Metabolomic profile of aggressive meningiomas by using high-resolution magic angle spinning nuclear magnetic resonance.

Laura Bender MD 1*, François Somme MD 2, Elisa Ruhland Msc 2,3, A. Ercümunt Cicek PhD 4,5, Caroline Bund MD, Msc 2,3,6, Izzie Jacques Namer MD PhD 2,3,6

1. Oncology Department, University Hospitals of Strasbourg, Hôpital de Hautepierre, 1 avenue Molière, Strasbourg, France, laura_2708@hotmail.fr
2. Biophysics and Nuclear medicine Department, University Hospitals of Strasbourg Hôpital de Hautepierre, 1 avenue Molière, Strasbourg, France
3. MNMS-Platform, University Hospitals of Strasbourg, Hôpital de Hautepierre, 1 avenue Molière, Strasbourg, France
4. Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh, USA
5. Computer Engineering Department, Bilkent University, Ankara, Turkey
6. ICube, Université de Strasbourg/CNRS, UMR 7357, Strasbourg, France

*corresponding author: Laura Bender, same address, laura_2708@hotmail.fr
Tel: + 33 (0)3 88 12 76 65
Fax: + 33 (0)3 88 12 89 54
Abstract

Background: Meningiomas are in most cases benign brain tumors. The WHO 2016 classification defines three grades of meningioma. This classification had a prognosis value since grade III meningiomas have a worse prognosis value compared to grades I and II. However, some benign or atypical meningiomas can have a clinical aggressive behavior. There are currently no reliable markers which allow distinguishing between the meningiomas with a good prognosis and those which may recur. High-resolution magic angle spinning (HRMAS) spectrometry is a noninvasive method able to determine the metabolite profile of a tissue sample. Results: We retrospectively analyzed 62 meningioma samples by using high-resolution magic angle spinning (HRMAS) spectrometry (43 metabolites). We described a metabolic profile defined by a high concentration for acetate, threonine, N-acetyl-lysine, hydroxybutyrate, myoinositol, ascorbate, scylloinositol, total choline and a low concentration for aspartate, glucose, isoleucine, valine, adenosine, arginine and alanine. This metabolomics signature was associated with poor prognosis histological markers (Ki-67 ≥ 40%, high histological grade and negative progesterone receptor expression). We also described a similar metabolomics spectrum between grade III and grade I meningioma. Moreover, all grade I meningioma with a low Ki-67 expression and positive progesterone receptor expression did not have the same metabolomics profile. Conclusions: Metabolomics analysis could be used to determine an aggressive meningioma in order to discuss a personalized treatment. Further studies are needed to confirm these results and to correlate this metabolic profile with survival data.

Keywords: Ex vivo spectrometry, HRMAS NMR, meningioma, metabolic signature

Introduction
Meningiomas are the most common adult primary central nervous system tumors. The Central Brain Tumor Registry of the United States (CBTRUS) reported 129,841 new cases between 2008 and 2012. In the United States, meningiomas represent 36.4% of all cases of primary central nervous system tumors. Meningiomas derive from arachnoid cap cells located in the arachnoid villi. These tumors arise in the majority of the cases from brain meninges but 10% derive from spinal cord meninges. Immunohistochemical analysis reveals an expression of vimentin, protein S100, epithelial membrane antigen and progesterone receptors. Ragel et al. described aberrant signaling pathways (mammalian Target of Rapamycin (mTOR), Phosphoinositide 3-kinase (PI3K), Mitogen Activated Protein Kinase (MAPK)) implicated in meningioma tumorigenesis. According to the World Health Organization (WHO) 2016 classification, meningiomas are divided into three grades: grade I or benign meningioma, grade II or atypical meningioma and grade III or malignant meningioma. The WHO 2016 classification did not undergo revisions concerning the classification and the grading of meningioma compared to the WHO 2007 classification. The only change was that brain invasion was added a criteria, which suffice to diagnose grade II meningioma. Grade I meningiomas (nine subtypes) represented the most common variant. These tumors had a good prognosis with 10-year Progression Free Survival (PFS) rate from 75 to 95% and 10-year overall survival (OS) rate from 80 to 90%. Grade II meningiomas (atypical, clear-cell and chordoid) had a poor prognosis with a 10-year PFS rate from 23 to 78% and 10-year OS rate from 50 to 79%. Grade III meningiomas (anaplastic, papillary, and rhabdoid) are a rare variant of meningioma. These tumors represent 1.2% of all meningioma. Grade III meningiomas are defined by 20 or more mitoses per ten high power fields and/or pathological examinations, which look like pseudo-carcinomas, pseudo-melanomas or high grade pseudo-sarcomas. These tumors had a worse prognosis with a 10-year PFS rate of 0% and 10-year OS rate from 14 to 34%. Patients with grade III meningioma had a worst prognosis
compared to patients with grade I and II meningioma, due to a higher risk of recurrence and to their capacity to develop brain and distant metastases. However, some grade I and grade II meningioma may present a clinical aggressive behavior. Cellular proliferation is based on protein synthesis and amino acids (AA) are the natural building blocks of protein. Monitoring AA expression by using HRMAS spectrometry is interesting to understand the physiological and pathological processes of cellular metabolism\(^9\). HRMAS nuclear magnetic resonance spectrometry is a nondestructive method that is used to determine the metabolomics profile of a tumor sample. A tissue sample preparation is essential\(^{10}\). This technique has already been evaluated in several fields, notably to explore acute rejection after tissue transplantation or to improve diagnosis and staging of tumor in oncology\(^{11–13}\). In the actual literature, two studies evaluated metabolite profile of meningioma by using HRMAS. Monleón et al. analyzed 10 metabolites according to HRMAS spectrometry in 30 meningioma samples\(^{14}\). Pfisterer et al. analyzed 68 meningioma samples (46 grade I, 14 grade II and 8 grade III) by using proton magnetic resonance spectroscopy (8 metabolites)\(^{15}\). Ex vivo spectroscopy analysis could permit to distinguish between meningioma with a good prognosis and those which tend to recur. Actually, there is a lack of data considering spectroscopy analysis of meningioma by using high-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR). The aim of the study was to correlate a metabolic profile with aggressive histological features for the meningioma.

**Material and methods**

**HRMAS NMR analysis**

**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. All tissue samples used in this study had a viable tumor/necrosis ratio and were quantitatively and qualitatively...
adequate to perform satisfactory NMR HRMAS analysis. In order to wait for this goal, 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. Only samples containing more than 80% of tumor cells were kept for the study. Each brain biopsy sample was prepared at −20°C by introducing a 15- to 18-mg biopsy into a disposable 30-µL KelF insert. To provide a lock frequency for the NMR spectrometer, 10 µL of D₂O was also added to the insert.

**HRMAS NMR data acquisition**

All HRMAS NMR spectra were acquired on a Bruker (Karlsruhe, Germany) Avance III 500 spectrometer operating at a proton frequency of 500.13 MHz and equipped with a 4-mm triple-resonance gradient HRMAS probe (¹H, ¹³C and ³¹P). The temperature was maintained at 4°C throughout the acquisition time in order to reduce the effects of tissue degradation during the spectrum acquisition. A one-dimensional (1D) proton spectrum using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was acquired with a 285-µs 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 heteronuclear experiments (¹H – ¹³C) were also recorded immediately after ending the 1D spectra acquisition.

**HRMAS NMR data processing**

Metabolite assignment and quantification was done with Chenomx software (Edmonton, AB, Canada), using a database of NMR spectra of 76 metabolites acquired in our laboratory under the same CPMG pulse sequence as the tissue samples. We could detect and quantify 43 metabolites in meningioma samples: acetate, adenosine, alanine, allocystathione, arginine,
ascorbate, asparagine, aspartate, betaine, choline, creatine, ethanolamine, fumarate, gamma-aminobutyric acid (GABA), glycine, glucose, glutamate, glutamine, glutathione (GSH), glycerol, glycerophosphocholine (GPC), hydroxybutyrate (HB), 2-hydroxyglutarate (2HG), hypotaurine, isoleucine, lactate, lysine, methionine, myoinositol, N-acetyl-aspartate (NAA), N-acetyl-lysine (NA-lysine), ornithine, phenylalanine, phosphocholine (PC), phosphocreatine, proline, serine, scylloinositol, succinate, taurine, threonine, tyrosine and valine. The results are expressed in nmol mg\(^{-1}\) of tissue. We also used total choline (choline + GPC + PC) and total creatine (creatine + phosphocreatine) as additional parameters in a network analysis.

**Network analysis**

The algorithm to determine the expected metabolite level alterations (ADEMA) network analyses using mutual information were applied to the metabolite quantification value. ADEMA includes information on the metabolic pathway in a unidirectional or bidirectional manner. The network was constructed using the Kyoto Encyclopedia of Genes and Genomes and Salway's work. Using the metabolic network topology, 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, methionine, allocystathione, serine
- Aspartate, asparagine, acetate, threonine, NAA
- Aspartate, lysine, N-acetyl-lysine
- Acetate, threonine, allocystathione, methionine
- Glucose, acetate, hydroxybutyrate
- Aspartate, threonine, isoleucine
- Glucose, glycine, serine
• Glucose, glycerol, phenylalanine, tyrosine
• Glucose, valine, isoleucine
• Glucose, lactate
• Valine, lactate, alanine
• Glucose, myoinositol, ascorbate, GSH, glycine, glutamate
• Myoinositol, scylloinositol
• Glutamate, GABA, proline
• Aspartate, adenosine, succinate, fumarate, 2HG
• Glutamate, glutamine, glycine, 2HG
• Glutamate, arginine, glycine, creatine, ornithine
• Aspartate, arginine, ornithine
• Ethanolamine, choline, GPC, PC, total choline
• Choline, betaine, glycine

Histological analysis

We studied 62 surgical samples. Hematoxylin and eosin staining was done for histological typing. Tumors were graded according to the WHO 2016 (world health organization) classification. Immunohistochemical staining was done to determine Ki-67 (clone SP6) and progesterone receptor (clone 16).

Written patient’s consent has been obtained. The Ethics Committee of Strasbourg approved the study (CARMEN Project, Ethics Committee no. 2003-100, 09.12.2003).

Statistical analysis

The receiver Operating Characteristic (ROC) curve was used to define the optimal threshold of Ki-67 to distinguish between grade I and grade II/III meningioma in our cohort (Youden index). Thus, the optimal cut-off obtained was 12.5% (AUC 88.3, sensitivity 87.5%, specificity 97.8%).
Results

Patient population

We retrospectively included 62 meningiomas from 50 patients treated by surgery in the Department of Neurosurgical at University hospitals of Strasbourg between October 2002 and September 2010. There were 45 grade I, 8 grade II and 6 grade III. The histological grade was statistically associated with overall survival (p<0.0001).

Forty-three meningiomas had a Ki-67 rate under 5% while eight tumors had a Ki-67 rate over 40%. For three patients, a Ki-67 expression was not available. A high Ki-67 rate (> 40%) was statistically associated with a worser overall survival (p=0.0017). The progesterone receptor (PR) expression was gathered for 54 meningiomas; rather than, 33 were positive and 21 negative. A positive PR expression was statistically associated a longer overall survival (p<0.0001). Among the 62 tumor samples, 58 (93%) were primary tumor and four meningioma relapsed.

Metabolic spectrum according to histological grade

Meningiomas with a high histological grade (II and III) according to the WHO 2007 classification, were associated with elevated concentration for acetate, threonine, NA-lysine, glycine, myoinositol, ascorbate, scylloionitol, HB, succinate, choline, GPC, PC, total choline and glycerol; and a low concentration for taurine, aspartate, serine, glucose, isoleucine, valine, alanine, adenosine, glutamine, arginine, ethalonamine and betaine.

Metabolic spectrum according to Ki-67 expression

A high Ki-67 rate was statistically associated with an elevated concentration of allocystathionine, methionine, acetate, threonine, NAA, NA-lysine, HB, glycine, myoinositol, ascorbate, scylloinositol, GABA, succinate, ornithine and choline and a low concentration of
taurine, hypotaurine, aspartate, serine, glucose, alanine, adenosine, arginine, creatine, phosphocreatine and total creatine.

**Metabolic spectrum according to progesterone receptor expression**

A positive progesterone receptor was statistically associated with an elevated concentration of aspartate, serine, lysine, glucose, isoleucine, valine, alanine, proline, adenosine, arginine, creatine and total creatine; and a low concentration of taurine, acetate, threonine, NAA, NA-lysine, HB, myoinositol, ascorbate, scylloinositol, fumarate, 2HG, choline, GPC, PC, total choline and betaine.

We were then able to describe a metabolic profile associated with aggressive meningioma (according to histological markers). Indeed, a high concentration of acetate, threonine, NA-lysine, HB, myoinositol, ascorbate, scylloinositol, total choline and a low concentration of aspartate, serine, glucose, adenosine, arginine, alanine and creatine were associated with a grade II/III meningioma and a Ki-67 rate ≥ 40% and a negative progesterone receptor expression (Fig. 1). Aside from threonine (p=0.01), none metabolite is associated with overall survival. There was no significant metabolic pathway (Fig. 2).

**Figure 1.** Summary of ADEMA network analysis conducted for specified groups comparison based on metabolite concentration obtained by HRMAS NMR spectroscopy. Red and blue boxes indicate, respectively, higher and lower metabolite concentrations between groups, and gray boxes no significant difference
**Figure 2.** Metabolic pathways considering aggressive meningioma (grade II/III and K-67 > 40% and negative progesterone receptor expression).
We proposed a management strategy by using metabolic analysis according to HRMAS NMR spectrometry (Table 1). Indeed, metabolite profile could permit to distinguish between
meningioma which will relapsed and those which did not, in order to adapt the therapeutic strategy.

**Table 1.** Management strategy of meningioma using high-resolution angle spinning spectrometry

Moreover, we showed that all meningioma with a low histological grade and a low Ki-67 proliferation index did not have a similar metabolomics profile (Fig.3). Indeed, some benign
meningiomas had a comparative metabolomics spectrum to the high grade meningiomas (Fig.4).

**Figure 3.** Three representative spectra of grade I meningioma with low Ki-67 rate (respectively 4, 2 and 3%) and positive progesterone receptor expression.
Discussion

We retrospectively studied the metabolic profile for 62 meningiomas by using HRMAS NMR. In our study, we correlated ex vivo spectrometry data with three histological features (histological grade, Ki-67 and progesterone receptor expression). We defined an aggressive meningioma as a tumor with a high histological grade and a Ki-67 rate \( \geq 40\% \) and a negative progesterone receptor expression. In our study, these three histological markers were associated with overall survival. According to the WHO 2007 classification (revised in 2016), high grade meningioma (II/III) is associated with a poor prognosis and a high risk of recurrence \(^7,^{20,21}\). Two studies described Ki-67 proliferative index as a prognosis factor of recurrence \(^22,^{23}\). Moreover in a retrospective study including 48 patients, Iplikcioglu et al.
showed that a positive progesterone receptor expression was statistically associated with low histological grade. In our study, a significantly elevated concentration of acetate, threonine, NA-lysine, hydroxybutyrate, myoinositol, ascorbate, scylloinositol and total choline and a low concentration of aspartate, glucose, isoleucine, valine, alanine, adenosine and arginine were associated with meningioma which had poor histological prognosis markers (high histological grade and Ki-67 ≥ 40% and negative PR expression). NA-lysine is produced by the acetylation of lysine. The impact of NA-lysine in tumor cell remains poorly understood for now. Myoinositol is a membrane component which participates in several cellular processes such as metabolic homeostasis, mRNA export, stress response. This role in the carcinogenesis remains unknown. Ascorbate is an essential nutrient. This metabolite is an inhibitor factor of the Hypoxia-Inducible Factor (HIF) system and it decreased VEGF expression. Ascorbate had also a cytotoxic activity due to an oxidative-related mechanism. The production of phosphorylcholine is an essential component in the induction of DNA synthesis and is therefore responsible of cell proliferation. An increased total choline expression reflects a tumoral hypoxia and the GPC/PC ratio is related to the tumoral aggressiveness. These findings are consistent with our result. Aspartate decreased tumor proliferation but the physiopathology remains unknown. This finding is also in consistent with our result. Glucose and alanine produce pyruvate which is transformed into lactate in the cell without oxygen such as tumor cell. In our study, we observed a low concentration of glucose and alanine was associated with an aggressive tumor.

There is actually a lack of data considering ex vivo spectroscopy for meningioma. Monleón et al. studied 30 meningioma samples by using HRMAS NMR. The authors analyzed only 10 metabolites. Among the 30 meningiomas, 23 were classed grade I and 7 grade II. The authors showed that the concentration of phosphocholine (3.2 ppm) (p=0.040) and
phosphoethanolamine (4.01 ppm) (p=0.016) were statistically higher for atypical meningioma compared to benign meningioma. These metabolites are implicated in the synthesis and degradation of phospholipids. Glutamine (2.44 ppm) (p=0.045), glutamate (2.35 ppm) (p=0.008), glutathione (2.55 ppm) (p=0.019) were statistically present in a higher concentration for grade II meningioma compared to grade I. Glutathione is an antioxidant and plays a role in free radicals protection. Moreover, the concentration of taurine (3.42 ppm) (p=0.0041) was statistically higher for atypical meningioma compared to benign meningioma.

Aside from phosphocholine, the findings were in opposite with our results. These differences could be explained by the fact that we included all grade of meningioma (grade I to III). Moreover, our work study included twice as many tumor samples. Pfisterer et al. analyzed 68 meningioma samples (46 grade I, 14 grade II and 8 grade III) by using proton magnetic resonance spectroscopy (8 metabolites). The means concentrations of alanine and creatine were statistically higher in benign meningioma compared to grade II and III meningioma (p= 0.002). Furthermore, the authors noted that the means concentrations of creatine and alanine were statically lower in tumors which rapidly recurred compared to those that did not (p<0.001) 15. To complete these results, Pfisterer et al. studied the metabolic profile by using proton magnetic resonance spectrometry for 30 benign meningiomas to distinguish between clinically-aggressively benign meningiomas and those with a good prognosis. The authors analyzed 6 metabolites. The creatine concentration was statistically lower for benign meningioma which rapidly recurred (p<0.05). Alanine tended to be lower for grade I tumors which recurred (p=0.05). No metabolite was statistically associated with Ki-67 expression or brain invasion 31. We described similar results in our study, indeed an elevated concentration of alanine was also associated with benign meningioma. Moreover, we described a correlation between a high concentration of alanine and a low Ki-67 expression.
However, in our study creatine was not associated with the tumor grade and a high concentration of this metabolite was statistically associated with a high Ki-67 expression.

Moreover, in our study, we described three different spectra representative of a meningioma with non-aggressive histological features (grade I and a low Ki-67 expression and a positive progesterone receptor expression). The concentration of the metabolites was different for these tumors, mainly total choline, glucose and creatine. One grade I meningioma with a low Ki-67 expression had no expression of glucose; this suggests an aggressive tumor behavior. Moreover, one grade I meningioma had a low concentration of creatine; this suggests a tumor hypoxia and an aggressive behavior. Despite, different metabolite concentrations, all grade I meningioma had a very low concentration of myoinositol and scylloinositol and a positive glucophosphocholine/phosphocholine (GPC/PC) ratio.

Furthermore, we described a similar metabolic spectra between a grade I meningioma (Ki-67 rate 4%, positive progesterone receptor expression) and a grade III meningioma (Ki-67 rate 80%, negative progesterone receptor expression). We obtained similar glutamate, glutamine and total choline concentration between both tumors. However, the ratio GPC/PC was different. Indeed, the ratio was positive for the grade I and negative for the grade III meningioma. Negative GPC/PC ratio is associated with malignant tumors. Furthermore, the creatine concentration was also lower for grade III meningioma compared to grade I meningioma. This suggests that hypoxia is higher for grade III meningioma. Glycine, myoinositol, scylloinositol and ascorbate concentrations were also higher for the grade III meningioma compared to the grade I meningioma. Taurine and hypotaurine concentrations were lower for the grade III meningioma in comparison with the grade I meningioma.

Finally, we proposed a metabolic profile associated with poor histological prognosis markers. This signature could be used to discuss a personalized therapeutic management. However,
futures studies are needed to confirm these results in order to include spectrometry and histological analysis to improve the classification of the meningioma.

Conclusion

Meningiomas are the most common benign brain tumors. However, some tumors can have a clinical aggressively behavior. Histological markers such as Ki-67, progesterone receptor expression or tumor grade are correlated to prognosis. We described a metabolic profile associated with poor histological prognosis markers for meningioma. Metabolic analysis could be useful to distinguish between clinical aggressively meningioma and those which did not recur in order to adapt the initial treatment. Futures studies are needed to determine an association between this metabolic signature and survival data.

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