Effects of reactive oxygen species on metabolism monitored by longitudinal $^1$H single voxel MRS follow-up in patients with mitochondrial disease or cerebral tumors

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Abstract. Free radicals, or Reactive Oxygen Species (ROS), have an effect on energy and glycolytic metabolism, mitochondrial function, lipid metabolism, necrosis and apoptosis, cell proliferation, and infiltration. These changes could be monitored longitudinally (every 4 months over 6 years) in humans with glial brain tumors (low and high grade) after therapy, using conventional magnetic resonance imaging (MRI) and spectroscopy (MRS) and MR perfusion. Some examples of early clinical data from longitudinal follow-up monitoring in humans of energy and glycolytic metabolism, lipid metabolism, necrosis, proliferation, and infiltration measured by conventional MRI, MRS and perfusion, and positron emission tomography (PET) are shown in glial brain tumors after therapy. Despite the difficulty, the variability and unknown factors, these repeated measurements give us a better insight into the nature of the different processes, tumor progression and therapeutic response.

1. Introduction
Diagnosis of cancer and mitochondrial diseases is challenging despite the availability of large numbers of imaging and biochemical investigational techniques and data. The conventional magnetic resonance imaging (MRI) provides information on tumor anatomy, its extent, and pathology; however the specificity of diagnosis distinguishing between benign and malignant disease is still not optimal. Furthermore, MRI does not provide information on the underlying biochemical processes associated with tumor progression and regression. Thus, the biochemical information from tissues may be useful for specific diagnosis and accurate prognosis when used as an adjunct to anatomic details obtained from MR images. This can be achieved through in vivo Magnetic Resonance Spectroscopy.
(MRS) that provides unique metabolic information, at the molecular level, of diagnostic and prognostic importance for tumor typing, and grading. The tissues contain several biologically important metabolites (biochemicals) in addition to water and fat hydrogen atoms that could be followed by MRS. It also provides information on the alterations in metabolic pathways during disease processes by detection and quantification of metabolites. MRS is also a useful tool in the planning and evaluation of treatments and in predicting tumor progression and treatment response. It is known that tumor growth is not a simple regulated phenomenon. There are variations in metabolism and cell density, and in the presence of cystic and necrotic regions (particularly in malignant tumors). Thus, the MR spectrum obtained from a tumor tissue can be a mixture of different tissue types, including those of different grades, which complicates the spectral interpretation. Most MRS measurements are based on the use of proton (1H) nuclei, thanks to its great sensitivity. The integration of 1H MRS with clinical MRI investigation is relatively straightforward and easy to implement. Addition of in vivo 1H MRS protocol increases the overall acquisition time by approximately 10 to 20 minutes but it drastically improves the diagnostic and prognostic accuracy. This method has generated considerable interest in recent years and the added value of the in vivo 1H MRS is in process of evaluation in clinical application. In particular it would allow quantification of metabolites from a well-defined volume of interest (VOI) or volume element (voxel). Moreover, the heterogeneous nature of tumors could be understood and detected, which would be of great interest for biopsy guidance and planning, monitoring, and evaluation of treatments such as surgery, radiotherapy, and chemotherapy. Therefore, the possibility to obtain in vivo quantitative biochemical information has revolutionized the field of clinical neurooncology. In the present work, metabolic information obtained in MRS measurements, associated with anatomic data, allows us to establish an accurate prognosis based on a spectral and metabolic classification system. In addition, in longitudinal follow-up studies, multimodality (with Positron Emission Tomography (PET) and perfusion) allow us to study the relationship between MRS, PET data, MRI segmentation and perfusion, together with some biologic parameters (eg, proliferation, necrosis, lactate, glutamine, some Reactive Oxygen Species (ROS) and their consequences, and antioxidants) thus helping to characterize sensitive biomarkers and to detect early changes in order to assess rapidly and more accurately therapies. Moreover, MRS data to be evaluated have to be related to data such as the response to therapy, histology, and genetic prognostic mutations (Isocitrate Dehydrogenase 1 IDH1, 1p19q, Methyle guanine methyl transferase MGMT).

1.1 Contribution of MRS in management of brain tumors

Numerous studies reported in the literature have shown that 1H MRS contributes significantly to the management of brain tumors. The major metabolites seen at long, intermediate and short echo times are N-Acetyl-aspartate (NAA; 2.02 ppm), total creatine (tCr; 3.03 ppm) and choline containing compounds (tCho; 3.2 ppm). NAA is found predominantly in neurons. The tCr level is reduced in astrocytomas and is nearly absent in meningiomas and schwannomas, indicating changes in energy metabolism. Gliomatosis cerebri is an uncommon and infiltrative type of glioma which is difficult to diagnose but has higher levels of tCr, thus distinguishing it from low-grade gliomas [1]. The tCho/tCr ratio increases with the grade of the glioma [2] but the large overlap of ratios between grades prevents its individual use as a reliable grading index. The level of tCho increases from grade II to grade III in astrocytomas, but grade IV tumors show wide variation due to the heterogeneity in cell density and necrosis. In low-grade pediatric gliomas, citrate was first found to be elevated [3] followed by an increase in tCho as the tumor progressed. The ratio tCho/NAA was shown to correlate with long-term survival independent of tumor type [4]. In addition to the above predominant metabolites observed at long echo times, additional metabolites such as Glx (glutamate (Glu) and glutamine (Gln), at 3.6-3.9 ppm and 2.1 - 2.6 ppm) and myo-inositol (mI, at 3.56 ppm) are seen at shorter echo times and these also have diagnostic and prognostic potential. Myo-inositol is a major metabolite found in glia but not in neurons [5] and has a role as an osmolyte. Elevated mI is seen in low-grade gliomas, especially in oligodendrogliomas, however it is decreased in high-grades. Higher Glx and glutathione (GSH, multiplet at 3.8 ppm) are seen in meningiomas compared to astrocytomas [6]. Alanine (Ala, doublet at
1.47 ppm) is also seen, which is very suggestive of meningioma [7]. These observations may indicate that energy metabolism is mainly carried out by partial oxidation of glutamine rather than glycolysis, with alanine being the end product instead of lactate. Elevated Gln is also found in some oligodendrogliomas, which could help (in conjunction with high ml) discriminate them from astrocytomas [8]. Lactate (a doublet at 1.33 ppm) is observed in tumours resulting from glycolysis [9], and lipids (1.3 and 0.9 ppm) are seen in high-grade tumors [10;11;12].

1.2 ROS and mitochondrial tumor suppressor genes roles in tumor development

The metabolic adaptation in tumors, the Warburg effect, induces a high rate of glycolysis in tumors, and sometimes a concurrent defect in mitochondrial respiration. These changes are hypothesized to be due to mitochondrial tumor suppressor genes. For example, succinate dehydrogenase and fumarate hydratase [13] are mitochondrial proteins of the tricarboxylic acid (TCA) cycle and the respiratory chain, and when mutated lead to tumors (for succinate dehydrogenase); it is known as paragangliomas and pheochromocytomas. Recently, a novel mitochondrial protein, SDHAF2 (SDH5), has also been shown to be a paraganglioma-related tumor suppressor gene. Another mitochondrial and TCA cycle related protein, isocitrate dehydrogenase 1(IDH1), uses Nicotinamide adénine dinucléotide phosphate hydrated (NADPH) as a cofactor and is frequently mutated in glioblastomas [14]. It should be noticed that an accumulation of 2-hydroxyglutarate (2-HG) is associated with this IDH1 mutation [15]. There are currently many competing hypotheses concerning the role of these genes in tumorigenesis, but frequently recurring themes are the stabilization of hypoxia-inducible factor 1-alpha (HIF1-alpha) and upregulation of genes involved in angiogenesis, glucose transport, and glycolysis. Other postulated mechanisms include the inhibition of developmental apoptosis, altered gene expression due to histone deregulation, and the acquisition of novel catalytic properties. Effects of these gene mutations on metabolism are not clear, but they can be studied by MRS. It has been suggested in particular that oxidative stress and thus ROS formation could be actively involved in these processes (cf. figure 1). ROS play a role in the regulation of cell survival or death by mediating the apoptosis, but surprisingly ROS can also favor an opposite effect such as the tumor cell proliferation. Tumor cells manage to control high extracellular ROS production and this prevent apoptosis induction by ROS but still maintain a sufficient level of ROS for proliferation stimulation. [16]

1.3 Studying the relationship between the MRS measures, ROS and antioxidants

Detection of the metabolites and processes using MRS is crucial to study oxidative stress and redox balance. We suggest that using MRS to identify metabolites (especially those from the Krebs cycle seen in blue in figure 1) involved in the formation of ROS and glutathione (GSH), could inform about the balance between ROS and antioxidant formation involved in some diseases and tumor processes and thus helps to better understand the role of ROS particularly in cancer [17,18,19]. It is known that succinate oxidation can support the higher rate of ROS production in non-phosphorylating mitochondria. Accumulation of succinate [13] during the hypoxic period might also contribute to increase mitochondrial ROS generation upon reoxygenation. The quantification of succinate and acetate in vivo can be correlated with the ROS production. However, in vivo succinate comes also from bacteria or from impaired tissue respiratory chain. Similarly, ROS could come from impaired tissue respiratory chain, from macrophages or from neutrophils.

MRS enables us to characterize pathologic processes such as proliferative potential, which correlates with a high level of choline, particularly in gliomas. These data could make possible the study of processes put into place by the cells in response to oxidative stress and the involvement of ROS in the proliferation of tumor cells, in particular by activation of HIF and angiogenesis [20]. Necrosis can also be characterized in MRS by the quantification of free phospholipids. This knowledge is useful in the study of lipid peroxidation and necrosis/apoptosis induced by ROS and therapies. Lactate is observed in tumors due to the metabolic switch that occurs when the Krebs cycle is altered and glycolysis increases. It is known that ROS are involved in these changes through the
regulation of glycolysis enzymes, and studying lactate by MRS is thus used to better understand this ROS-induced metabolic switch. A correlation was not always found between lactate levels and tumor grade or metabolic rate. Lactate accumulation occurs in both intra- and extra-cellular spaces and its overall level is a function of metabolic rate and clearance; hence in higher-grade tumors lactate may pool in necrotic or cystic regions independent of increased glycolysis. In addition, this type of study gives some insight into pH change in tumors, which is important for therapeutic efficacy.

It would be of interest to do non invasive longitudinal follow-up studies of these metabolites as well as pathological processes such as proliferation, hyperperfusion, necrosis, ROS, and disease processes.

**Figure 1.** Metabolites (measurable by MRS, in blue) and metabolism involved in ROS and antioxidant balance.
2. Examples of potential effects of ROS on the metabolism in different pathologies

MRS is a non-invasive and reproducible technique for monitoring this energy metabolism in vivo in normal and diseases states. In this study, the aim of this technique was to try to better understand the potential relationship between free radicals and metabolism on these processes in some diseases such as mitochondrial disease, abscesses or cancer.

2.1 Mitochondrial-dependent diseases

Mitochondrial-dependent diseases are a group of disorders caused by dysfunctional mitochondria. In these diseases, MRS is a tool which allows us to better understand the metabolism changes such as lactate production and evolution (figure 2) and their relationships with genetic, biochemical and free radical abnormalities. However, these diseases present some difficulties for MRS study. In fact, abnormalities and processes are heterogeneous and multifocal. In addition, there is a fair amount of temporal variation.

![Figure 2. Evolution of mitochondrial-dependent disease: lactate (at 1.3 ppm) increase in left occipital region (a); one of the first exams at short TE (b); after stroke-like episodes at short TE (c) and long TE (d); with lactate resonance TE modulation: positive in (c) and negative in (d)](image)

2.2 Gliomas (with and without treatment)

The World Health Organization (WHO) classification is based on the presumed cell origin, distinguishes astrocytic, oligodendrocytic, and mixed gliomas. A grading system is based on the presence of the following criteria: increased cellular density, nuclear atypia, mitosis, vascular proliferation, and necrosis. The main histological subtype of grade I gliomas are pilocytic astrocytomas, which are benign. Diffuse astrocytomas, oligodendrogliomas and oligoastrocytomas are low-grade (II) or high-grade (III and IV) tumors. Glioblastomas correspond to grade IV astrocytomas.
Characteristics of spectroscopic profiles of glioma often show an increase in Choline/N-Acetyl-Aspartate (Cho/NAA, choline at 3.22 ppm and NAA at 2.02 ppm), Cho/Cr linked to proliferation and Myoinositol/Creatine (mI/Cr) ratios, a decrease in NAA/Cr, correlate better with infiltration. Sometimes the spectral profile presents an increase in lactate and lipids which correlates with necrosis (figure 3).

![Figure 3](image)

**Figure 3.** Example of spectral variation in glioma with SCI-MRS-LAB.

Cho (containing metabolites glycerophosphocholine (GPC) and phosphocholine (PC) at 3.22 ppm) elevated: proliferation. Lactate (at 1.33 ppm): glycolysis. NAA/Cr (NAA at 2.02 ppm and Cr at 3.03 ppm) decreased: infiltration.

Cho/Cr and mI/Cr decrease under chemotherapy (Temozolomide ®). NAA/Cr ratios are variable and have a tendency to improve under Temozolomide in gliomatosis. None or very little change in NAA/Cr occurs in oligodendrogliomas. A study of the contralateral side is very important after therapy to assess effects of these therapies on the remaining non-tumoral neuronal tissues. MRS can also contribute to the differential diagnosis between a tumoral and non tumoral lesion, especially in abscesses.

3. Methods and Results

3.1 - MRI acquisition

Measurements of Sagittal T1, axial proton density, T2, FLAIR, diffusion, perfusion, 3D T1 and 3D T1 planes after gadolinium were acquired. Total tumoral volume, volume of FLAIR and T2 hypersignal, contrast enhancement, hyperperfusion and necrosis volumes are available on the MRI. A late enhancement should be taken into account. A segmentation allows a more quantitative multispectral MRI analysis to estimate tumor volumes, oedema and necrosis (figure 4, [21,22]). These data allow us to show that treated tumor volumes, observed on MRI, change little between two measurements while spectroscopic profiles and Cho/Cr or mI/Cr ratios decrease (see below section 3.5).
3.2 MRS acquisition, data processing and results

Single voxel (6 to 12 cm³) from 1.5 T machine were done on most aggressive area (figure 5) and in contra-lateral side (figure 6) with PRESS MRS sequence and with multiple TE (35, 144, 288, 432 ms). Measured volume size ranged from 1 cm³ to 27 cm³, Concentrations ranged from 0.25 mM to 100 mM, TE influence on the detected resonance number. The lowest statistical significant estimated concentration is in general around 1mM.

Figure 4. Manual segmentation and tissue classification of the different compartments from different weighted (T1, T2 and proton density) images from an oligodendroglioma

Figure 5. Example of spectra from glial tumor patients at 1.5 Tesla in PRESS sequences at different TE: 35 ms (left) and 144 ms (right)

Figure 6. Magnetic Resonance Spectroscopy single voxel short TE 35 ms spectrum from white matter contra-lateral side close to a normal spectrum
MRS acquisition is sensitive to some parameters and some instrumental problems: localization quality, saturation bands and homogeneity [23]. This is even more evident in large volumes like CSI. SA/GE software (figure 7-a) and a home-written SCI-MRS-LAB [24,25] (at CHU and Caen University and in Scilab INRIA-ENPC open source code) automatic processing software yield amplitudes, areas, ratios, and relative concentrations. Quantification based on amplitude and area (proportional to concentration and relaxation) estimation in spectral domain needs solvent resonance(s) suppression and presents some difficulties such as unknown resonances and molecules metabolism. This process requires normalization with relative scale with ratio to another metabolite such as water, NAA, or Cr or absolute metabolite concentration (absolute scale).

**Figure 7.** Example and comparison of SCI-MRS-LAB (a) and SAGE (b) processing for short TE spectra from normal healthy volunteer.
There are few quantification methods (SA/GE, JMRUI, SCI-MRS-LAB, sparse representation [30]), which include different processing steps like water suppression, baseline estimation, fitting and analysis [26-29]. Figure 7 shows an example of quantification methods comparison between Vendor tools for example SAGE (Spectroscopy Analysis by General Electric) (b) and SCI-MRS-LAB (a) Usually, we can obtain much better water signal suppression and baseline estimation with SCI-MRS-LAB than with some wavelets or SAGE. SCI-MRS-LAB gives also better results than SAGE for overlapped coupled spins as with glutamate and glutamine, citrate or an usual additional resonances to fit.

Acquisition of the spectral signatures in vitro of metabolites requires accurate protocol in order to identify and measure them (as for example GSH in figure 8) in patients. This protocol, optimized by Gabriela Hossu [29], optimizes the accuracy for frequency shift (eg, due to temperature or pH) of measurement and also the reproducibility. It is of great interest to be able to detect and measure directly or indirectly tissular antioxidant level with different therapies [34].

![Figure 8](image)

**Figure 8.** In vitro spectrum of glutathione (GSH): GSH resonances TE modulation:
positive in a) at TE 35 ms, partially negative in b) at TE 144 ms and again positive at TE 288 ms.

3.3 Perfusion
This NMR technique allows the study of cerebral microcirculation with a very fast slice acquisition after gadolinium injection (first past perfusion). The evaluation of new methods of perfusion in clinical conditions involves Spin labelling, first passage (figure 9), dynamic relaxometry, modelization of index size of vessels, evaluation of variability (reserve Vaso Constriction, Vaso Dilatation (Diamox, CO2)).

![Figure 9](image)

**Figure 9.** Glial tumor: Map (right) of relative (left) CBV ratio proportional to area under the curves to evaluate tumoral vascularization after intravenous gadolinium injection and showing hyperperfusion (in red) and hypoperfusion (in blue).
The data show some tumoral angiogenesis with important lesions of the BBB (blood-brain barrier). The extravasation of radiocontrast agents induces an increase in the curve of first passing above the baseline. The relative Cerebral Blood Volume (rCBV) in glial tumors is often elevated between 2 and 6 and shows hyperperfusion.

### 3.4 PET and multimodality

These techniques allow to study the relationship between MRI (FLAIR, T1, perfusion) and MRS. Two examples of relationships in the proliferation study and in the hypoxia and necrosis study with free radicals are given in figures 10 and 11 respectively.

### Anatomical

| FLAIR | T1w post inj |
|-------|-------------|

### Perfusion

| 11C-Met | 18F-FLT |

### Metabolism PET

#### Proliferation in MRS: Cho/Cr elevated

**Figure 10.** Proliferation study in PET and MRS (Cho/Cr): on this patient we can detect contrast enhancement on T1 post injection, hyperperfusion, hyperfixation on Met and FLT PET images and very important Cho/Cr increase on MRS (Cho at 3.2 ppm and Cr at 3.03 ppm) showing proliferation.

There is a large variability, but the repetition and the modelization of spectroscopic measurements during longitudinal follow-up allowed us to improve the prognostic evaluation. Studying the relationship between MRS measures, methionine and FLT PET quantitation, segmentation and perfusion parameters could lead to a better understanding of the therapeutic response, especially with regard to chemotherapy, antiangiogenic molecules, and, in the future, hypoxia modulators and other new therapies.
3.5 Interest, objectives and example of the longitudinal MRS study

The longitudinal study allowed us to better understand therapeutic response in neurosciences or cancer, especially with regard to oxidative stress metabolic steps and therapies. The aim was to try to detect MRS differences due to in infiltration, proliferation, and relevant metabolite before any change in perfusion or contrast enhancement. The aim was also to see the tissue response to therapy. These studies could enable us to improve our understanding concerning the relationships between the different stages of tumoral processes with free radicals production and key metabolites measured by MRS like pyruvate, glutathione, citrate, succinate, acetate or hydroxyglutarate. In addition, those studies could improve the different prognostic classifications and the assessment of tumoral responses to different therapies and their optimal combinations.

Figure 11. Hypoxia, lactate and necrosis study: in the same patient we can detect necrosis on the T1 post injection, on diffusion image and lactate, glycolysis and probably hypoxia on MRS

Figure 12 is an example of a longitudinal study to demonstrate glial tumor metabolism and post chemotherapy variation and to determine cerebral variation in MRI and MRS area, amplitude, ratios of metabolites and spectral profiles during a 5 years prospective longitudinal follow-up of 24 patients (12 oligodendrogial tumours and 12 gliomatosis cerebri) treated with Temodal ® [31]. The aggregation of different data on the box plot are not significant and not adequate. Therefore, the best way is to
analyze the data longitudinally to be able to decrease variability and detect small changes earlier. The Cr concentration is quite stable in most of the oligodendrogliomas and in the majority of gliomatosis.

**Figure 12**: Follow-up of Cho/Cr over at least 40 months during chemotherapy in spectra at echo time 144 ms: a) the first patient (a oligodendroglioma) is stable (Cho/Cr<2) until a progression with high proliferation (Cho/Cr>6) b) the second patient (also a oligodendroglioma) has aggravation (increase of Cho/Cr ratio until 3) and after treatment a decrease c) the third patient (a gliomatosis) have a very high and short period of proliferation (Cho/Cr>7) and then a decrease under treatment with some variability (Cho/Cr<3) and then again a high and short period of proliferation (Cho/Cr>7) d) the fourth patient has initial proliferation (Cho/Cr around 3) and then stability.

4. Discussion and future works
ROS-induced processes and metabolic switches in cancer could be measured by MRS. The aim is to enable us to study spontaneous ROS effects and other specific effects under therapy.

4.1 Antioxidants
Exposure to oxidative species has led organisms to develop a series of defence mechanisms, eg, antioxidants playing a protective role against oxidative stress by scavenging ROS. Enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) [32]. Non-enzymatic antioxidants include ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E) or glutathione (GSH) [34]. Under physiologic conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. The introduction of antioxidants can inhibit tumor cell proliferation by modulating Receptor Tyrosine Kinase (RTK)-related intracellular signaling pathways [33] while ROS can stimulate neoangiogenesis, phospholipase c, protein kinase (PKC), gene regulation, and cell proliferation [20].

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Anticancer drugs have been shown to induce apoptosis. Deficiencies in the apoptotic pathways may lead to chemoresistance. Studies report that glutathione (GSH) plays a critical role in the activation of apoptosis pathways due to anticancer drugs and conclude that dominant apoptosis resistance could depend on intracellular GSH levels with low GSH leading to apoptosis [35]. The GSH amount measured in vivo by MRS may help estimate antioxidant capacity and, indirectly, oxidative stress in tumors. Pyruvate is used as a precursor for glutathione synthesis and favors the restoration of the glutathione pool. These antioxidant properties of pyruvate and GSH (figure 8), while improving the cellular response and resistance to hypoxia might, however, interfere with anti-tumor treatment. In such cases, their measurement in vitro and in vivo using MRS might be an advantageous way to try to understand the processes induced by ROS in tumor cells and perhaps increase the effectiveness of some cancer therapies by predicting the resistance to anti-tumor treatment [36]. In addition, the measurement of glutathione levels in vivo may also be of potential relevance for measurements related to neurological disease processes and also viral infections.

4.2 Krebs cycle catabolites involved in ROS regulation
Measurement of metabolites and processes by MRS could allow the study of oxidative stress and the redox balance. In fact, oxidized glutathione (GSSG) is reduced back to GSH by the enzyme glutathione reductase, which uses NADPH as the electron donor, cytosolic enzymes such as isocitrate dehydrogenase (IDH1) [37], and Glucose-6-Phosphate dehydrogenase [38] (figure 1). Mitochondrial enzymes with modified activity (by mutations as with alpha-ketoglutarate dehydrogenase [39]) are coupled with NAD+/NADH, which can regulate ROS formation in the respiratory chain. We could then suggest that using MRS to identify and measure the quantity of metabolites involved in the formation of ROS and GSH (especially those from the Krebs cycle seen in blue in figure1) could reflect the balance between ROS and antioxidant formation involved in some diseases and tumor processes. This would contribute to our understanding of the role of ROS, in particular in cancer. Therefore, MRS makes possible in vivo, non invasive and reproducible quantification of these metabolites indirectly involved in the regulation of ROS and the quantification of antioxidant (GSH) production. In addition, it is known that succinate oxidation can support the highest rate of ROS production and that accumulation of succinate due to gene mutation (e.g. succinate dehydrogenase (SDH)) [13] might also contribute to an increase in mitochondrial ROS generation. Therefore the quantification of succinate in patients could also be correlated with the ROS production, especially in abscesses.

4.3 Mitochondrial tumor suppressor genes
The gene roles in tumorigenesis are critical. Their mutations are involved in the stabilization of HIF-1, in the up-regulation of genes involved in angiogenesis, in glucose transport, glycolysis or inhibition of developmental apoptosis. Recently, mutations of some enzymes such as isocitrate dehydrogenase (IDH1-IDH2), succinate dehydrogenase and fumarate hydratase were observed which could lead to accumulation of some substrates (succinate and fumarate) [13] to regulate NADH followed by changes in the production of ROS and antioxidant.

4.4 Relationship between ROS, HIF and hypoxia
It has been suggested that ROS, via changes in the cellular redox state, may trigger signalling pathways resulting in the activation of HIF-1 transcription factor. Some research is supported by the fact that tumors often have hypoxic areas that activate HIF1. This HIF-1 activation plays a key role in cancer development including metabolic switch by glycolytic enzyme activation, angiogenesis, or proliferation. However some studies show that tumor cells activate HIF-1 despite the absence of hypoxia. Involvement of ROS may explain this. Some studies have shown that mitochondria could regulate the stability of HIF through the increased production of ROS [40,41]. In addition, following radiotherapy, tumor reoxygenation leads to nuclear accumulation of HIF-1 in response to ROS, regulated by cytokines that could enhance cell radioresistance [42].
4.5 HIF involvement in tumoral metabolism

Induction of HIF-1 leads to adaptive mechanisms to reduce ROS, especially uncontrolled mitochondrial generation of ROS. Under hypoxic conditions, perturbation in electrons flows from the respiratory chain results in increased ROS production due to ineffective electron transfer in the mitochondria. It would be toxic to cells if the TCA cycle flow would not be attenuated. The hypoxia inducible pyruvate dehydrogenase kinase 1 (PDK1) [43] is critical for the attenuation of mitochondrial ROS production and maintenance of ATP levels. The gene encoding for PDK1 is a direct target of HIF-1. PDK1 inactivates the pyruvate dehydrogenase (PDH) enzyme complex that converts pyruvate to acetyl-coenzyme A, thereby inhibiting pyruvate metabolism via the TCA cycle. HIF-1 plays three critical roles in the hypoxia-induced metabolic switch from oxidative to glycolytic metabolism [43,44,45]: (1) HIF-1 induces expression of upstream glucose transporters and glycolytic enzymes to increase flow from glucose to pyruvate, (2) HIF, via PDK1, blocks the conversion to acetyl CoA, and, (3) stimulates lactate dehydrogenase A to convert pyruvate to lactate. The induction of PDK1 seems necessary to prevent excessive mitochondrial ROS production and to shunt pyruvate transformation into lactate and regenerate NAD+, which allows glycolysis and ATP production under hypoxia. Concentrations of lactate, pyruvate, succinate, glucose and other metabolites involved in the TCA cycle can be estimated by MRS and allow better understanding of these processes in vivo with all the pathways regulations in a repeatable non destructive way.

Lactate (a doublet at 1.33 ppm) is observed in tumors resulting from aerobic and anaerobic glycolysis. Correlation was not always found between lactate levels and the grade of the tumor or metabolic rate because lactate occurs both in intra- and extra-cellular spaces and its overall level is a function of metabolic rate and clearance. In addition, lactate may pool in necrotic or cystic regions.

The presence of fatty acid synthesis (e.g., in necrosis and some membrane turnover) implied the need for two supporting pathways: (1) a source of NADPH (the electron donor for fatty acid synthesis), and, (2) an anaplerotic mechanism to replenish the TCA cycle intermediates during citrate export in order to meet energy needs during tumoral growth [46]. Glutamine metabolism can potentially fulfill both these needs in proliferating glioblastomas. In addition, HyperGln is easily detectable by MRS in high-grade gliomas, increased cell cytolysis, mitochondrial disease, hyperammonemia, and osmotic and hepatic encephalopathy.

4.6 ROS and angiogenesis

Studies found formation of excessive amounts of ROS after radiation treatment (actually it is the aim of this treatment) and activation of HIF-1 during the reoxygenation, which leads to upregulation of VEGF and other HIF-1 regulated pro-angiogenic factors.

Many studies have shown that ROS production occurs as a result of activated growth factor receptor signalling such as receptor-tyrosine kinase. ROS generated by these pathways can function as true second messengers and mediate important cellular functions such as proliferation and programmed cell death [47]. Following radiotherapy, tumor reoxygenation leads to nuclear accumulation of HIF-1 in response to ROS, which might generate enough oxidative stress to stabilize HIF-1. The resulting increase in HIF-1 regulated cytokines enhances cell radioresistance because the HIF-1 complex regulates the expression of more than 40 genes involved in tumor metabolism, growth, and angiogenesis, including VEGF [48]. The effect of ROS on angiogenesis could be illustrated by MR perfusion data.

4.7 Proliferation

HIF-1 activation via ROS production may promote tumor proliferation by angiogenesis and metabolic switches. The oxidative stress also plays a role in the activation of PKC isoforms and cell proliferation [20]. The tCho peak is seen in vivo due to rapid membrane synthesis of dividing cancer cells and is predominantly due to PC (a precursor for cell membrane synthesis) and GPC (involved in cell membrane degradation). tCho levels were shown to correlate with proliferative potential in gliomas.
In addition to its main roles, methionine could have an anti-oxidant role by protecting cells from free radical damage [49]. Methionine could protect against lipid peroxidation, membrane damage, and changes in the glutathione system. On the other hand, 11C methionine PET tracer (as seen p. 9 in *PET and Multimodality*) [50,51] could be used to study tumor proliferation in relation with the Cho/Cr MRS parameter.

4.8 ROS – Necrosis - Apoptosis
Lipids (1.3 and 0.9 ppm) are seen in high-grade tumors [11]. Kuesel et al., from ex vivo studies, showed that lipids correlate with necrosis, a histological characteristic of high-grade tumors [12]. Increased lactate and lipids are seen with glioma grades that represent the progression from viable tumor cells to severe hypoxia, followed by necrosis. Recent studies showed that MRS spectral lipid profiles contain diagnostic information and may be used to differentiate glioblastomas from metastases [10]. Absolute lipid concentration could also help to differentiate toxoplasmosis from lymphoma at certain stages. ROS can be considered as mediators of cell death (by apoptosis or necrosis) and ROS can activate anti-apoptotic pathways leading to increased cellular survival. For example Bcl-2 family members are involved in control of ROS or ROS-related processes [52]. MRS could improve our understanding of the physiopathologic mechanisms of necrosis and apoptosis, determine whether their origin is infectious or tumoral, or provide information about the rate of development of these processes.

4.9 Infiltration
Reduction of NAA (found in neurons) in adult brain tumors is non-specific, as it also occurs in other brain pathologies with neuronal damage such as strokes or MS. The presence of NAA in a tumor may indicate viable neurons within an infiltrative tumor such as a glioma. The parameter, choline-NAA-index (CNI), very robust and related to high grade, is used with some success for defining the regional abnormalities in multivoxel spectroscopy of gliomas [4]. NAA/Cr is also an important ratio to follow in the contralateral side for the assessment of therapeutic effectiveness.

4.10 Under therapy
Radiation and chemotherapy and antiangiogenic therapies are widely used in the treatment of these malignant brain tumors. The limitation of brain radiation therapy and chemotherapy in humans not only comes from the inability to kill certain types of tumor cells and to induce cellular death in different types of environmental conditions (eg, pH, hypoxia, perfusion, energy, necrosis, cell density, and edema) but also from excessive damage to normal appearing brain which increases cognitive impairment. Among the types of radiation-induced brain toxicities, late delayed effects together with the original perturbations [53] can lead to severe and irreversible neurological dysfunctions (assessed by NAA/Cr). Following radiation, late delayed effects of exposure within the central nervous system have been attributable to both parenchymal and vascular damage involving oligodendrocytes, glial cells, neural progenitors, neurons and endothelial cells. This dynamic process, involving radiation-induced damage of target cells, could sometimes involve secondary reactive neuroinflammatory and glial processes that could lead to cell loss, tissue damage, metabolic changes, and functional deficits. The progressive, delayed processes and damage to the brain and the tumors after high-dose radiation and chemotherapy can be caused by radiation-induced long-lived processes induced by free radicals, reactive oxygen species, and pro-inflammatory cytokines. To better understand and follow longitudinally the metabolic effect of ROS after clinical radiotherapy (conventional, gamma-knife, protontherapy and heavy ions) is important.

New drug types, their design and combination therapies, especially those that could exert their effects by blocking pro-inflammatory cytokines and reactive oxygen species (eg, glutathione) and avoid irreversible metabolic switches, could be studied in future clinical trials.
5. Conclusion

(MRI) and spectroscopy (MRS) and perfusion could be useful for monitoring the Reactive Oxygen Species (ROS) effects on energy and glycolytic metabolism, mitochondrial function, lipid metabolism, necrosis, apoptosis, cell proliferation, and infiltration. These changes, as shown, could be studied longitudinally and non-invasively in humans with glial brain tumors, abscesses, or mitochondrial disease after therapy. Despite the difficulty, the variability, and unknown factors, these repeated measures could give us more insights into the evolution at different times of the pathological processes, stages, tumor progression and response to therapy.

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