



An integrated genomic and metabolomic approach for defining survival time in adult oligodendrogliomas patients

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Abstract

Introduction The identification of frequent acquired mutations shows that patients with oligodendrogliomas have divergent biology with differing prognoses regardless of histological classification. A better understanding of molecular features as well as their metabolic pathways is essential.

Objectives The aim of this study was to examine the relationship between the tumor metabolome, six genomic aberrations (isocitrate dehydrogenase 1 [IDH1] mutation, 1p/19q codeletion, tumor protein p53 [TP53] mutation, *O*⁶-methylguanine-DNA methyltransferase [MGMT] promoter methylation, epidermal growth factor receptor [EGFR] amplification, phosphate and tensin homolog [PTEN] methylation), and the patients' survival time.

Methods We applied ¹H high-resolution magic-angle spinning (HRMAS) nuclear magnetic resonance (NMR) spectroscopy to 72 resected oligodendrogliomas.

Results The presence of IDH1, TP53, 1p19q codeletion, MGMT promoter methylation reduced the relative risk of death, whereas PTEN methylation and EGFR amplification were associated with poor prognosis. Increased concentration of 2-hydroxyglutarate (2HG), *N*-acetyl-aspartate (NAA), myo-inositol and the glycerophosphocholine/phosphocholine (GPC/PC) ratio were good prognostic factors. Increasing the concentration of serine, glycine, glutamate and alanine led to an increased relative risk of death.

Conclusion HRMAS NMR spectroscopy provides accurate information on the metabolomics of oligodendrogliomas, making it possible to find new biomarkers indicative of survival. It enables rapid characterization of intact tissue and could be used as an intraoperative method.

Keywords HRMAS-NMR spectroscopy · Metabolomics · Oligodendroglioma · Overall survival · IDH · PTEN

1 Introduction

Oligodendrogliomas, divided into low-grade (grade II) and high-grade (grade III) (Louis et al. 2007), are one of the most difficult therapeutic challenges in neurosurgery and neuro-oncology, because of their sensitivity to radio- and chemotherapy, which improve survival in some subtypes. These tumors are frequently diagnosed after convulsive

seizures, although the patients remain asymptomatic, often for several years even if the tumor continues to progress slowly. However, after this period of lull, the tumor inevitably turns to aggressive growth. In contrast to this classical evolution, in clinical practice, it is not unusual to observe that some low-grade cases present a poor prognosis with rapid malignant transformation and conversely that some high-grade cases survive beyond 10 years. Although EORTC trial showed the radiotherapy can delay the low-grade tumor progression, this trial failed to demonstrate an overall survival improvement. Consequently, in lack of progression, and in absence of aggressive tumor feature, radiotherapy is often postponed at progression time. Recently a new trial has been open to inclusions for the patients with high-grade oligodendrogliomas to compare chemotherapy with PCV and

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immediate radiotherapy and the same chemotherapy with a radiotherapy delayed at progression time. Furthermore, early treatment could expose asymptomatic patients to functional risks. So, the choice and the time of the therapeutic intervention are a real issue and obviously are linked to the expected survival.

Over the last few years, the identification of frequent acquired mutations shows that patients with oligodendrogliomas have divergent biology with differing prognoses. Consequently, since 2016, molecular characteristics are included in the WHO classification (Louis et al. 2016) (Banan and Hartmann 2017), including two new entities: IDH-mutant and 1p19q codeleted oligodendrogliomas and anaplastic oligodendrogliomas, IDH-mutant and 1p/19q codeleted. These molecular features provide prognostic and/or predictive data, which make it crucial for treatment choice because of their relation to survival (Chen et al. 2015) (Wirsching and Weller 2016). Other genetic mutations are being studied in clinical routine, but their impact is not well understood. A better understanding of molecular features as well as their metabolic pathways is essential.

Metabolomics is a technique that has been maturing over the past few years, making it possible to relate genotype and phenotype, providing a better understanding of tumor metabolism by measuring the metabolite concentration impacted by down- or up-regulation of a specific gene transcript (Griffin and Shockcor 2004) (Mörén et al. 2015). HRMAS NMR spectroscopy enables identification and quantification of metabolites in intact tissue samples with excellent resolution (Battini et al. 2016) and can be used in clinical routine (samples are quickly transported by a pneumatic tube system in our hospital).

The aim of this study was to examine the relationship between the tumor metabolome, six genomic aberrations (IDH1 mutation, 1p/19q codeletion, TP53 mutation, MGMT promoter methylation, EGFR amplification, PTEN methylation), and the survival time of patients.

2 Materials and methods

2.1 Patient population

Between February 2002 and December 2016, 72 patients with an oligodendroglioma, 23 grade II and 49 grade III (WHO 2007), were included. WHO 2016 classification is also provided taking into account molecular biology. Thus, we included 19 low-grade- WHO 2016 and 53 high grade—WHO 2016. All of them underwent surgical subtotal resection followed by temozolomide adjuvant chemotherapy. Only high-grade (according to WHO 2007) oligodendroglioma patients underwent brain radiotherapy. One patient was excluded from survival analysis, because of

a multimetastatic bronchial cancer development leading to death 3 years after the diagnosis of oligodendroglioma grade II. All patients gave their written informed consent. The local Ethics Committee approved the study.

2.2 HRMAS NMR analysis

2.2.1 Sample preparation

Tissue specimens were collected with minimum ischemic delays after resection (average time 2 ± 1 min) and snap-frozen in liquid nitrogen before being stored at -80 °C. Each brain biopsy sample was prepared at -20 °C by introducing approximately 18 mg biopsy into a disposable Kelf insert.

2.2.2 HRMAS NMR data acquisition

All HRMAS NMR spectra were acquired on a Bruker (Karlsruhe, Germany) Avance III 500 spectrometer (installed in our University Hospital) operating at a proton frequency of 500.13 MHz. The temperature was maintained at 4 °C throughout the acquisition time. A one-dimensional (1D) proton spectrum using a Carr–Purcell–Meiboom–Gill pulse sequence was acquired with a 285- μ s inter-pulse delay and a 10-min acquisition time for each tissue sample. The number of loops was set at 328, giving the CPMG pulse train a total length of 93 ms. The chemical shift was calibrated to the peak of the methyl proton of L-lactate at 1.33 ppm. To confirm resonance assignments in a few representative samples, two-dimensional (2D) ^1H – ^{13}C experiments were also recorded. Metabolites were assigned using standard metabolite chemical shift tables (Wishart et al. 2013).

2.2.3 HRMAS NMR data processing

The region between 4.50 and 0.70 ppm of each 1D HRMAS NMR spectrum was automatically bucketed into integral regions of 0.01 ppm, using AMIX 3.9.14 software (Bruker).

The metabolites were quantified using the PULCON method, a very accurate quantification method (Dreier and Wider 2006; Imperiale et al. 2013). Twenty-eight metabolites were quantified using an external reference standard of lactate (3 μ mol) scanned under the same analytical conditions as the tissue samples. The spectra were normalized according to sample weight. Peaks of interest were automatically defined using an in-house program under MATLAB 7.0 (MathWorks, Natick, MA, USA). The peak integration for each metabolite was then compared to the peak integration of the lactate reference and corrected according to the number of protons. The results are expressed in nmol mg^{-1} of tissue.

2.2.4 Histopathology

After NMR HRMAS analysis, the inserts were cut, and for half the content of each sample, the percentage of tumor cells in the total sample of cells with regard to the total surface were calculated based on frozen hematoxylin & eosin-stained sections.

2.2.5 Biomolecular analysis (detailed in supplemental digital content)

Genetic analysis of *IDH1* mutation status was determined on genomic DNA extracted from formalin-fixed paraffin-embedded tumor tissue from the other half of the content of inserts. For the other genetic analyses, the mirror sample of the insert material was used.

MGMT promoter methylation was also studied with a fluorescent semi-quantitative methylation-specific PCR (FSQ-MS-PCR) developed in-house (Nguyen et al. 2015). Genomic alterations of the other genes (*IDH1*, *1p19q*, *TP53*, *EGFR* and *PTEN*) were assessed using PCR by means of allelotyping of microsatellites located in or flanking each of the different genes or chromosomal regions studied.

Genomic DNA was isolated using the usual techniques with the QIAamp[®] DNA purification kit (Qiagen, Courtaboeuf, France). A Nanodrop[®] (ThermoScientific, Wilmington, DE, USA) spectrophotometer was used to assess the DNA quality and quantification. The genomic DNA concentrations from tumor or blood paired samples ranged from 50 to 400 ng/ μ L and from 50 to 150 ng/ μ L, respectively.

2.3 Statistical analysis

The normality of the distribution of the metabolite results was estimated using the Wilks-Shapiro test. The differences between two independent groups were estimated using the Mann-Whitney U-test. The differences between more than two groups were assessed using the Kruskal-Wallis one-way analysis of variance test. The correlation between continuous variables was assessed using the Spearman R calculation, a nonparametric comparison based on the ranks of the observations, rather than on the values themselves ("Hmisc" package). The association between mutations was evaluated using the Chi square statistics on the contingency tables generated for each pair of genes.

Survival analysis between groups of patients was performed using the Kaplan-Maier method and significance was estimated based on the log-rank test. Single markers as well as combining metabolites and genetic markers were assessed using the Cox proportional hazards model and the

significance level was set as 0.05 using the likelihood ratio test.

All statistical analyses were performed with the statistical software R (R Development Core Team 2008) (www.cran.r-project.org). To uncover all significant associations between the genetic context of a tumor and the steady-state level of the metabolites, we performed association rules based on data-mining (Hastie et al. 2009). Association rules between metabolites and genetic markers were extracted from a binarized data set, where the presence of mutation in a patient's tumor was labeled 1 and the concentration levels of each metabolite were discretized in two bins applying different thresholds. The association rules were generated using KEM[®] software (Jullian and Afshar 2008) (Liquiere and Sallantin 1998) and filtered using the four usual interestingness metrics: support, confidence, lift and *p* value (Lallich et al. 2006).

2.4 Network analysis

The algorithm to determine expected metabolite level alterations (ADEMA) network analyses using mutual information were applied to the metabolite quantification values (Cicek et al. 2013). The network was constructed using the Kyoto Encyclopedia of Genes and Genomes (Kanehisa and Goto 2000; Minoru Kanehisa et al. 2014) and Salway's work (Salway 2014). The ADEMA algorithm evaluates the change in groups of metabolites between concentration data from two experimental groups instead of analyzing metabolite concentrations one by one. Based on mutual information, the algorithm determines whether some metabolites are biomarkers when considered together, and it can predict the direction of the expected change per metabolite depending on the metabolic network topology considered. Various groups of metabolites related to the metabolic pathways involved were compared:

- Taurine, hypotaurine, aspartate, serine, methionine
- Aspartate, asparagine, acetate, threonine, NAA
- Aspartate, threonine, isoleucine
- Glucose, serine, glycine
- Glucose, valine
- Glucose, isoleucine
- Glucose, acetate
- Glucose, lactate
- Valine, lactate, alanine
- Glucose, myo-inositol, ascorbate, glutathione, glycine, glutamate
- Glutamate, histidine, glutamine, 2HG
- Aspartate, succinate, fumarate, 2HG
- Glutamate, histidine, glutamine, glycine
- Glutamate, glycine, creatine
- Choline, GPC, PC, total choline

3 Results

All statistical analysis was done on all oligodendrogliomas: 72 tissue samples for studying the relationship between genetic mutations and metabolomics and 71 for survival analysis without taking into account the histopathological grading of oligodendrogliomas. According to WHO 2016 classification, 19 low-grade and 53 high-grade were included.

For all patients, we measured the concentration of 28 metabolites and also used the ratio between glycerophosphocholine and phosphocholine (GPC/PC) and total choline (choline + GPC + PC) as additional parameters in the statistical analyses.

Figure 1 shows the Kaplan–Meier survival curves according to WHO 2007 (Fig. 1a) and WHO 2016 (Fig. 1b).

3.1 Impact of genetic mutations on tumor metabolomics

The presence of IDH1 mutation (Fig. 2a) was associated with an elevated concentration for glucose, myo-inositol, GPC, histidine, creatine and 2HG and a low concentration for lactate, serine, glycine, alanine, isoleucine, threonine, aspartate, acetate, methionine, hypotaurine, PC, choline, GSH and glutamate. The accumulation of 2HG, as measured using HRMAS, was strongly dependent on the presence of the IDH1 mutation ($p = 1.8 \times 10^{-7}$). In the absence of the IDH1 mutation, the level of 2HG was on the order of 0.42 ± 0.71 nmol/mg, with only three tumors showing levels higher than 1 nmol/mg, whereas in the presence of the IDH1 mutation, the average level of accumulated 2HG in the tissue was found to be more than 2.5 times higher (1.05 ± 1.0 nmol/mg).

The 1p19q codeletion was associated with elevated concentrations of glucose, myo-inositol, ascorbate, valine, isoleucine, asparagine, GPC, PC, choline, taurine, histidine, glutamate, glutamine, 2HG and creatine. Alanine, aspartate and acetate were inversely correlated to the presence of this codeletion.

The presence of MGMT was associated with elevated concentrations of GPC, PC, asparagine, acetate, ascorbate, histidine and 2HG and low levels of glucose, aspartate, glutamate, alanine and hypotaurine.

Glucose and threonine concentrations were elevated for TP53-mutated oligodendrogliomas, and lactate, isoleucine, asparagine, aspartate, fumarate, methionine, GPC, PC, choline, serine, histidine, glutamate, glutamine and creatine had low rates.

The presence of a PTEN mutation (Fig. 2b) was associated with a rise in alanine, lactate, isoleucine, threonine,

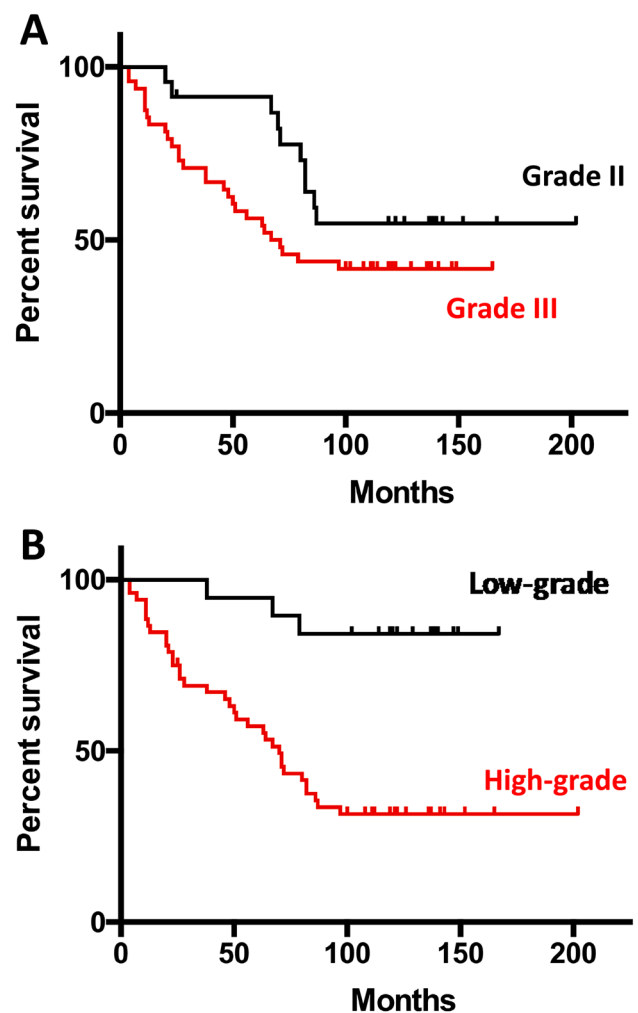


Fig. 1 Kaplan–Meier survival curves according to WHO 2007 (a) and WHO 2016 (b) classifications: low-grade oligodendrogliomas in black lines and high-grade oligodendrogliomas in red lines

aspartate, methionine, fumarate, PC, choline, serine, hypotaurine, glycine, GSH and glutamate and a decrease in acetate, NAA, glucose, myo-inositol and creatine and a reduction in glucose, myo-inositol, creatine, acetate and NAA.

The presence of EGFR was associated with a high concentration for alanine, serine, glycine, lactate, threonine, aspartate, methionine, fumarate, choline, hypotaurine, GSH, glutamate and glutamine and a low concentration for 2HG, creatine, GPC, NAA, glucose and myo-inositol.

3.2 Relationship between genetic mutations and overall survival

In Fig. 3, Kaplan–Meier survival curves show the relationship between genetic mutations and overall survival. ‘28 of the 71 patients died during the follow-up period (mean,

66 ± 34 months; range, 1–125 months). In the univariate survival analysis, all six genetic markers measured on the samples were found as significant prognostic predictors. Presence of a mutation on EGFR and PTEN, which were strongly associated (Chi square test of $p = 1.4 \times 10^{-10}$), increased the relative risk (RR) (2.675 and 3.078, respectively) of the patients dying. Inversely, the presence of a mutation on IDH1, TP53, 1p/19q codeletion and MGMT methylation reduced the relative risk for the patients (RR = 0.316, $p < 0.0001$; RR = 0.902, $p = 0.406$, RR = 0.457, $p = 0.0045$; RR = 0.574, $p = 0.0003$ respectively). In this patient set we found that the presence of the EGFR mutation is strongly anti-correlated to the presence of the IDH1 mutation ($p = 8.9 \times 10^{-8}$). The IDH1 mutation also frequently co-occurred with MGMT methylation ($p = 0.0008$), TP53 mutation ($p = 0.005$) and 1p/19q codeletion ($p = 0.03$).

3.3 Relationship between metabolomics and overall survival

When using the measured concentration of the metabolites as independent continuous variables in the Cox proportional hazards model, 11 out of the 30 metabolite parameters measured were found to be significant prognostic predictors. For eight of them (GPC/PC, 2HG, Myo-inositol, NAA, Glycine, Glutamate, Alanine, Serine) the concentrations measured allowed a precise estimate of the relative risks (RR) to be calculated. We also compared patient survival by establishing thresholds (in nmol/mg) for the concentrations of each metabolite (Table 1). Increased concentration of 2HG (> 0.3 nmol/mg, RR = 0.396, $p < 0.0001$), myo-inositol (> 3.12 nmol/mg, RR = 0.415, $p = 0.0016$), NAA (> 1.35 nmol/mg, RR = 0.438, $p = 0.101$) and the GPC/PC ratio (> 0.42 nmol/mg, RR = 0.388, $p < 0.0001$) were good significant prognostic factors. In contrast, increasing the concentration of serine (> 0.55 nmol/mg, RR = 2.242, $p < 0.0001$), glycine (> 1.36 nmol/mg, RR = 1.788, $p = 0.0097$), glutamate (> 1.48 nmol/mg, RR = 1.991, $p = 0.0007$) and alanine (> 0.52 nmol/mg, RR = 2.044, $p = 0.0001$) led to an increased relative risk of death for patients. When using binary values for the metabolites, we obtained the Kaplan–Meier survival curves shown in Fig. 4.

3.4 Relationship between genomics, metabolomics and overall survival

To summarize the associations between tumor genetic background and the metabolite levels in the tumor tissues, we performed unsupervised data mining using association rules in the entire data set and graphically represented the rules obtained as two networks linked to the overall survival (Fig. 5).

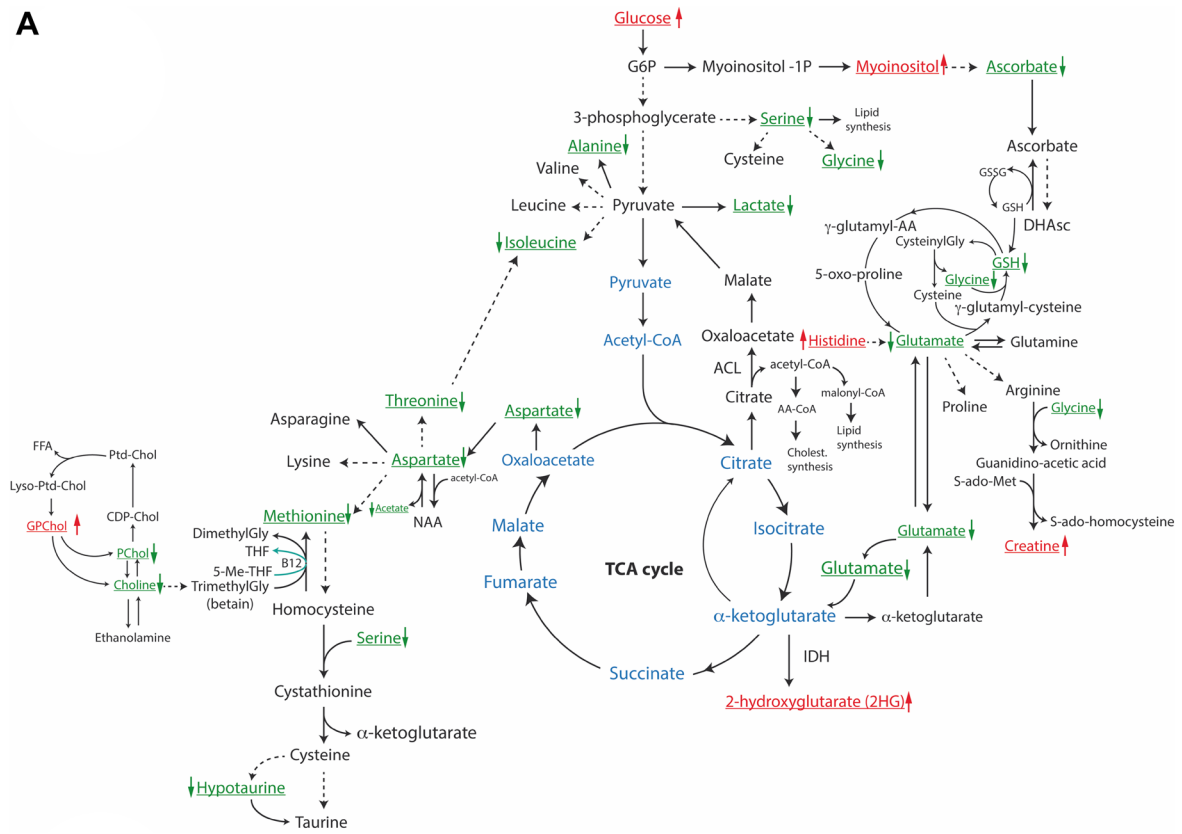
4 Discussion

The high demand of proliferating cells from catabolites in order to meet the increased demand of bioenergetics, maintain homeostasis and generate biomass, produces increased consumption and alterations on the metabolism of two major nutrients, glucose and glutamine. These complex metabolic changes occur as a function of the tumor oncogenotype and the microenvironment in different tissues. The main objective of the present study was to evaluate the relationship between different genetic mutations, tumor metabolism and patient outcome in oligodendrogliomas in order to find potential metabolic biomarkers to malignancy. In fact, adding biomolecular information led to a better classification of oligodendrogliomas, which is why biomolecular features are now used in WHO 2016 classification (Louis et al. 2016). It allows a better correlation with survival. Moreover, the metabolic classification seems to provide a closer correlation with the patient evolution than the histopathological analysis (Erb et al. 2008).

In agreement with the literature (Wang et al. 2014; Kong et al. 2015; Ducray et al. 2011; Zou et al. 2013; Cairncross et al. 1998; Smith et al. 2000; Karayan-Tapon et al. 2010; Sanson et al. 2009) the results presented in this study demonstrate that the presence of a mutation on IDH1, TP53, 1p19q codeletion and MGMT methylation slow down cellular metabolism and increase overall survival, particularly with IDH1, which presents a relative risk of 0.316. Since the first report of Parsons et al. (2008), many studies demonstrated that IDH mutations facilitate reduction of α -ketoglutarate to 2HG, reduce nicotinamide adenine dinucleotide phosphate, which is necessary for regeneration of reduced glutathione (Ducray et al. 2011; Dang et al. 2009; Cairns and Mak 2013) and generate competitive inhibition for enzymes that regulate DNA methylation and histone demethylations (Siegal 2015). This novel enzymatic activity with overproduction of 2HG does not participate in any known productive metabolic pathway, but does not seem to be an epiphenomena (Koivunen et al. 2012; Losman et al. 2013) and alters cellular genetic and epigenetic programs. The 2HG is involved in oncogenesis through the hypoxia inducible factor 1- α (HIF1- α) in gliomas and sensitizes tumor cells to chemotherapy, increasing cellular oxidative stress (Wang et al. 2014; Dang et al. 2009; Molenaar et al. 2014). The results reported herein demonstrated several metabolic markers predictive of overall survival and in relation with IDH1 status. The most important are 2HG, GPC, myo-inositol, threonine, alanine, methionine, acetate and glutamate.

Inversely, PTEN and EGFR mutations were associated with up cellular metabolism (glycolysis, lactic acid fermentation, amino acid metabolism, lipid metabolism) and

A



B

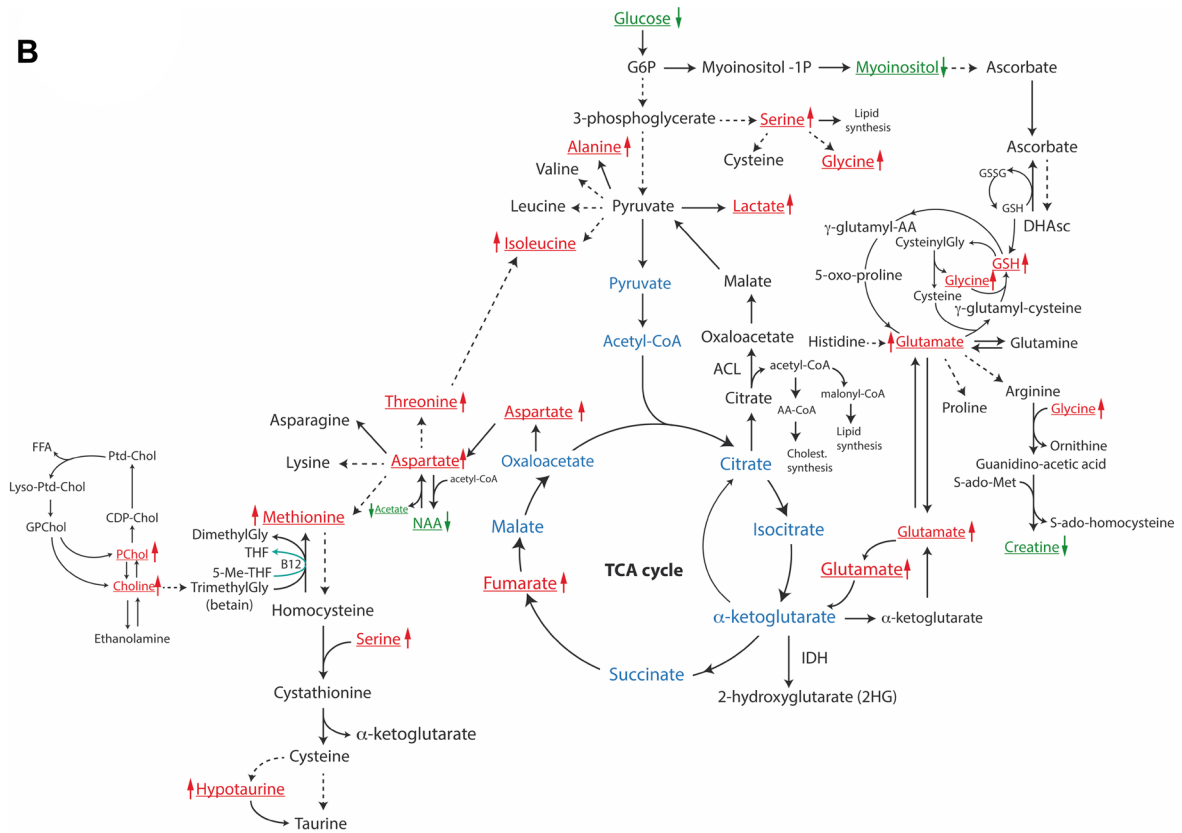


Fig. 2 a Association of the level of metabolite accumulation in oligodendroglioma tumors and the presence of the IDH1 mutation. The metabolites underlined and written in *red* and *green* with an arrows for each one, respectively, indicate the metabolites that are predicted to increase and decrease in oligodendrogliomas, in presence of the IDH1 mutation. The metabolites in blue are present in the mitochondria. **b** Association of the level of metabolite accumulation in oligodendroglioma tumors and the presence of the PTEN mutation. The metabolites underlined and written in *red* and *green* with an arrows for each one, respectively, indicate the metabolites that are predicted to increase and decrease in oligodendrogliomas in presence of the PTEN mutation. The metabolites in blue are present in the mitochondria

a poor prognosis: increasing the relative risk for death to 3.078 and 2.675, respectively, in accordance with previous reports for the association of these two genes with an adverse prognosis in multiple cancers (de Muga et al. 2010) and gliomas (Altieri et al. 2014; Han et al. 2016). The loss of PTEN function leads to the activation of the AKT/PKB signaling pathway and the activation of HIF1- α , thereby promoting tumor cell growth, survival, migration and neoangiogenesis (Yamada and Araki 2001; Jiang and Liu 2008).

In the present study, we observed that several metabolic markers were in relation with IDH and/or PTEN status (Fig. 5). Among them, six metabolic markers are closely related to overall survival: 2HG, alanine, myo-inositol, glycine, glutamate and the GPC/PC ratio (Fig. 4). The relation

Table 1 Univariate survival analysis using genomics markers as binary variables and metabolites concentrations as continuous variables

Parameter	Status	Relative risk	95% CI	P-value
IDH1	+	0.316	0.05–0.3	<0.0001
1p19q	+	0.457	0.15–0.92	0.0045
MGMT	+	0.574	0.11–0.53	0.0003
TP53	+	0.902	0.08–0.97	0.406
EGFR	+	2.675	3.49–18.64	<0.0001
PTEN	+	3.078	3.51–22.98	<0.0001
Threshold in nmol/mg				
GPC/PC	>0.42	0.388	0.027–0.22	<0.0001
2HG	>0.3	0.396	0.23–0.97	<0.0001
Myo-inositol	>3.12	0.415	0.554–0.92	0.0016
NAA	>1.35	0.438	0.245–1.03	0.101
Glycine	>1.36	1.788	1.021–1.24	0.0097
Glutamate	>1.48	1.991	1.1–1.953	0.0007
Alanine	>0.52	2.044	1.5–3.26	0.0001
Serine	>0.55	2.242	1.79–15.82	<0.0001

between the 2HG level and overall survival confirms the large clinical studies that support the hypothesis that IDH mutations occur early during oncogenesis and subsequently other mutations determine the progression of disease (Ichimura 2012).

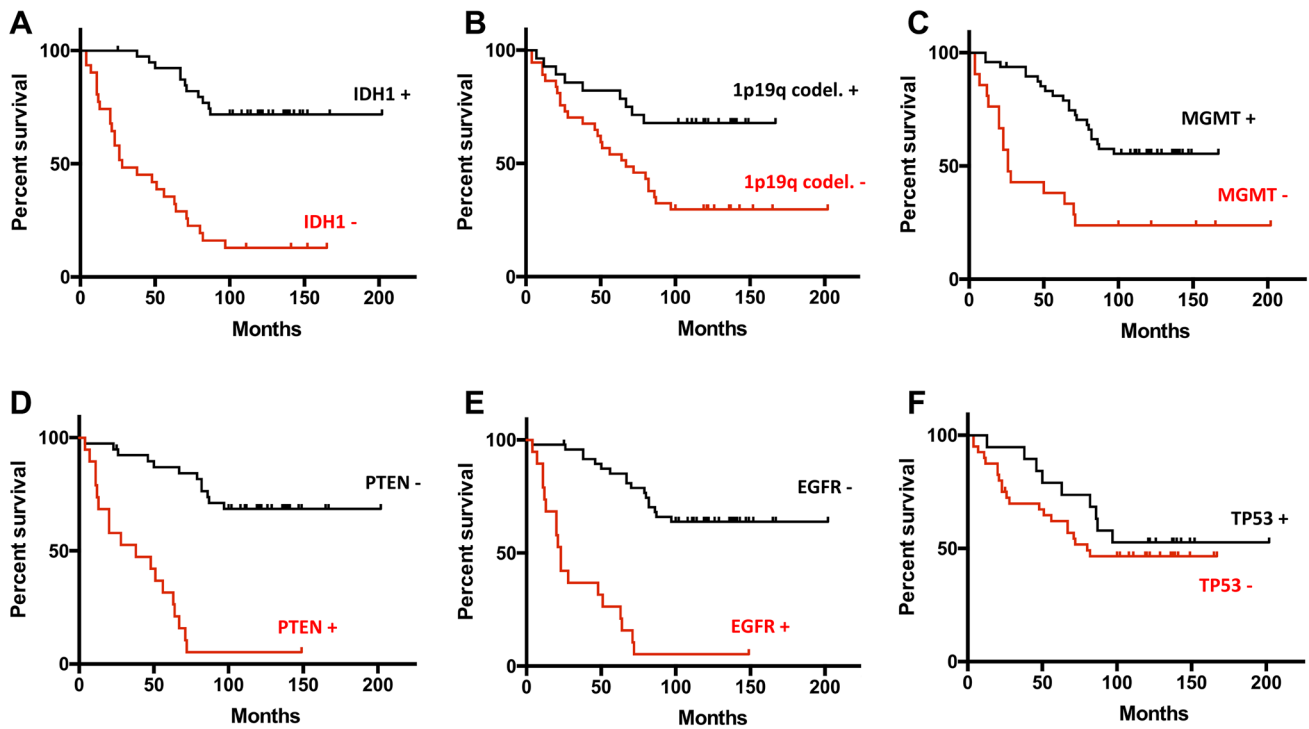


Fig. 3 Comparison of the survival rates between groups of patients with different status of genomics markers (a vIDH1, b 1p19q, c MGMT, d PTEN, e EGFR, f TP53) measured in the tumor tissues.

Survival in the presence of the mutation is shown as black lines for genomic aberrations associated to a good prognosis and as red lines for genomic aberrations associated to a poor prognosis and vice versa

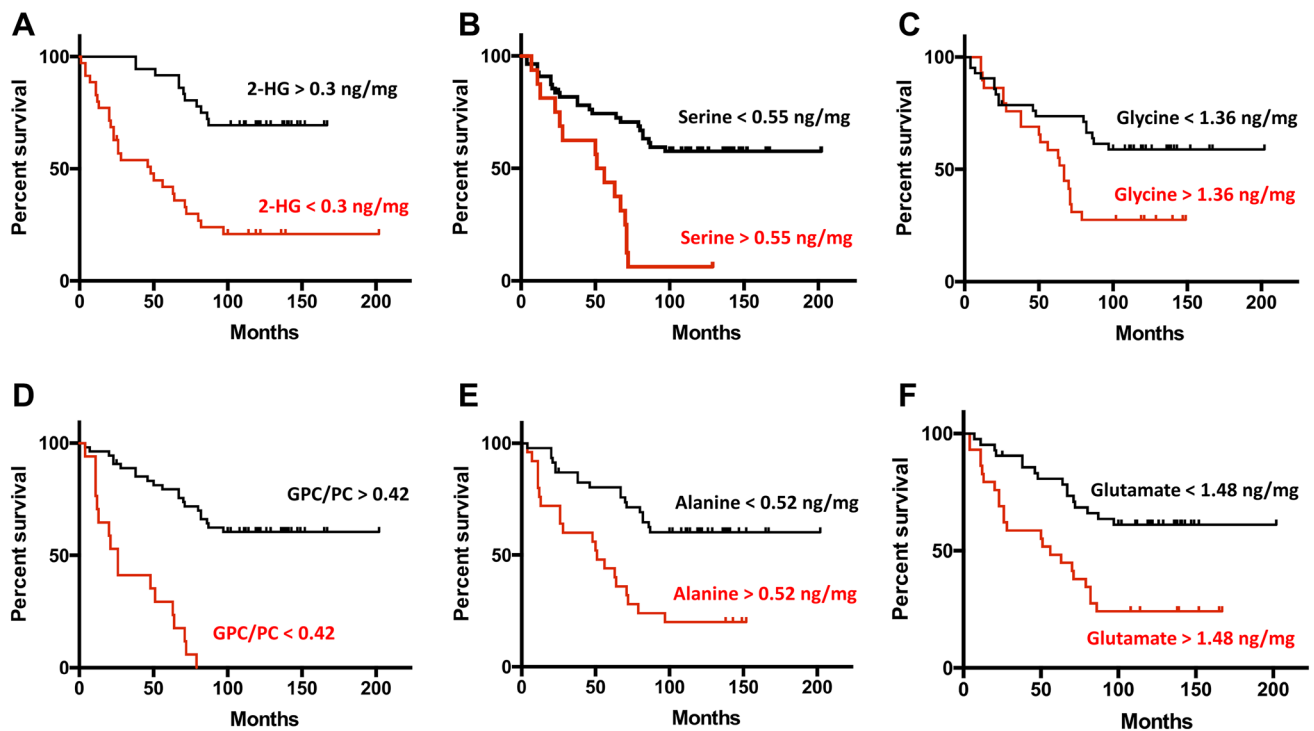


Fig. 4 Comparison of the survival rates between groups of patients with different levels of accumulation of six metabolites (a 2HG, b Alanine, c Glycine, d GPC/PC, e Serine, f Glutamate) measured in the tumor tissues

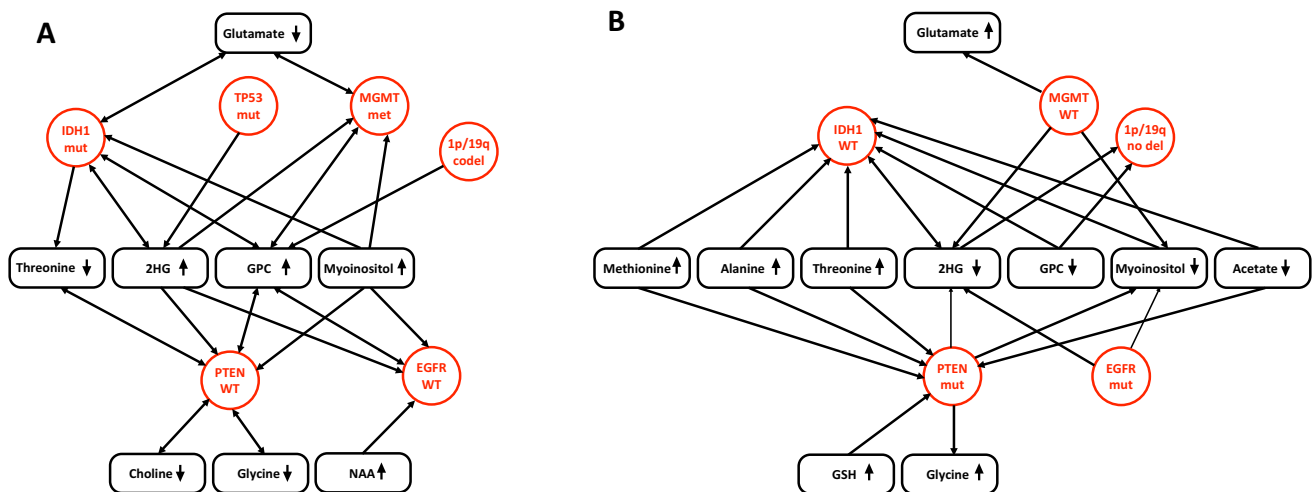


Fig. 5 Associations between tumor genetic background and the metabolite levels in the tumor tissues for patients having mutations associated to a good prognosis (a) and a poor prognosis (b)

Myo-inositol, a precursor of phosphatidylinositol, GPC and PC make up the phospholipid membrane involved in cell signaling (Tzika et al. 2007). A high level of GPC is associated with a good prognosis and was observed in low-grade gliomas (Sabatier et al. 1999). The GPC/PC ratio has been reported to positively correlate with survival time and associated with the expression of the histone deacetylase genes

HDAC1 and HDAC4, influencing tumor aggressiveness in human gliomas (Dali-Youcef et al. 2015).

Glycine, a nonessential amino acid, is a precursor to make proteins, lipid and nucleic acids, and is synthesized through several pathways, principally via intermediate glycolysis (with serine) and from choline (Giskeødegård et al. 2012). Many cancer cells exhibit increased flux from glucose

into serine and glycine biosynthesis (Locasale and Cantley 2010).

Finally, the glutamate–glutamine cycle played a major role in cancer cell metabolism. We have known for some time that cancers used glutamine, whose uptake and metabolism are regulated by the MYC oncogene. MYC-transformed cells become addicted to glutamine through increased expression of glutamine transporters and glutamine catabolic enzymes (Dang 2010; Lunt and Vander Heiden 2011). Glutamine is the major substrate that is oxidizable by tumor cells (Baggetto 1992) and a major source of nitrogen for nucleotide and amino acid synthesis (Vander Heiden 2011). This amino acid can be used as a precursor for lipid synthesis, especially in hypoxic environments.

In conclusion, HRMAS NMR spectroscopy provides accurate information on the metabolomics of oligodendrogliomas, making it possible to find new biomarkers indicative of survival. It enables rapid characterization of intact tissue and could be used as an intraoperative method, opening the perspectives of real-time metabolic profiling during surgery and therefore could influence the operative strategy.

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Author contributions CB: writer, interpretation of data; MGK: writer, statistical analysis; AEC: network analysis; FMM: NMR data acquisition, spectra analysis; NDY: participation of biochemical construction of network model, manuscript review; MP: spectra analysis; PS: statistical analysis; RH: IDH1 analysis; NEW: genomics analysis; BL: pathological analysis; MPC: pathological analysis; RS: patient oncological management; FP: patient surgical management; GN: patient radiotherapy management, manuscript review; IJN: writer, experimental design, data analysis.

Data availability The datasets generated during and/or analysed during the current study are available through EMBL-EBI MetaboLights, with the accession number MTBLS869.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval The Ethics Committee of Strasbourg approved the study (No. 100/2003, 09.12.2003 and No. 2013-37, 12.11.2013).

Informed consent A written informed consent was obtained from all the included patients.

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