiat Oncol Biol Phys* **68**:151–158.
159. Zhu H, Barker PB (2011) MR spectroscopy and spectroscopic imaging of the brain. *Methods Mol Biol* **711**:203–226.
160. Ziegler A, von Kienlin M, Decorps M, Remy C (2001) High glycolytic activity in rat glioma demonstrated in vivo by correlation peak 1H magnetic resonance imaging. *Cancer Res* **61**:5595–5600.
161. Zoula S, Herigault G, Ziegler A, Farion R, Decorps M, Remy C (2003) Correlation between the occurrence of 1H-MRS lipid signal, necrosis and lipid droplets during C6 rat glioma development. *NMR Biomed* **16**:199–212.