Interrogating Metabolism in Brain Cancer

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KEYWORDS

• Metabolic imaging • Hyperpolarization • MRS • NMR • MRI • GC/MS • DNP • CEST

KEY POINTS

- Many existing and emerging techniques of interrogating metabolism in brain cancer are at an early stage of development.
- A few clinical trials that employ these techniques are in progress in patients with brain cancer to establish the clinical efficacy of these techniques.
- It is likely that in vivo metabolomics and metabolic imaging is the next frontier in brain cancer diagnosis and assessing therapeutic efficacy.

MR SPECTROSCOPY/NMR SPECTROSCOPY–BASED METABOLOMICS IN BRAIN TUMOR

Metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles.[1] Recently, metabolomics in cancer research is gaining considerable importance. The application of Nuclear Magnetic Resonance (NMR)-based and Magnetic Resonance Spectroscopy (MRS)-based metabolomics as applied to brain cancer is an evolving area of clinical use and investigation. In brain tumors, diagnosing tumor type and grade non-invasively has been a clinical challenge. ^{1}H MRS/NMR–based metabolomics has been explored to identify elevated metabolites in malignant tissue specifically in contrast with normal brain. Interestingly, most of the ^{1}H MRS in vivo studies to date have been done on the brain. This is primarily owing to the reduced effects of motion and lipid contamination in the brain. The global metabolic profile of live cells or cell extracts from established glioma models has been determined using standard NMR methods, and validated using tumor biopsies obtained from animal models or patients that have been imaged using high-resolution magic angle spinning spectroscopy.[2,3] Such approaches complement data to the in vivo findings.

Detected Metabolites Using ^{1}H MR Spectroscopy

^{1}H MRS is used extensively to monitor the steady-state levels of major endogenous cellular metabolites. For a full review of in vivo ^{1}H MRS-detectable

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metabolites, see De Graaf.[4] In the field of neurooncology, the most prevalent metabolites in the ^{1}H MR spectrum are *N*-acetylasparate (NAA), total choline-containing metabolites (Cho), lactate (Lac), mobile lipids, creatine (Cre), glutamate (Glu), glutamine (Gln), Gln and Glu, glycine, glutathione, and 2-hydroxyglutarate (2-HG). The largest signal in normal healthy brain tissue is NAA and the NAA level typically decreases in gliomas.[5] The Cho signal is a composite of free choline, phosphocholine and glycerophosphocholine, 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 increased in cancer.[6] Lactate is the end product of aerobic glycolysis and is enhanced in cancer as part of the Warburg effect.[7] Lipids (long chain fatty acids), especially lipid droplets known as mobile lipids or triglycerides, are rarely observed in the normal brain, but are often increased in glial tumors and are associated with cell death and increased necrosis.[8] The Cre signal is a composite of Cre and phosphocreatine, which are involved in energy metabolism via the creatine kinase reaction. Cre levels vary within normal brain regions and in some cases with tumorigenesis.[9] The amino acid Glu is the most abundant amino acid in the brain and an essential neurotransmitter. In gliomas, glutaminolysis is often required for tumor growth as an anaplerotic source of carbon complementary to glucose metabolism.[2] Finally, with the recent discovery of the isocitrate dehydrogenase (IDH) mutation, the most common mutation in oligodendroglioma and astrocytoma tumors,[10] increased levels of 2-HG, which is produced from α-ketoglutarate (α-KG) 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 TE (<50 ms), most metabolites can be observed, but overlappings between resonances often hampers proper quantification; on the other hand, when using a long TE (>120 ms), only a few metabolites remain visible, but their respective resonances can be readily identified and quantified.[9]

Detected Metabolites Using ^{13}C MR Spectroscopy

Early ^{13}C MRS/NMR investigations were used to monitor glucose metabolism and Lac turnover during steady-state hyperglycemia with stable isotopically labeled, [1-^{13}C] glucose in C6 glioma-bearing rats.[11] Labeling of glucose-derived [3-^{13}C] Lac, [4-^{13}C] Glu, [4-^{13}C] Gln, and [1-^{13}C] glycogen could all be detected. More important, increased labeled Lac with reduced labeling in Glu and Gln were observed when comparing tumor with normal contralateral brain, consistent with the Warburg effect and a reduction in flux into the tricarboxylic acid (TCA) cycle. ^{13}C MRS studies investigating glioblastoma (GBM) cells and a combination of ^{13}C-labeled glucose and ^{13}C-labeled Gln have also shed light on the possible role of Gln in high-grade brain tumors. Conversion of Gln to Lac via glutaminolysis was found to be sufficient to produce NADPH required for fatty acid synthesis. More recently, studies of primary human GBM models in mice infused with ^{13}C-labeled glucose further demonstrated not only increased glycolysis, but also active glucose metabolism via the TCA cycle to Glu and Gln, confirming that flux via pyruvate dehydrogenase was not suppressed in GBM. However, this study showed limited glutaminolysis.[12] Using ^{13}C MRS to probe the fate of ^{13}C-labeled acetate in orthotopic brain tumors, a recent investigation demonstrated that acetate is oxidized via the TCA cycle, together with glucose, to generate labeled Gln and Glu. This identifies an additional metabolite that could help to meet the high biosynthetic and bioenergetic demands of GBM tumor growth.[13] Future studies using additional ^{13}C-labeled substrates could be envisaged to shed further light on the metabolism of GBM and, as models are being developed, on the metabolism of lower grade brain tumors.

Clinical ^{1}H MR Spectroscopy

Numerous studies have highlighted the potential benefits of using ^{1}H MRS to estimate metabolite levels in brain tumors in the clinic.[14] When combined with similar spatial localization techniques that are used in generating anatomic MR images, this strategy can be used to produce maps of the variations in levels of choline containing compounds, Cre, NAA, Lac, and mobile lipids. 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 to 10 minutes and the spatial resolution of the voxels obtained is typically 0.5 to 1 cm^{3}.[15] More recent advances in pulse sequence development and spectral editing schemes have facilitated the detection of metabolites with shorter T_2 relaxation times and lower signal-to-noise ratios such as Glu, Gln, Gln and Glu , and 2-HG, expanding the investigation