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MR molecular imaging of brain cancer metabolism using hyperpolarized ^{13}C magnetic resonance spectroscopy

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Abstract

Metabolic reprogramming is an important hallmark of cancer. Alterations in many metabolic pathways support the requirement for cellular building blocks that are essential for cancer cell proliferation. This metabolic reprogramming can be imaged using magnetic resonance spectroscopy (MRS). ^{1}H MRS can inform on alterations in the steady-state levels of cellular metabolites, but the emergence of hyperpolarized ^{13}C MRS has now also enabled imaging of metabolic fluxes in real-time, providing a new method for tumor detection and monitoring of therapeutic response. In the case of glioma, preclinical cell and animal studies have shown that the hyperpolarized ^{13}C MRS metabolic imaging signature is specific to tumor type and can distinguish between mutant IDH1 glioma and primary glioblastoma. Here, we review these findings, first describing the main metabolic pathways that are altered in the different glioma subtypes, and then reporting on the use of hyperpolarized ^{13}C MRS and MR spectroscopic imaging (MRSI) to probe these pathways. We show that the future translation of this hyperpolarized ^{13}C MRS molecular metabolic imaging method to the clinic promises to improve the noninvasive detection, characterization, and response-monitoring of brain tumors resulting in improved patient diagnosis and clinical management.

Keywords

glioma; metabolic reprogramming; hyperpolarized ^{13}C; magnetic resonance spectroscopy

Introduction

Gliomas are the most common type of brain tumor, representing 80% of all diagnosed malignant central nervous system tumors in the United-States [1]. They are classified into three categories – astrocytoma, oligodendroglioma and glioblastoma – and four World Health Organization grades – I to IV – based on clinical and pathological criteria [1,2]. Grade I tumors are typically benign. Grade II tumors are referred to as low-grade in contrast to grades III and IV tumors that are considered higher-grade. Glioblastoma (GBM) is a grade IV glioma that accounts for more than 50% of all diagnosed gliomas [1]. It is the most aggressive type of glioma and is associated with a very poor prognosis with a median

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survival time of 15 months [1]. 90% of all GBM develop rapidly *de novo* and are referred to as primary GBM. The rest progress from grade II or III astrocytoma and are referred to as secondary or upgraded GBM [3,4]. Oligodendroglioma tumors present only as grade II or III and do not evolve to secondary GBM. Grades II and III astrocytoma and oligodendroglioma have a relatively better prognosis than GBM [2]. Current standard of care for brain tumors is a combination of treatments that depends on tumor type and grade and includes surgical resection, radiation therapy and chemotherapy, with immunotherapy and targeted therapies presenting new therapeutic approaches.

Independent of their histology or prognosis, oligodendroglioma, astrocytoma and secondary GBM differ significantly in their genetic and epigenetic signatures from primary GBM. Primary GBM are driven by multiple genetic alterations such as loss of the phosphatase and tensin homolog (PTEN) gene, amplification or mutation of the epidermal growth factor receptor (EGFR), and increased signaling via the phosphatidylinositol-3-kinase (PI3K)/Akt pathway [2,5]. In contrast, 70–90% of grade II/III glioma and secondary GBM harbor a mutation in the cytosolic isocitrate dehydrogenase 1 gene (IDH1) [6–9]. This recently discovered mutation, identified as a single amino acid substitution at arginine 132 [6–9], is one of the earliest genetic events in low-grade tumors, and has been shown to drive tumor development. All mutations are heterozygous leading to the retention of the wild-type form of the isocitrate dehydrogenase enzyme, responsible for the conversion of isocitrate to alpha-ketoglutarate (α-KG). However, mutant IDH1 inhibits the wild-type form and establishes a new function converting α-KG to 2-hydroxyglutarate (2-HG). Production of elevated levels of 2-HG leads to epigenetic alterations which, in turn, lead to the development of oligodendroglioma and astrocytoma tumors [10]. In addition to the IDH1 mutation, over 70% of grade II/III astrocytomas and oligodendrogliomas have a mutation in the TP53 tumor suppressor gene and a co-deletion of chromosome arms 1p/19q respectively [2,11].

Magnetic resonance imaging (MRI) is the main imaging modality used to diagnose and grade brain tumors. In the clinic, T1-weighted imaging pre- and post-injection of gadolinium, T2-weighted imaging, fluid-attenuated inversion recovery imaging, diffusion-weighted imaging and dynamic susceptibility-weighted contrast-enhanced imaging are the most commonly used MRI methods [12–14]. However, although they provide crucial information on the structure and perfusion of the tumor as well as the integrity of the blood-brain barrier (BBB), these methods do not provide metabolic information on the lesions. ^{1}H magnetic resonance spectroscopy (MRS) *in vivo* or in biopsy samples allows probing steady-state metabolite levels, and several studies have demonstrated the potential of ^{1}H MRS to differentiate tumors from normal brain and non-neoplastic lesions [13,15–19]. Tumors are usually associated with high levels of choline-containing metabolites (comprised of choline, phosphocholine (PC) and glycerophosphocholine (GPC)) and low levels of N-acetyl-aspartate as well as an increase in lactate level in high-grade tumors [13,17]. Recently, using ^{1}H MRS, the oncometabolite 2-HG produced by mutant IDH1 was detected *in vivo* in glioma patients harboring the IDH1 mutation [20–23] as well as *ex vivo* in glioma biopsies [24,25]. ^{13}C MRS can provide additional information in the study of brain tumor metabolism by monitoring metabolic fluxes [26]. However, ^{13}C MRS studies have been more challenging due to the significantly lower intrinsic sensitivity of the technique. The natural abundance of ^{13}C is only 1.1% and its gyromagnetic ratio, γ_{13C}=10.705 MHz/T, is ~ 4 times

lower than γ_{1H}. As a result, even when using ^{13}C-labeled compounds long acquisition times are required limiting the application of this method for patient studies. The emergence of hyperpolarized ^{13}C MRS has opened a range of new possibilities for novel metabolic imaging studies that are translatable to the clinic and can serve to characterize brain tumors and their response to therapy [27–30].

Here, we will first describe the main metabolic pathways that are altered in brain cancers. We will focus primarily on the pathways that have been investigated using hyperpolarized ^{13}C MRS. Then, we will briefly describe the principles of hyperpolarized ^{13}C MRS and discuss the main agents developed to monitor the metabolism of brain tumor cells. Tables 1 and 2 and Figure 1 summarize the ^{13}C-labeled probes that have been hyperpolarized using the DNP technique and applied to the study of metabolic reprogramming in cell models and *in vivo* preclinical models of brain tumors.

Metabolic reprogramming in brain tumors

Cancer cells have the ability to adapt their metabolism to enhance survival. Most notably, they alter their glucose metabolism, up-regulating glucose uptake to produce elevated lactate levels even under aerobic condition [31,32]. This phenomenon is known as the “Warburg effect”, and was first described by Otto Warburg in 1926 [31]. Elevated glucose uptake and glycolytic activity serve to acidify the environment, promoting metastasis [33], and to generate the building-blocks necessary to support rapid cell proliferation and tumor survival, such as nucleotides, amino acids and lipids [34,35]. Importantly, and contrary to Warburg’s initial hypothesis, the glycolytic switch does not result from defective mitochondria but from metabolic reprograming that has recently been recognized as one of the ten hallmarks of cancer [36]. This metabolic reprogramming depends upon the activation of several oncogenic signaling pathways, proto-oncogenes and tumor suppressors [32]. As mentioned above, the activated pathways vary significantly between brain tumor subtypes resulting in metabolic reprogramming that is also unique.

In the case of primary GBM, the PI3K/Akt pathway is activated in more than 88% of cases [5,37]. The increased activity of this pathway is associated with tumor progression and resistance to cancer therapies [38]. At the same time, activation of the PI3K/Akt pathway leads to an increase in glucose transporter expression and up-regulation of glycolysis [32]. It also promotes lipid synthesis facilitating conversion of mitochondrial citrate to acetyl-coA by activating the expression of the enzyme citrate lyase [32]. Downstream of PI3K/Akt, mammalian target of rapamycin complex 1 (mTORC1) facilitates anabolic processes including protein synthesis and lipid synthesis, and limits autophagy [32]. mTORC1 also regulates the activity of hypoxia-inducible factor-1 alpha (HIF-1α), a major player in mitochondrial metabolism [32]. Stabilization of HIF-1α in cancer cells drives the expression of pyruvate dehydrogenase kinase 1 (PDK1) that leads to phosphorylation and inhibition of pyruvate dehydrogenase activity (PDH). Inhibition of PDH, in turn, blocks the entry of pyruvate into the tricarboxylic acid (TCA) cycle and therefore limits glucose-dependent TCA metabolism [32]. Another key role of HIF-1α in cancer cells is to up-regulate monocarboxylate transporters (MCTs) [39]. Over-expression of MCTs is crucial to help maintain the hyper-glycolytic and acid-resistant phenotypes [40]. Whereas MCT1 and MCT2

have a high-affinity for the influx of pyruvate, MCT4 is mostly associated with the export of lactate protecting the intracellular environment from acidification while reducing the extracellular pH [40]. Thus, although lactate production can vary significantly amongst brain tumors, GBM typically exhibit elevated lactate levels [31,41,42]. The PI3K-Akt pathway and HIF-1α also modulate choline metabolism in GBM. Similar to other cancers [43] PC, which is generated by choline phosphorylation via choline kinase and is the precursor of the main membrane phospholipid phosphatidylcholine (PtdCho), is significantly increased in GBM [44]. This reflects the requirement of proliferating cancer cells for membrane synthesis, and the interplay between choline metabolism and oncogenic processes [43,45]. Finally, a large proportion of GBM over-express the oncogenic transcription factor Myc, which also impacts mitochondrial metabolism [46,47]. Myc stimulates glutamine uptake and glutamine mitochondrial utilization by increasing the expression of glutaminase, the enzyme that converts glutamine to glutamate [32,48,49]. Recent studies also reported an increase in acetate uptake and conversion to acetyl-CoA correlating with up-regulation of acetyl-CoA synthase enzyme 2 in GBM [50–52].

As mentioned above, primary GBM differ genetically from mutant IDH1-driven low-grade gliomas (astrocytoma and oligodendroglioma) and secondary GBM. As a result, the metabolic reprogramming of these tumors is also different. Similarly to primary GBM, low-grade gliomas have been characterized by an elevated total choline pool. However, more recent studies looking at events specifically associated with the IDH1 mutation, have demonstrated that GPC levels are elevated in these cells and that PC levels are reduced compared to wild-type IDH1 cells [44,53–55]. The link to mutant IDH1 remains to be determined. Other metabolic alterations detected in mutant IDH1 gliomas have been directly linked to elevated 2-HG levels. It has been shown that accumulation of 2-HG leads to hypermethylation of the branched chain amino acid transferase (BCAT) 1 promoter reducing the activity of the enzyme responsible for the conversion of α-KG to glutamate [56]. The IDH1 mutation also leads to the hypermethylation and consequently silencing of lactate dehydrogenase A (LDHA), the enzyme that converts pyruvate to lactate [42]. Additionally, reduced expression of MCT1 and MCT4 is observed in mutant IDH tumors [42,57]. We also recently observed a reduction in PDH activity that was associated with increased PDK3 expression downstream of HIF-1α stabilization by 2-HG [58]. Finally, as a result of the reduced activity of wild-type IDH1, and the increased activity of NADPH-dependent mutant IDH1, levels of NADPH are also diminished in mutant IDH1 glioma cells as are the levels of glutathione (GSH), leading to elevated levels of reactive oxygen species (ROS) [59].

Hyperpolarized ^{13}C MRS: imaging brain tumor status

The emergence of hyperpolarized ^{13}C MRS enables monitoring of metabolic pathways and their alterations in a non-invasive and non-ionizing manner. Molecules containing NMR-visible nuclei, such as ^{13}C or ^{15}N, can be hyperpolarized using dissolution dynamic nuclear polarization (DNP). The dissolution DNP method allows hyperpolarization and dissolution of ^{13}C-labeled compounds resulting in an increase in their signal-to-noise ratio (SNR) by 10,000 to 50,000-fold as compared to thermal equilibrium [27,30,60]. To achieve this, the labeled compound, mixed with a free radical, is placed at low temperature (<2K) and at high magnetic field (~3–5T). Microwave irradiation then saturates the electron spin resonance

and polarization is transferred from the radical electron to the labeled nucleus [27,2]. This leads to an increase in polarization from parts per million to 10–50%. However, a limitation of hyperpolarized agents is their lifetime, or the longitudinal T1 relaxation time of the polarized carbons, which determines how fast the polarization is lost after dissolution. Relaxation times are typically less than a minute. A meaningful brain study therefore requires rapid dissolution and injection of the hyperpolarized agents, as well as rapid transport across the BBB and a fast metabolic rate. In addition, a rapid data acquisition strategy is required with minimal excitation of the injected hyperpolarized labeled compound and optimal excitation of the downstream metabolic products, as each excitation results in an accelerated and non-renewable decay of the hyperpolarization [30,61–65]. This has led to a trade-off between spatial resolution and acquisition time [30]. In spite of these challenges, several hyperpolarized ^{13}C agents as well as novel imaging methods have been developed over the past decade to specifically image metabolism including the aforementioned metabolic pathways that are reprogrammed in brain tumors (see Figure 1 and Tables 1 and 2).

Pyruvate at the “cross-roads” of several metabolic pathways

[1-^{13}C]-pyruvate is the poster-child of hyperpolarized ^{13}C probes. It has a relatively long T1 (~67s at 3T), a high polarization level (up to 40%) [66], and is also highly biologically relevant. Pyruvate is the end product of glucose degradation and at the intersection of several metabolic pathways that are altered in brain tumors. As mentioned previously, GBMs are characterized by their up-regulation of aerobic glycolysis (also known as the “Warburg” effect) [31] via increased expression of LDHA, the enzyme that converts pyruvate into lactate, and decreased activity of PDH, the enzyme responsible for pyruvate flux into the TCA cycle. In contrast, in low-grade mutant IDH1 tumors, LDHA is silenced [42]. Hyperpolarized [1-^{13}C]-pyruvate can be used to monitor LDHA and PDH status by monitoring the production of [1-^{13}C]-lactate and [^{13}C]-bicarbonate respectively (Figure 1). During the past decade there has also been a growing interest in using hyperpolarized [2-^{13}C]-pyruvate to monitor TCA cycle flux by probing for [5-^{13}C]-glutamate formation simultaneously with the production of [2-^{13}C]-lactate (Figure 1). Similarly to [1-^{13}C]-pyruvate, [2-^{13}C]-pyruvate fulfills the technical requirements for hyperpolarized studies (T1~40s at 3T and polarization level up to 27%) [30]. However, its use in the study of brain tumors *in vivo*, as well as other organs, has been limited due to the relatively low SNR of [5-^{13}C]-glutamate and the requirement for a wide spectral window compared to studies with [1-^{13}C]-pyruvate (~24ppm between [2-^{13}C]-pyruvate and [5-^{13}C]-glutamate or 136ppm between [2-^{13}C]-pyruvate and [2-^{13}C]-lactate compared to only ~12 ppm between [1-^{13}C]-pyruvate and [1-^{13}C]-lactate) [30].

[1-^{13}C]-pyruvate

Different acquisition strategies have been developed and used to monitor the metabolic conversion of [1-^{13}C]-pyruvate as previously reviewed in detail [30]. Briefly, dynamic data sets from live cells have been obtained by acquiring sequential spectra using a pulse-acquire sequence with a low flip angle [57,67–69]. *In vivo* animal brain tumor studies have been performed using either dynamic or single time point 2D chemical shift imaging (CSI) with low flip angle [70,71]. An alternate approach used is single time point 2D MR spectroscopic

imaging using a double spin echo RF pulse with variable flip angles [72,73] (see Tables 1 and 2).

Elevated hyperpolarized [1-^{13}C]-lactate production following the injection of hyperpolarized [1-^{13}C]-pyruvate was detected in several preclinical orthotopic GBM models compared to normal brain [67,70,72-74]. In contrast, in a low-grade IDH1 mutant orthotopic tumor model where LDHA is silenced [42], very low production of hyperpolarized [1-^{13}C]-lactate was observed [69]. Other studies showed a decreased level of hyperpolarized [^{13}C]-bicarbonate production as a result of decrease PDH activity in a preclinical GBM model [74]. Additionally, in a recent study, hyperpolarized [1-^{13}C]-pyruvate was used as an imaging marker to monitor decreased expression of MCT1 (responsible primarily for pyruvate cellular influx) and MCT4 (responsible primarily for lactate cellular efflux) in immortalized normal human astrocytes (NHAs) harboring the IDH1 mutation as compared to the IDH1 wild-type NHAs [57].

Response to treatment was also studied using hyperpolarized [1-^{13}C]-pyruvate in GBM. A decrease in hyperpolarized [1-^{13}C]-lactate/[1-^{13}C]-pyruvate ratio was correlated with a drop in LDHA expression and HIF-1α activity in response to Everolimus, a first-generation mTOR inhibitor, and in response to LY294002, a PI3K inhibitor, in GS-2 GBM cells [37,67] and in a GS-2 rat orthotopic tumor model [73]. A similar observation was made following treatment with Voxtalisib, a second-generation dual PI3K/mTOR inhibitor in GS-2 and U87 GBM models in mice [70]. In response to treatment with Temozolomide (TMZ), the current standard of care for GBM, a decrease in pyruvate kinase M2 (PKM2) in orthotopic U87 and GS-2 GBM models in rats also led to a decrease in hyperpolarized [1-^{13}C]-lactate production [70,75,76]. Importantly, a drop in hyperpolarized [1-^{13}C]-lactate production in all these models was an early event that occurred prior to tumor shrinkage and was associated with increased animal survival. Recently, treatment with dichloroacetate (DCA), a PDH activator, resulted in a drop in hyperpolarized [1-^{13}C]-lactate/[^{13}C]-bicarbonate ratio in rat C6 GBM [74]. The [1-^{13}C]-lactate/[1-^{13}C]-pyruvate ratio was also used as an index of response to radiotherapy in C6 GBM [71]. Finally, one study reported the potential of hyperpolarized [1-^{13}C]-pyruvate to evaluate response to the histone deacetylase inhibitor SAHA in a GBM cell model (GBM14), and, more importantly, to serve as a biomarker of acquired resistance to treatment [68]. In contrast, in mutant IDH1 tumors, recent studies in our lab demonstrate that response to TMZ-treatment and tumor shrinkage did not lead to a detectable drop in hyperpolarized [1-^{13}C]-lactate production [69].

[2-^{13}C]-pyruvate

Recently, one study reported the first application of hyperpolarized [2-^{13}C]-pyruvate in a rat C6 GBM model [77]. Using a volumetric spiral chemical shift imaging method, reduced hyperpolarized [5-^{13}C]-glutamate and increased [2-^{13}C]-lactate were observed compared to normal brain. Upon DCA injection, these changes were reversed. Similarly, in models of mutant IDH1 cells, lower hyperpolarized [5-^{13}C]-glutamate production following [2-^{13}C]-pyruvate injection was observed in two genetically engineered cell models (U87 and NHAs) expressing the IDH1 mutation as compared to cells expressing wild-type IDH1 associated with a HIF-1α-mediated decrease in PDH activity [58]. In the same study, treatment with

DCA also resulted in an increase in hyperpolarized [5-^{13}C]-glutamate. This points to one metabolic event that is similar in primary GBM and mutant IDH1 glioma cells.

Specific biomarker of IDH1 mutation: hyperpolarized [1-^{13}C]-alpha ketoglutarate

As mentioned previously, the IDH1 mutation leads to elevated levels of 2-HG production. Monitoring IDH1 mutational status using hyperpolarized ^{13}C MRS is therefore of great value. Recently, following the optimization of [1-^{13}C]-α-KG as an hyperpolarized agent with sufficiently long T1 and adequate polarization (T1~52s at 3T and polarization level~16% [30,78]), our group showed the accumulation of [1-^{13}C]-2-HG (Figure 1) both *in vitro* and in an orthotopic preclinical model engineered to express mutant IDH1 [78]. Additionally, a drop in the conversion of [1-^{13}C]-α-KG to [1-^{13}C]-glutamate (Figure 1) was also detected in the same orthotopic glioma model and was correlated with a drop in the activity and expression of several enzymes (BCAT1, AST1/2, GDH1/2) that catalyze the α-KG to glutamate conversion, associated with their 2-HG-induced promoter methylation and silencing [79]. *In vivo* detection of [1-^{13}C]-α-KG conversion was possible thanks to a recently developed hyperpolarized ^{13}C acquisition scheme that combines the use of a multiband spectral-spatial RF pulse sequence to optimize substrate and products excitations with a variable flip angle strategy [62].

Other promising hyperpolarized probes

Other promising probes have been developed for the study of metabolism in physiological condition *in vitro* and *in vivo* [28,30,80]. These have not yet been applied to brain tumor studies, but could be of interest (Figure 1).

[1-^{13}C]-ethyl pyruvate

In hyperpolarized studies, time is a limiting factor. The faster the hyperpolarized agent can be delivered to the site of interest, the higher the SNR and chances of observing metabolic conversion. In studies of normal brain or low-grade glioma, where the BBB is not broken, agents must diffuse rapidly through the BBB. To respond to this constraint, Hurd et al. showed the advantage of using hyperpolarized [1-^{13}C]-ethyl pyruvate (T1~45s at 3T and polarization level~28–35%), an analog of hyperpolarized [1-^{13}C]-pyruvate that diffuses faster through the BBB [81]. This agent could potentially provide a better reading of tumor glycolysis. However, the requirement for an extra metabolic step, namely the conversion of ethyl pyruvate into pyruvate, prior to its subsequent metabolism, could limit the expected advantage of this probe.

[U-^{13}C]-alpha-ketobutyrate

Another analog of pyruvate, alpha-ketobutyrate (α-KB), is also reduced by LDH but has a higher specificity towards LDHB, the enzyme that is typically responsible for the conversion of lactate into pyruvate (as opposed to pyruvate which has a higher specificity for LDHA) [82]. The conversion of hyperpolarized [U-^{13}C]-α-KB (T1~52s at 3T and polarization level~10%) into [U-^{13}C]-α-hydroxybutyrate was observed in the liver, heart and kidney of a

normal rat [82]. LDHB is highly expressed in the brain; therefore following changes in LDHB could be of great interest, particularly in low-grade tumors expressing the IDH1 mutation and wherein LDHA is silenced [42].

[1-^{13}C]-acetate

Acetate is a specific biomarker of glial metabolism as it is solely taken up by astrocytes. As such, several thermal studies of ^{13}C-labeled acetate demonstrated the value of this metabolite in the study of normal brain metabolism [83,84]. Additionally, the importance of acetate as a source to fuel tumor cells has recently been demonstrated in several studies [50–52]. In the normal brain, the conversion of [1-^{13}C]-acetate or [1,2-^{13}C]-acetate (T1~40s at 9.4T and polarization level~9–17%) into [5-^{13}C]-α-KG or [4,5-^{13}C]-α-KG respectively was successfully detected using direct or polarization transfer detection techniques [85,86]. However, studies in tumors have not been performed to date.

[1-^{13}C]-dihydroascorbate

Redox balance in the tumor is correlated with aggressiveness and resistance to treatment [59,87]. Redox reactions involve multiple agents, including glutathione, thioredoxin, NADPH, dehydroascorbic acid (DHA) and vitamin C, that are all implicated in controlling the level of ROS. Hyperpolarized [1-^{13}C]-DHA has now been reported as a probe for imaging redox status (T1~57s at 3T and polarization level~6–8%) [88,89]. The detection of the conversion of hyperpolarized [1-^{13}C]-DHA into [1-^{13}C]-vitamin C in normal rat brain as well as the modulation of this reaction in a model of prostate cancer illustrated the potential of this agent [88]. DHA is transported into cells through the glucose transporter [90], making it a good candidate for *in vivo* brain studies and particularly interesting for the study of mutant IDH1 tumors wherein redox status is likely altered.

[5-^{13}C]-glutamine

[5-^{13}C]-glutamine is of interest in brain cancer metabolism due to the aforementioned importance of Myc in high-grade GBM. However, studies of the conversion of hyperpolarized [5-^{13}C]-glutamine into [5-^{13}C]-glutamate have been limited due to the short T1 of this substrate (T1~8s at 3T and polarization level~28%), the rapid degradation of glutamine into glutamate, which is also the metabolic product of interest, and the apparently slow uptake of glutamine by cells [91–94]. Currently, three studies performed on hepatoma cells and prostate cancer cells, and one *in vivo* study performed in a rat model of liver cancer, reported the use of hyperpolarized glutamine [91–94] but no other studies have been successful in using this compound.

[^{13}C]-bicarbonate

As aforementioned, acidification of the extracellular environment plays an important role in tumor development. Rapid exchange between bicarbonate and carbon dioxide depends upon pH. Therefore, monitoring the fate of hyperpolarized [^{13}C]-bicarbonate allows mapping of the extracellular pH (pHe) and studies in murine lymphoma [95] or prostate cancer [96] models reported the use of hyperpolarized [^{13}C]-bicarbonate (T1~10s at 9.4T and polarization level~16%) to image pHe. More recently, a new pH probe, [1-^{13}C]-1,2-glycerol carbonate

was developed and was used in a prostate cancer model [97]. These probes could therefore be used to monitor pH in brain tumors, where the extracellular pH is likely acidic in GBM, but likely not in the case of mutant IDH-driven tumors that have silence LDH-A and reduced lactate production [42,69].

[1,4-$^{13}C_2$]-fumarate

Increased permeability of necrotic cell plasma membrane within the tumor facilitates the entry of fumarate within necrotic cells or release of the fumarase enzyme within the tumor microenvironment, resulting in increased conversion of fumarate, an intermediate of the TCA cycle, into malate. In contrast, in normal cells where the integrity of the plasma membrane has not been compromised, the entry of fumarate is limited over the lifetime of the hyperpolarized probe. Hyperpolarized [1,4-$^{13}C_2$]-fumarate (T1~24s at 9.4T and polarization level~26–35%) has been used to monitor treatment response *in vivo* in an implanted EL-4 murine lymphoma [98,99] and human breast adenocarcinoma [100]. To date, no studies of hyperpolarized [1,4-$^{13}C_2$]-fumarate have reported in brain tumors. However, in the case of GBM, where the entry of fumarate is facilitated by the disruption of the BBB, hyperpolarized [1,4-$^{13}C_2$]-fumarate could potentially detect cell death.

[2-^{13}C, 1,2-$^{2}H_4$]-choline chloride

Monitoring choline metabolism in high- and low-grade gliomas is of significant interest due to their abnormal choline metabolism. A recent study showed that [2-^{13}C, 1,2-$^{2}H_4$]-choline chloride can be hyperpolarized, fulfills the technical requirements for hyperpolarized studies (T1~60s at 7T and polarization level~28–35%) and its conversion into [2-^{13}C, 1,2-2H_4]-acetylcholine can be detected in an *in vitro* acetyltransferase enzyme experiment [101,102]. *In vivo*, distribution of the hyperpolarized substrate was observed in the inferior vena cava, heart, aorta and kidneys [103]. However, metabolism into PC has not been reported to date in cells or *in vivo.*

Hyperpolarized [^{13}C]-urea: beyond metabolism, probing brain tumor perfusion

In addition to tumor metabolism, measuring tumor vascularity and perfusion provide crucial information to monitor tumor evolution and response to treatment. Hyperpolarized [^{13}C]-urea has been well studied in the context of prostate, liver and kidney cancers [104–106]. However, permeability of brain tissue to urea is very low limiting its application to the study of brain perfusion [107]. Recently, two novel hyperpolarized agents were developed, [^{13}C]-hydroxymethyl cyclopropane (HMCP) and [^{13}C]-t-butanol, with the latest diffusing freely into normal brain tissue [107]. In a recent study, [^{13}C]-HMCP was used to investigate tumor perfusion in an orthotopic human glioblastoma model (G55 MG) [108]. The strong correlation between hyperpolarized findings, conventional perfusion imaging and level of vascular staining detected by immuhistochemistry illustrates the potential of this new agent.

Conclusion

Over the past decade, studies using the dissolution DNP method combined with ^{13}C MRS have demonstrated the potential of this technique to change the future of brain cancer diagnosis. By enhancing the signal from ^{13}C-labeled compounds, this new metabolic imaging approach enables probing in real-time, non-invasively, and in a non-ionizing manner, major metabolic pathways and their reprogramming in cancer. So far, several agents have been developed to target different pathways and monitor response to therapies, and more will likely emerge in the years to come. Importantly, the potential of this technique is not limited to the study of brain tumors but is applicable to many other cancers and diseases [30]. Finally, the recent first-in-human study performed on prostate cancer patient [29] as well as the beginning of a clinical trial in brain tumor patients at the University of California, San Francisco, confirms the potential of this technique for translation to the clinic.

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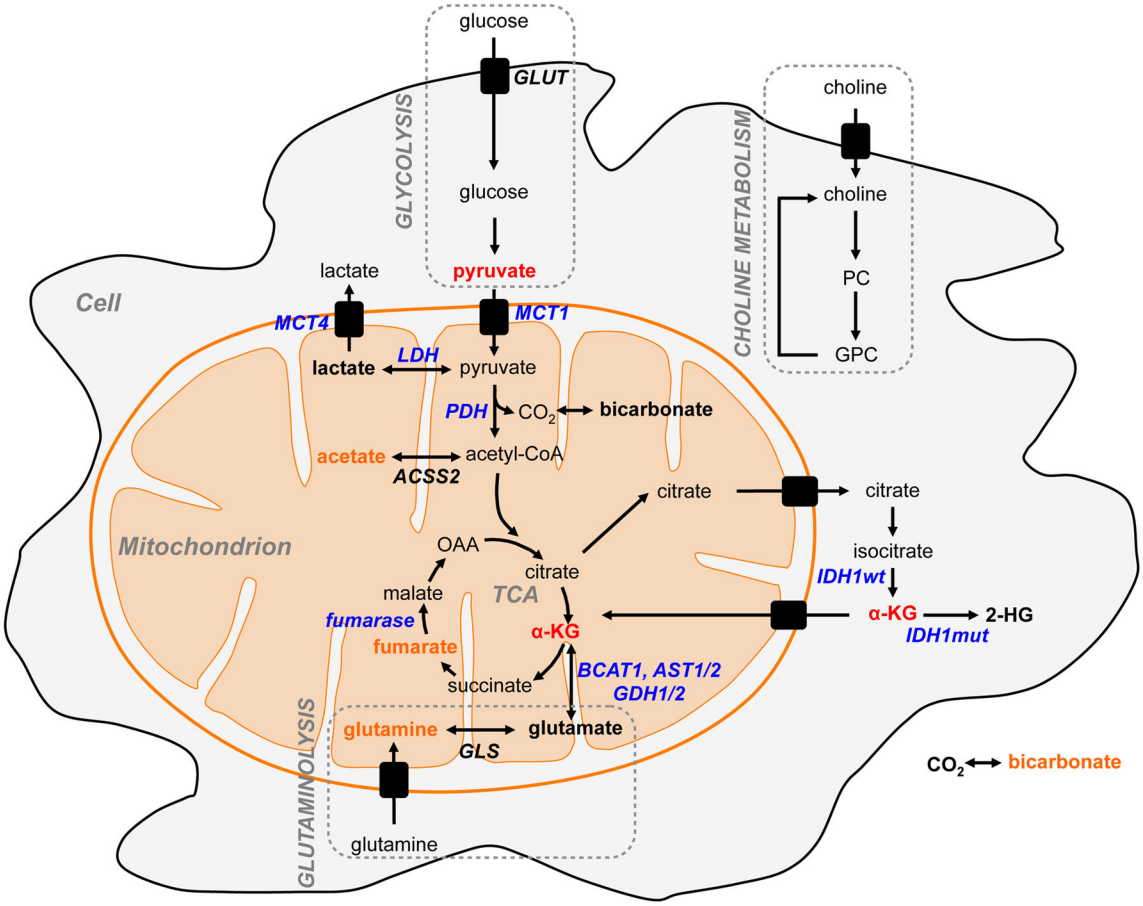

Figure 1.

Illustration of the metabolic pathways with their associated DNP probes applied to the study of GBM and low-grade mutant IDH1 glioma (red) and potential future probes (orange).

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Table 1

List of ^{13}C DNP probes that have been polarized and MR sequences applied to the study of altered metabolic pathways in orthotopic GBM model and cells.

	Altered Pathways		REF	HP ^{13}C MRS					
				HP Probe	Sequences		Use		REF
Primary GBM	•	***Pyruvate metabolism*** *through activation of PI3K/Akt/mTORC1 and stabilization of HIF1-α:* ✓ ↑ glucose transporter ✓ ↑ glycolysis ✓ ↑ PDK1 enzyme ✓ ↓ PDH activity ✓ ↑ LDHA ✓ ↑ MCTs	31, 32, 39, 40	***[1-^{13}C]-pyruvate*** T1~67s at 3T P_{hp}~14–40%	• *In vivo*: ✓ dynamic and single time point 2D CSI with low flip angle ✓ single time point 2D MR spectroscopic imaging using double spin echo RF pulse with variable flip angle • In cells: ✓ dynamic non localized acquisition with low flip angle	• *Conversion to [1-^{13}C]-lactate*: ✓ ↑ in several preclinical orthotopic GBM models (GS-2, U87, c6 glioma) ✓ ↓ after treatment with Everolimus, LY294002, Voxtalisib, Temozolomide, DCA (in GS-2, U87, c6 glioma models) • *Conversion to [^{13}C]-bicarbonate*: ✓ ↓ in rat c6 glioblastoma model ✓ ↑ with DCA treatment in rat c6 glioma model	37, 57, 66–76
				[2-^{13}C]-pyruvate T1~40s at 3T P_{hp}~27%	• *In vivo*: ✓ volumetric spiral CSI	• Conversion to [2-^{13}C]-lactate ✓ ↑ in rat c6 glioma model ✓ ↓ following DCA treatment in same model • Conversion to [5-^{13}C]-glutamate ✓ ↓ in rat c6 glioma model ✓ ↑ following DCA treatment in same model	30, 77
				[1-^{13}C]-ethyl pyruvate T1~45s at 3T P_{hp}~28–35%		• Not yet applied to the study of GBM	81
				[U-^{13}C]-α-KB T1~52s at 3T P_{hp}~10%		• Not yet applied to the study of GBM	82
	•	***Glutamine metabolism*** *by an increase of Myc activity* ✓ ↑ glutaminase	32, 46–49	***[5-^{13}C]-glutamine*** T1~8s at 3T P_{hp}~28%		• Not yet applied to the study of GBM: rapid degradation into two by-products, low glutamine uptake by cells and short T1	91–94
	•	*Abnormal* ***choline metabolism*** *through activation of PI3K/Akt/mTORC1 and stabilization of HIF1-α*: ✓ ↑ PC ✓ ↓ GPC	43–45	***[2-^{13}C, 1,2-$^{2}H_4$]-choline chloride*** T1~60s at 3T P_{hp}~28–35%		• Not yet applied to the study of GBM	101–103

Altered Pathways		REF	HP ^{13}C MRS					REF
			HP Probe	Sequences			Use	
•	***Acidification*** *of the extracellular microenvironment* ✓ ↑ glucose transporter ✓ ↑ glycolysis ✓ ↑ MCTs	33,40	***[^{13}C]-bicarbonate*** T1~10s at 9.4T P_{hp}~16%				• Not yet applied to the study of GBM	95–97
•	***Necrosis****: increase permeability of the necrotic cell membrane* ✓ ↑ uptake fumarate ✓ ↑ release fumarase enzyme	98–100	***[1,4-$^{13}C_2$]-fumarate*** T1~24s at 9.4T P_{hp} ~26–35%				• Not yet applied to the study of GBM	98–100
•	*Increased* ***acetate*** *uptake* ✓ ↑ acetyl-CoA synthase enzyme 2	50–52	***[1-^{13}C]-acetate and [U-^{13}C]-acetate*** T1~40s at 9.4T P_{hp}~9–17%	• *In vivo*: ✓ direct or polarization transfer detection technique			• *Conversion to [5-^{13}C]-α-KG and [4,5-^{13}C]-α-KG* demonstrated in normal rat brain • Not yet applied to the study of GBM	85

Table 2

List of ^{13}C DNP probes that have been polarized and MR sequences applied to the study of altered metabolic pathways in low-grade mutant IDH1 glioma orthotopic model and cells.

Altered Pathways			REF	HP ^{13}C MRS					
				HP Probe	Sequences			Use	REF
Low-grade mutant IDH1 glioma	•	Alterations linked to 2-HG accumulation	10, 42, 56–58, 79	***[1-^{13}C]-α-KG*** T1~52s at 9.4T P_{hp}~16%	• *In vivo*:	✓ Multiband spectral-spatial RF pulse with variable flip angle	•	*Conversion to [1-^{13}C]-2-HG:* ↑ both in cells and in an orthotopic glioma model harboring IDH1 mutation	62, 78,79
		✓ ↑ 2-HG					•	*Conversion to [1-^{13}C]-glutamate:* ↓ in an orthotopic U87 glioma harboring IDH1 mutation	
		✓ ↓ BCAT1, AST1/2, GDH1/2 activities			• In cells:	✓ dynamic non localized acquisition with low flip angle			
		✓ LDHA silencing							
		✓ ↓ PDH							
		✓ ↓ MCT1 and MCT4		***[1-^{13}C]-pyruvate*** T1~67s at 3T P_{hp}~14–40%	• *In vivo*:	✓ dynamic 2D CSI with low flip angle	•	Conversion to [1-^{13}C]-lactate:	69, 57
								✓ low production in an orthotopic tumor model	
					• In cells:	✓ dynamic non localized acquisition with low flip angle		✓ ↓ in cells harboring IDH1 mutation as compared to the IDH1 wild-type cells	
				[2-^{13}C]-pyruvate T1~40s at 3T P_{hp}~27%	• In cells:	✓ dynamic non localized acquisition with low flip angle	•	Conversion to [2-^{13}C]-lactate	30, 77
								✓ ↑ in U87 and NHAs cells	
								✓ ↓ following DCA treatment in same cell models	
							•	Conversion to [5-^{13}C]-glutamate	
								✓ ↓ in U87 and NHAs cells	
								✓ ↑ following DCA treatment in same cell models	
				[1-^{13}C]-ethyl pyruvate T1~45s at 3T P_{hp}~28–35%			•	Not yet applied to the study of low-grade brain tumor	81
				[U-^{13}C]-α-KB T1~52s at 3T P_{hp}~10%			•	Not yet applied to the study of low-grade brain tumor	82
	•	Elevated levels of ROS linked to reduce activity of wild-type IDH	59	***[1-^{13}C]-dihydroascorbate*** T1~57s at 3T P_{hp}~6–8%			•	*Conversion to [1-^{13}C]-vitamin C* illustrated in normal rat brain	88, 89
		✓ ↓ NADPH					•	Not yet applied to the study of brain cancer	

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Altered Pathways			REF	HP ^{13}C MRS			
				HP Probe	Sequences	Use	REF
	✓	↓ glutathione					
•	Abnormal choline metabolism associated with IDH1 mutation		43, 53–55	***[2-^{13}C, 1,2^{2}H$_4$]-choline chloride*** T1~60s at 3T P_{hp}~28–35%		•	Not yet applied to the study of low-grade brain tumor 101–103
	✓	↓ PC					
	✓	↑ GPC					
•	***Necrosis****: increase permeability of the necrotic cell membrane*		98–100	***[1,4-^{13}C$_2$]-fumarate*** T1~24s at 9.4T P_{hp} ~26–35%		•	Not yet applied to the study of GBM 98–100
	✓	↑ uptake fumarate					
	✓	↑ release fumarase enzyme					