Imaging tumor metabolism to assess disease progression and treatment response

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Financial support: Work in KMB's laboratory is supported by a Cancer Research UK Programme grant (17242) and the CRUK-EPSRC Imaging Centre in Cambridge and Manchester (16465). Clinical studies are funded by a Strategic Award from the Wellcome Trust (095962). KNT was in receipt of MRC and Cancer Research UK studentships and B.W.C. Kennedy a Cancer Research UK studentship

Conflict of interest: KMB and BWCK have patents on some aspects of hyperpolarized ^{13}C technology and KMB has research agreements with GE Healthcare.

Running title: Imaging tumor metabolism

Keywords: **tumor, metabolism, imaging, DNP, PET**

Word count: 2901

Abstract

Changes in tumor metabolism may accompany disease progression and can occur following treatment, often before there are changes in tumor size. We focus here on imaging methods that can be used to image various aspects of tumor metabolism, with an emphasis on methods that can be used for tumor grading, assessing disease progression and monitoring treatment response.

Introduction

Reprogramming of metabolism is a recognized hallmark of cancer (1), with altered fluxes in both anabolic and catabolic pathways. Recent work has shown, for example, that some breast cancers are dependent upon increased serine biosynthesis (2) and that human lung tumors show high levels of both glycolysis and glucose oxidation (3). Tumors often preferentially reduce pyruvate to lactate, rather than oxidize it in the tricarboxylic acid (TCA) cycle. This failure to oxidize pyruvate, even in the presence of oxygen (the "Warburg Effect"), is not because mitochondria in cancer cells are necessarily dysfunctional, but rather because flux of pyruvate carbon into the TCA cycle is down regulated (4). A major function of the TCA cycle in tumors is to generate building blocks for macromolecular biosynthesis, such as acetyl-CoA for fatty acid synthesis and amino acids for protein synthesis. As in other tissues, tumors can utilize fuels other than glucose for energy generation, such as glutamine, fatty acids, ketone bodies and amino acids, with tumors adapting to nutrient availability and other micro-environmental factors (4). Tumor cells can also exist in a metabolic symbiosis, whereby hypoxic tumor cells import glucose and export lactate, while nearby normoxic cells import and catabolize this lactate (5).

Altered metabolic fluxes in cancer cells are intimately linked to control of redox state. NADPH, which maintains a major cellular anti-oxidant, glutathione, in a reduced state, can be generated by flux of glutamine carbon through the TCA cycle to malate, with the production of NAPDH in the reaction catalyzed by NADP+-dependent malic enzyme (ME, EC 1.1.1.40), and by flux of glucose carbon into the pentose phosphate pathway (PPP) (4). Decreased glycolytic flux resulting from oxidation of a sensitive sulfhydryl residue in the pyruvate kinase (PK) isoform PKM2, promotes diversion of glucose carbon into the PPP (6). Flux is also diverted into the PPP by TP53-induced glycolysis and apoptosis regulator (TIGAR), which possesses fructose 2,6-bisphosphatase activity, and re-routes glycolytic flux into the PPP by decreasing the amount of fructose 2,6-bisphosphate, a potent allosteric activator of the glycolytic enzyme phosphofructokinase 1 (PFK1) (7). Flux of serine and glycine carbon through the tetrahydrofolate (THF) pathway also generates NADPH (2). Increased uptake of glucose and glutamine by cancer cells drives the hexosamine pathway, which yields metabolites involved in protein and lipid glycosylation. Increased *O*-glycosylation of proteins with N-acetylglucosamine promotes cancer cell metabolic reprogramming and cell survival and sialylation of cell-surface carbohydrates is associated with increased metastasis (8).

The differences in metabolism between tumors and normal tissues and the changes in tumor metabolism during disease progression and in response to treatment make metabolic imaging techniques important tools for detecting and grading tumors and for guiding therapy in individual patients (9) (see Figure 1 and Table 1).

PET

Positron Emission Tomography (PET) with the glucose analogue, 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) is the most widely used metabolic imaging technique in the clinic for tumor staging and assessment of treatment response (10). FDG is trapped by phosphorylation in the reaction catalyzed by the first enzyme in the glycolytic pathway, hexokinase. Many tumor cells overexpress the glucose transporters (GLUT) 1 and 3 and therefore take up large amounts of FDG. However, FDG-PET suffers from a lack of specificity as increased glucose uptake also occurs in inflammation (11). Moreover, sensitivity is low in tumors with a low glycolytic rate, such as prostate tumors, or where the tumors are located in a tissue with high glucose uptake, such as the brain (9). The Royal College of Radiologist's guidelines recommend that imaging tumor treatment response with FDG-PET should commence weeks to months after chemo- or radiotherapy (12), which hampers rapid assessment of a therapy's effectiveness. Recently, a PET tracer for imaging PKM2 expression has been described, ^{11}C]DASA-23, which may be more useful for detecting the increased aerobic glycolytic activity of tumors than FDG. The tracer showed clear delineation of implanted patient-derived glioblastoma tumors in mouse brain (13). [^{18}F]-2-deoxy-2-fluoroarabinose has been developed as a potential marker of PPP flux, accumulating predominantly in the liver, where PPP flux is high (14). Most cells take up fatty acids for oxidation and energy production and for biosynthesis of membrane components, but tumors are unusual in that they can derive fatty acids predominantly via *de novo* synthesis (15). However, hypoxic and Ras transformed cells have been shown to bypass *de novo* lipogenesis by scavenging exogenous fatty acids (16). PET measurements with ^{11}C-acetate have been used to measure *de novo* fatty acid synthesis in a range of cancers (17). ^{11}C-acetate trapped by acetyl-CoA synthetases may also be used for non-lipogenic pathways, such as acetylation of histones (18). In prostate cancer it is unclear whether ^{11}C-acetate PET adds more clinical information than measurements of prostate-specific antigen (PSA) levels in plasma (19). Glutamine metabolism in many cancers is reprogrammed by oncogenes such as *MYC* and *RAS*, redirecting glutamine into biosynthetic pathways to promote proliferation and redox homeostasis as well as fuelling the hexosamine pathway (4). The PET tracer, ^{18}F-(*2S,4R*)4-fluoroglutamine (^{18}F-FGln) showed a high tumour-to-brain uptake ratio in glioblastoma-bearing mice as well as in glioblastoma patients with progressive disease (20), which was reduced following chemo- and radiotherapy in the mice. As there is no uptake in models of neuro-inflammation or in mice with a compromised blood brain barrier this makes ^{18}F-FGln a specific PET tracer for glutamine-addicted brain tumours. Although a wide range of PET tracers have been developed for interrogating other aspects of tumor metabolism, including uptake of other amino acids and tracers reporting on tissue redox state and pH (19, 21), few have translated into routine clinical practice (21).

A drawback of PET is that only tracer uptake can be assessed, although this may be related to underlying metabolism. Information on true metabolic conversion requires blood sampling to obtain dynamic information on metabolite formation (21). This is not a problem for magnetic resonance spectroscopy (MRS) since the spectrum can identify the observed molecule. For example, glycolytic flux cannot be assessed from PET measurements of FDG uptake, which is rather an indication of glucose uptake than glycolytic flux to pyruvate and lactate. In a study of canine tumors undergoing sequential imaging with hyperpolarized [1-^{13}C]pyruvate and FDG PET there was not always concordance between FDG uptake and [1-^{13}C]lactate labeling from hyperpolarized [1-^{13}C]pyruvate (22). An advantage of PET, however, is that very low concentrations of tracer can be used, which leaves the metabolism of the cell undisturbed.

Magnetic resonance spectroscopy

^{1}H MRS has been used clinically in the brain, where resonances from choline, lipids and N-acetylaspartate (NAA) have been observed (23) and in the prostate, where resonances from citrate and choline predominate (24). Metabolite ratios can help define malignant *versus* benign lesions in both brain and prostate, however it is questionable how useful these metabolite signals are. ^{1}H MRS data can help to identify regions for biopsy, but it is the histopathological findings rather than MR spectra that guide clinical decision making (25), and in prostate cancer MRS data adds little to multi-parametric magnetic resonance imaging (MRI) measurements (26). However, some recently discovered ^{1}H MRS biomarkers may be more useful. The "oncometabolite", 2-hydroxyglutarate (2HG), which results from a mutation in isocitrate dehydrogenase 1/2 (IDH1/2), can be detected in grade 2/3 gliomas by ^{1}H MRS (27). Although the use of 2HG levels to grade gliomas remains contentious (27), ^{1}H MRS measurements of 2HG can also be used to monitor treatment response (28). Glycine may provide another marker of brain tumor malignancy, as the ratio of glycine in tumor *versus* normal brain increases with glioma grade (29). Germline mutations in the TCA cycle enzyme succinate dehydrogenase (SDH) can be found in certain renal cancers, phaeochromocytomas, gastrointestinal stromal tumors and paragangliomas. The resulting accumulation of succinate, which can be observed in ^{1}H spectra (30), may act as an "oncometabolite" by inhibiting hypoxia-inducible factor 1 (HIF-1) degradation (31).

^{13}C MRS has been used in clinical studies of brain (32) and lung (3) tumors, where ^{13}C-labeled substrates were infused prior to surgery and tumor samples were frozen immediately after resection and extracted for ^{13}C MRS analysis. ^{13}C resonances from glutamine, glutamate, aspartate, γ-aminobutyric acid (GABA) and NAA have been detected in human brain *in vivo* at high magnetic field (7T) following infusion of [2-^{13}C]glucose (33). However, low spatial resolution and long acquisition times would make these ^{13}C MRS measurements *in vivo* challenging to use in a routine clinical setting.

^{31}P MRS was used in an early study of a human tumor *in situ* (34), however a lack of sensitivity has inhibited its use in clinical oncology. The ^{19}F nucleus is almost as sensitive as the proton to NMR detection and has the advantage that there is no background signal in biological systems. ^{19}F MRS has been used mainly for tracking labeled cells (35) and for detecting probes sensitive to various biologically relevant parameters, such as tumor hypoxia (36). A recent advance is the use of combined PET/MR scanners (37). For example, simultaneous diffusion-weighted MRI and PET measurements of ^{18}F-FDG uptake in both pre-clinical models and patients (38, 39) allowed metabolic effects to be distinguished from changes in cell viability.

CEST

Metabolites can be detected in MR images of tissue water by exploiting the chemical exchange saturation transfer (CEST) effect. Resonances of labile metabolite protons that exchange with water are selectively saturated using long, low power r.f. pulses and the resulting decrease in the water signal is detected in a conventional MR image (40). The technique has been used to detect glycogen in mouse liver (41), glucose (glucoCEST) in tumors, where the signal was shown to correlate with FDG-uptake (42), and lactate in a mouse model of lymphoma and in exercising skeletal muscle of healthy volunteers (43). However, in the case of glucoCEST, the contributions of intracellular glucose, glucose metabolites and glucose in the vasculature and interstitial space to the signal are currently unknown.

Dynamic Nuclear Polarization

Signal from injected ^{13}C-labelled metabolites can be increased greatly ($>10^4$x) by dissolution dynamic nuclear polarization (DNP) (44). Carbon-13 labeled molecules are mixed with a stable radical and cooled to ~1 K in a high magnetic field. At this temperature the electron spins in the radical are almost completely polarized. Microwave irradiation at the resonance frequency of the electron spin transfers this polarization to the ^{13}C nuclei. Rapid dissolution with superheated, pressurized buffer brings the ^{13}C-labeled molecule to room temperature with substantial retention of the nuclear spin polarization. The hyperpolarized ^{13}C-labeled substrate can then be injected and the transient hyperpolarized signal, which *in vivo* can last for 2 – 3 minutes depending on the substrate, can be detected using ^{13}C MRS or ^{13}C magnetic resonance spectroscopic imaging (MRSI) (45). More than 60 hyperpolarized molecules have been used *in vitro* and/or in animal models *in vivo* (46). Hyperpolarized [1-^{13}C]pyruvate, which in tumors exchanges the hyperpolarized ^{13}C-label with the large endogenous lactate pool, has transitioned into the clinic. In prostate cancer patients, hyperpolarized [1-^{13}C]pyruvate allowed early detection of biopsy-proven tumor lesions that were unidentifiable by standard T_2-weighted MRI (47). Other metabolites, such as [1,4-$^{13}C_2$]fumarate (a marker of necrosis (48)) and [1-^{13}C]dehydroascorbic acid (DHA, a marker of cellular redox state (49)) also show promise for clinical translation (50). Hyperpolarized [U-^{2}H, U-^{13}C]glucose has been used to measure glycolytic flux in a tumor model *in vivo*, as well as flux into the pentose phosphate pathway (51). Short-chain fatty acid metabolism can be investigated with hyperpolarized [1-^{13}C]acetate and [1-^{13}C]butyrate (52, 53), which resulted in observable labeling of acetylcarnitine in heart muscle. Amino acids have so far shown little promise as hyperpolarized substrates, as their polarization lifetimes are too short to observe slow anabolic processes and even catabolic processes, such as the deamidation of glutamine, are too slow for MRSI (54).

Hyperpolarized ^{13}C-labeled serine and glycine could in principle be used to probe mitochondrial NADPH production via THF metabolism. However, we have not observed metabolism of hyperpolarized ^{13}C-labelled serine or glycine in EL4 murine lymphoma tumors, although small amounts of labeled bicarbonate were produced following injection of hyperpolarized [1-^{13}C]glycine when data were acquired from over the whole abdomen (unpublished data).

Cytosolic redox state can be assessed by measuring the concentrations of pyruvate and lactate, which are in near-equilibrium with NAD^+ and NADH in the reaction catalyzed by lactate dehydrogenase (LDH). Similarly mitochondrial redox state can be assessed from measurements of the near-equilibrium D-3-hydroxybutyrate/acetoacetate ratio (Figure 2a). Measurements of the hyperpolarized ^{13}C-labeled lactate/pyruvate ratio in cells incubated with hyperpolarized glucose has been used to estimate the cytosolic NAD^+/NADH ratio (55). However, in a rat Morris hepatocellular carcinoma model, administration of hyperpolarized [1,3-$^{13}C_2$]ethyl acetoacetate, which was rapidly hydrolyzed by intracellular esterases to produce [1,3-$^{13}C_2$]acetoacetate, resulted in no detectable production of labeled hydroxybutyrate (56). Addition of hyperpolarized [1,3-$^{13}C_2$]acetoacetate to an EL4 murine lymphoma cell suspension showed exchange of hyperpolarized ^{13}C label with co-injected unlabeled D-3-hydroxybutyrate, which appeared to be decreased in cells treated with the chemotherapeutic drug, etoposide [Figure 2 b, c]. This exchange was also observed in implanted EL4 murine lymphoma tumors, without co-injection of unlabeled D-3-hydroxybutyrate. Interestingly, D-3-hydroxybutyrate accumulates in some ovarian cancers (57). In non tumor-bearing mice ^{13}C label was also observed in acetyl-CoA in the liver/heart region following injection of hyperpolarized [1,3-$^{13}C_2$]acetoacetate [Figure 2 d]. Injection of DL-[1,3-$^{13}C_2$, 3-^{2}H]-3-hydroxybutyric acid into non tumor-bearing mice resulted in detectable labeling of acetoacetate, pyruvate and bicarbonate, when signal was acquired from over the heart/liver region [Figure 2 e] (58).

Current research in this area is focused on improving methods for fast imaging, analyzing kinetic data with [1-^{13}C]pyruvate, and translating new substrates to the clinic (50). Higher field-strength hyperpolarizers (59) and improved hyperpolarizer designs, with automated injection systems (60), will improve the levels of polarization and decrease the transfer time from dissolution to injection. Chemical derivatization to enhance tissue uptake (61) and prolongation of the polarization lifetime by deuteration (51) may allow the use of hyperpolarized substrates that so far have been limited by their slow cell uptake and/or short polarization lifetime. The polarization process, which for a substrate such as [1-^{13}C]pyruvate, can take over an hour, can be accelerated by first polarizing protons and then transferring this polarization to the ^{13}C nuclei (62).

Imaging cell surface glycosylation

Cell surface glycosylation is important for cell-cell communication and adhesion, and hyper-sialylation of cell surface glycans confers metastatic potential to a range of cancer cells (8). Glycosylation can be imaged using appropriately labeled probes that bind specific glycan moieties (63). However, probe-based techniques give only a static picture of cell surface glycosylation, whereas metabolic labeling can give information on glycan turnover. Cell surface glycans can be metabolically labeled with unnatural azido-sugars and the incorporated sugars can then be reacted *in vivo* with bioorthogonal probes (64), and visualized, for example, with fluorescence imaging (65), MRI (66) or radionuclide imaging (67). Further development of sensitive radionuclide-labeled probes may allow high contrast real-time metabolic imaging of cancer glycosylation *in vivo*, which in the longer term has the potential to be translated to the clinic, where it could be used to image cancer progression and assess metastatic potential.

The future of metabolic imaging in oncology

There are some aspects of tumor metabolism for which there is as yet no imaging probe. Metabolic pathways important for both proliferation and resistance to oxidative stress, such as the PPP and the THF pathway, which produce NADPH, are underexplored imaging targets.

Enzymes involved in serine synthesis are emerging drug targets (68) and an imaging technique that could visualize serine/glycine metabolism and one-carbon transfer *in vivo* could improve our understanding of this branch of metabolism. Hyperpolarized [U-^{2}H, U-^{13}C]glucose can be used to measure flux through the PPP (51) and may become relevant clinically with improved levels of polarization, potential further increases in polarization lifetime (by selective ^{13}C labeling) and by employing routes of administration that allow faster delivery to the tissue of interest. Hyperpolarized [1-^{13}C]DHA as a marker of the capability of a tissue to resist oxidative stress (49) also has some potential to reveal new cancer biology. ^{1}H MRS can be used to measure "oncometabolites", such as 2HG (27) and succinate (30), and the development of hyperpolarized ^{13}C-labeled substrates that could be used to explore the pathways leading to these oncometabolites could give more dynamic information on their metabolism (69). Hyperpolarized DL-[1,3-$^{13}C_2$, 3-^{2}H]-3-hydroxybutyric acid has shown some potential for exploring ketone body metabolism *in vivo* (58). Combining different imaging modalities such as PET/MR (37) with DNP (22) will increase the information content of the imaging examination, allowing more specific information to be obtained from the relatively few metabolic imaging tracers that have been approved for clinical use (70). A newly emerging health concern in the clinic is the potential toxicity of gadolinium contrast media used in MRI (71). In principle some of the roles of these contrast media could be replaced with hyperpolarized ^{13}C-labeled cell substrates, for example hyperpolarized ^{13}C urea, which has been used to assess tissue perfusion (72). The power of metabolic imaging with hyperpolarized ^{13}C-labeled cell substrates is that it gives information on dynamic processes. However, its weakness is that there is no information on the concentrations of the labeled substrates and therefore no quantitative information on flux (expressed, for example, as M s^{-1}). While measurements of changes in this apparent first order rate constant have been demonstrated to be sufficient to detect treatment response in lab based studies, this will need to be examined carefully in clinical studies. Nevertheless the technique has great clinical potential, not only for detecting disease, disease progression (73, 74) and treatment response (75) in cancer, but also in other pathologies such as neurodegenerative disorders and inflammation (50).

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Figure legends

Figure 1 Clinical imaging of cancer metabolism. Many cancer cells metabolize glucose preferentially to lactate, even in the presence of oxygen ("Warburg effect"). Glucose is taken up on the glucose transporters (GLUT), while pyruvate and lactate enter cells through the monocarboxylate transporters (MCT). The pentose phosphate pathway (PPP) is linked to glycolysis via glucose 6-phosphate (G6P), which is oxidized by glucose 6-phosphate dehydrogenase (G6PDH). Flux into the oxidative arm of the PPP is promoted by TP53-induced glycolysis and apoptosis regulator (TIGAR). Serine and glycine can be produced from 3-phosphoglycerate (3PG) and both amino acids can fuel the tetrahydrofolate (THF) pathway. Glutamine is deamidated by glutaminase (GLS), which can yield NADPH via NADP+-dependent malic enzyme (ME, EC 1.1.1.40) activity. NADPH generated from the oxidative PPP, ME flux and the THF pathway is used for lipid biosynthesis and redox balance while intermediates from the non-oxidative PPP and the THF pathway are used for nucleic acid synthesis. Mutant isocitrate dehydrogenase (IDH) in the TCA cycle generates the "oncometabolite" 2-hydroxyglutarate (2HG). IDH1 in the cytosol is mutated in most grade II/III gliomas whereas IDH2 in the mitochondria is less commonly mutated. The hexosamine pathway branches off from glycolysis at G6P and produces substrates for cell surface glycosylation. Imaging agents that are used clinically and explore some of the above mentioned metabolic features of tumors are shown in: black and yellow (positron emission tomography tracers), green (hyperpolarized substrates) and blue (metabolites detectable by ^{1}H MRS). Abbreviations: ^{18}F-FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; ^{18}F-FGln, ^{18}F-(*2S,4R*)4-fluoroglutamine; 6PG, 6-phosphogluconate; 6PGDH, 6-phosphogluconate dehydrogenase; 6PGL, 6-phosphogluconolactone; 6PGLase, 6-phosphogluconolactonase; αKG, α-ketoglutarate; ALT, alanine aminotransferase; ChT, choline transporter DHAP, dihydroxyacetone phosphate; F1,6BP, fructose 1,6-bisphosphate; F2,6BP, fructose 2,6-bisphosphate; FA, fatty acid; G3P, glyceraldehyde 3-phosphate; LDH, lactate dehydrogenase; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PFK1/2, phosphofructokinase 1/2; Ru5P, ribulose 5-phosphate; SDH, succinate dehydrogenase; TPI, triosephosphate isomerase.

Figure 2 Assessing ketone body metabolism with hyperpolarized ^{13}C-labeled cell substrates. (a) Metabolism of the ketone bodies acetoacetate and D-3-hydroxybutyrate. Abbreviations: AcAc, acetoacetate; BDH, D-3-hydroxybutyrate dehydrogenase; D-3-HB, D-3-hydroxybutyrate; OAA, oxaloacetate; AcAcCoA, acetoacetyl CoA; ACOAS, acetyl-CoA synthetase; ACOAT, acetyl-CoA thiolase; ACYL, ATP-citrate lyase. (b) Spectrum showing the resonances observed 30 s after injection of hyperpolarised [1,3-$^{13}C_2$]acetoacetate at a final concentration of 15 mM, into an EL4 murine lymphoma cell suspension (10^8 cells). Unlabeled D-3-hydroxybutyrate was added in an equimolar amount. There are resonances from C1 [1,3-$^{13}C_2$]acetoacetate at 177.3 ppm and D-3-[1-^{13}C]hydroxybutyrate at 183.0 ppm (indicated by the arrow). (c) Labeling of D-3-hydroxybutyrate decreased after the cells were treated with etoposide for 16 h (unpublished data). (d) Injection of hyperpolarized [1,3-$^{13}C_2$]acetoacetate *in vivo*. The spectrum was obtained by summing the first 30 s of non-localised spectra, where the surface coil was placed directly over the heart/liver region (58). The peak at ~212 ppm is C3 [1,3-$^{13}C_2$]acetoacetate. The spectrum also shows a resonance at ~204 ppm, which is most likely from [1-^{13}C]acetyl-CoA. (e) Representative spectrum obtained by summing the first 30 s of data acquired following administration of 0.2 mL of 60 mM hyperpolarized DL-[1,3-$^{13}C_2$, 3-^{2}H]-3-hydroxybutyric acid to a non-tumor bearing mouse *via* a tail vein catheter, where the surface coil was placed directly over the heart/liver region. The observed resonances correspond to: 1) C1 DL-3-[1,3-$^{13}C_2$, 3-$^{2}H_1$]hydroxybutyrate (183.0 ppm); 2) [1-^{13}C]acetoacetate (177.3 ppm); 3) [1-^{13}C]pyruvate (172.8 ppm); 4) ^{13}C bicarbonate (161.3 ppm) and 5) an unknown metabolite (144.9 ppm) (53).