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RESEARCH

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Metabolic remodeling in glioblastoma: a longitudinal multi-omics study

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Abstract

Monitoring tumor evolution and predicting survival using non-invasive liquid biopsy is an unmet need for glioblastoma patients. The era of proteomics and metabolomics blood analyzes, may help in this context. A case–control study was conducted. Patients were included in the GLIOPLAK trial (ClinicalTrials.gov Identifier: NCT02617745), a prospective bicentric study conducted between November 2015 and December 2022. Patients underwent biopsy alone and received radiotherapy and temozolomide. Blood samples were collected at three different time points: before and after concomitant radiochemotherapy, and at the time of tumor progression. Plasma samples from patients and controls were analyzed using metabolomics and proteomics, generating 371 omics features. Descriptive, differential, and predictive analyses were performed to assess the relationship between plasma omics feature levels and patient outcome. Diagnostic performance and longitudinal variations were also analyzed. The study included 67 subjects (34 patients and 33 controls). A significant differential expression of metabolites and proteins between patients and controls was observed. Predictive models using omics features showed high accuracy in distinguishing patients from controls. Longitudinal analysis revealed temporal variations in a few omics features including CD22, CXCL13, EGF, IL6, GZMH, KLK4, and TNFRSP6B. Survival analysis identified 77 omics features significantly associated with OS, with ERBB2 and ITGAV consistently linked to OS at all timepoints. Pathway analysis revealed dynamic oncogenic pathways involved in glioblastoma progression. This study provides insights into the potential of plasma omics features as biomarkers for glioblastoma diagnosis, progression and overall survival. Clinical implication should now be explored in dedicated prospective trials.

Key points

- Circulating omic features distinguishes patients with glioblastoma from healthy subjects.
- Plasma proteomic features change over time in patients undergoing radiochemotherapy.
- Certain plasma proteins are correlated with survival.

Keywords Glioblastoma, Proteomic, Metabolomic, Liquid biopsy, Mass spectrometry

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Introduction

Glioblastoma is the most common malignant brain tumor, representing approximately half of newly-diagnosed malignant brain tumors [1]. Despite a multimodal approach involving initial tumor resection if feasible, radiotherapy combined with temozolomide chemotherapy [2], supportive care, and more recently, when accessible, tumor-treating fields [3], the prognosis remains poor, with an overall survival of between 16 and 20 months. In the case of biopsy alone, the median overall survival drops to 9 months in the general population [4]. No significant improvement in patient care was found during the past decades. Although the diagnosis of glioblastoma is based on robust histo-molecular criteria [5], tumor biology is complex in its heterogeneity, its evolution under treatment and its interactions with the microenvironment. One of the keys to improving patient care and prognosis is the ability to longitudinally monitor tumor evolution. Circulating biomarkers could even predict treatment responses in order to personalize care for patients. In this context, the concept of liquid biopsy allows for the non-invasive detection of tumor material in the patient's biological fluids, such as blood or cerebrospinal fluid in glioblastoma patients [6]. The low sensitivity in detecting tumor-related nucleic acids (DNA, RNA, miRNA, etc.) in the plasma of patients with glioblastoma hinders its daily-practical use [7–9]. More recently, the simultaneous identification of multiple proteins or metabolites, namely proteomics and metabolomics, derived from the tumor and its microenvironment is a new approach for non-invasive tumor characterization [10–13]. Proteogenomics and metabolomics pave the way for new therapeutic approach in glioblastoma [14]. Concerning plasma features, proteomic or metabolomic analyses have shown the ability to distinguish patients with glioblastoma from other brain conditions [15]. Combined analysis of transcriptomic bulk tumor with post-therapy proteomic features in cerebrospinal fluid allows the identification of prognostic biomarker in glioblastoma [16]. Although promising, these results are limited by the often-complex clinical implications of serial cerebrospinal fluid sampling in patients. To date, no study has prospectively and longitudinally examined the evolution of circulating plasma proteomics and metabolomics biomarkers in a homogeneous population of glioblastoma patients. Given that neurosurgical tumor resection could falsely influence blood omics release, the selection of a patient population with unresected tumor could provide information about tumor evolution.

In this context, the objective of the presented work was to study the temporal evolution during the first-line treatment of circulating blood omics features in a population of unresected glioblastoma patients.

Material and methods

Patients and samples

A case–control study was conducted. Patients were selected from the GLIOPLAK trial (ClinicalTrials.gov Identifier: NCT02617745). GLIOPLAK is a prospective and bicentric study conducted between November 2015 and December 2022. Primary objective was to identify circulating biomarkers of temozolomide-induced hematological toxicities in glioblastoma patients receiving radiochemotherapy [2]. Comedication and patients' characteristics were collected in GLIOPLAK trial. Patients in the present study had undergone biopsy alone as part of the initial surgical procedure. Tumor non-resectability was due to tumor characteristics (size, location) and/or patient characteristics, according to surgeon choice. GLIOPLAK included patients aged equal or higher than 18 years-old of age, suffering from newly-diagnosed glioblastoma according to WHO CNS5 2021 classification [5]. Patients received concomitant radiotherapy and temozolomide followed by a maintenance phase for 6 or 12 cycles of temozolomide according to local guidelines and physician choice.

For each patient, blood samples were collected at three times along first-line treatment schedule: before (inclusion), after (W6) concomitant radiochemotherapy and at the time of disease progression (progression). The baseline time was collected after biopsy procedure. Blood samples were collected in EDTA tubes and plasma was collected as previously described procedure [7]. After collection, blood was double centrifugated and plasma was stored at − 80 °C within the three hours until use. Control plasma samples were purchased from BioIVT©. The overview of the study is presented in Fig. 1.

This study is ancillary to the prospective GLIOPLAK trial (ClinicalTrial.gov NCT02617745), which has been approved by the French National Committee for the Protection of Persons (RCB ID 2015-A00377-42). All patients provided written informed consent.

Targeted metabolomics

The kit for AbsoluteIDQ®p180 analysis, provided by Biocrates Life Science AG (Innsbruck, Austria) was used. This kit enables the measurement of 188 metabolites: amino acids (21), biogenic amines (21), hexoses (1), acylcarnitines (40), glycerophospholipids (90) and sphingolipids (15). Sample preparation followed the manufacturer's protocol. In brief, 10 µL of plasma were transferred to the upper 96-well plate and dried using a nitrogen stream. Subsequently, a 5% PITC solution (50 µL) was added to derivatize amino acids and biogenic amines. After incubation, the spots were dried, and the metabolites were extracted using

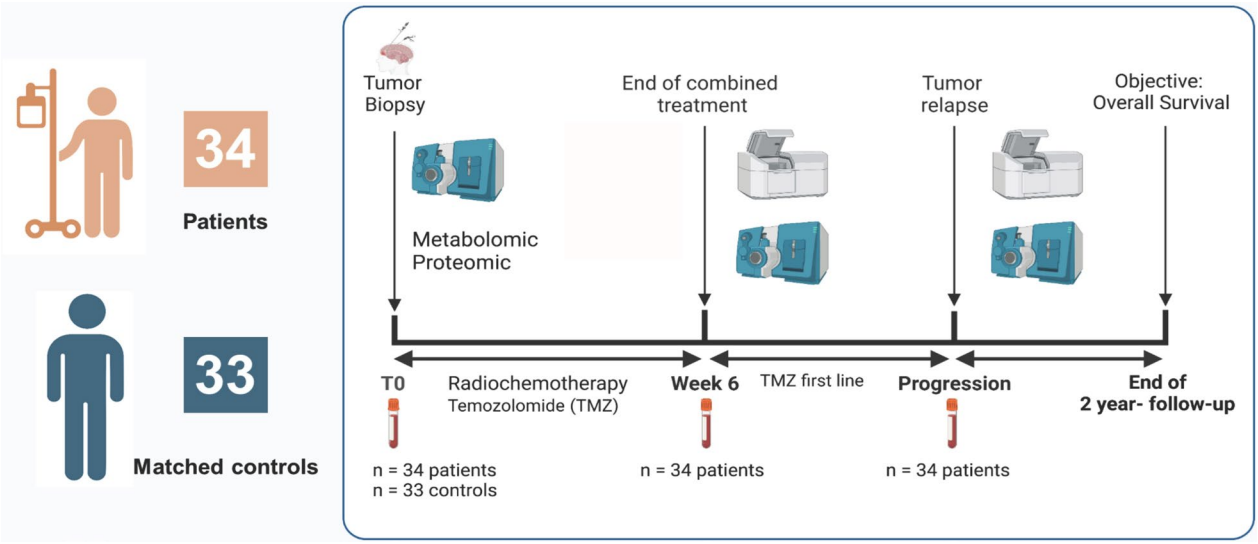

Fig. 1 Overview of the study protocol. Plasma samples and clinical data were collected from 33 control samples and 34 unresected glioblastoma patients before, during treatment, and at disease progression. Plasma samples were analyzed using metabolomics and proteomics, generating 371 omics features. This data was then used to compare controls with diseased samples and build predictive models to assess the relationship between plasma omics feature levels and disease status, patient outcome, and progression-free survival

5 mM ammonium acetate in methanol (300 µL) into the lower 96-well plate. Prior to analysis, the metabolites were further diluted using the mobile phase A. Quantification was performed using internal standards and a calibration curve as previously described [15, 17]. Liquid chromatography was performed on a HPLC system (SCIEX 4000, Dermstadt, Germany). The separation process took place at a temperature of 50 °C using a 3.0 × 100 mm Agilent Zorbax Eclipse C18 column with a 4 × 3 mm pre column (Phenomenex). The mobile phase A consisted of a mixture of ACN/H2O (40/60) containing 10 mM ammonium formate, while mobile phase B consisted of IPrOH/ACN (90/10). Both solvents were supplemented with 0.1% formic acid. The flow rate was set at 100 µL/min. A mobile phase gradient separation was performed over a duration of 20 min according to the following steps: 10% B at 0 min, 56% B at 2 min, 75% B at 10 min, 99% B at 12–15 min, and 10% B at 16–20 min. Acylcarnitines, lipids are analyzed using flow injection analysis-tandem mass spectrometry (FIA-MS/MS), while amino acids and biogenic amines are subjected to derivatization using phenylisothiocyanate and subsequently analyzed using liquid chromatography tandem-mass spectrometry (HPLC–MS/MS). The analysis used a SCIEX Api4000 QTrap mass spectrometer (AB SCIEX, Darmstadt, Germany) with electrospray ionization. The injection order was randomized before data acquisition.The concentration of each metabolite was measured in µM.

Targeted proteomics

The targeted analysis of 183 proteins was performed by Olink® Biomarker technology (Olink®, Uppssala, Sweden). The Olink Oncology II and Oncology III were used to measure proteins, the specific biomarkers included in each panel are described on the Olink website (https://olink.com/products-services/target/oncology-panel/). Each manipulation steps were performed according to the manufacturer protocol. The protocol is divided into three steps: Incubation, extension amplification, and detection. The Incubation step is where the antibody-pairs with attached DNA tags are added to the samples and allowed to bind to their target proteins during overnight incubation. The extension and amplification steps are performed in the following morning, during which unique DNA reporter sequences are generated for each target protein and pre-amplified through conventional PCR. The final Detection step quantifies the DNA reporters for each biomarker using high throughput real-time qPCR on the Olink Signature Q100 system. The samples were previously randomized on the plates. To account for intra- and internal variation, data underwent quality control and normalization using an internal extension and interpolate control. Protein measurements are expressed as normalized protein expression (NPX) values which are log-base-2 transformed values.

Data analysis

Descriptive and differential analysis

Quantitative variables were summarized by median, and range. Qualitative data are presented as absolute frequency, relative frequency, confidence interval of 95%, and percentage of missing data. These data are compared using the Chi2 test or a Fisher exact test in the case of failure to meet the Chi2 application rules. Quantitative data were presented as histograms, median, range, mean, standard deviation and percentage of missing data. The normality of those parameters was evaluated with frequency histograms and the Shapiro test. Correlation analysis was performed using the Spearman method. Quantitative data were compared using the Student T or Mann-Withney tests as appropriate. One way ANOVA was used for the time-related groups comparison. Correction for multiple testing using false discovery rate (FDR) through the Benjamini–Hochberg method was performed when appropriate. All data analyses were performed using R Software.

Cox regression and survival analysis

Overall survival (OS) was defined as the time from the completion of radiotherapy until death from any cause. Progression-free survival (PFS) was defined as the duration from the completion of radiotherapy until the date of tumor progression. A univariate Cox model was built for every protein and metabolite at each time point. Results are presented with a hazard ratio (HR) and 95% confidence interval (95% CI). To perform a comparative analysis, the median concentrations and expressions of metabolites and proteins of the control group were considered a threshold for the glioblastoma samples. For each metabolite and protein, this threshold separated the cohort into a "High" and a "Low" group. Survival analysis was performed based on this threshold derived groups. The association with overall survival was assessed in a univariate analysis by using Cox regression model. All tests were two-sided, and a p-value of 0.05 or less was considered statistically significant. The metabolites and proteins differentially expressed between Patients and Controls were compared to those previously published. Data published between 2000 and 2023 have been retrieved from PubMed using the following MESH: "glioblastoma", "Proteomics", "Metabolomics", "Lipidomics".

Predictive analysis

We performed a predictive glioblastoma diagnosis analysis using two machine learning algorithms. Metabolomics and proteomics datasets were concatenated into one data matrix. Values below the detection limit (LOD) have been replaced by their respective LOD. Subsequently, we developed classification models to distinguish patient samples from controls. These models were constructed using the 'caret' R package. Initially, we split the data from the disease at inclusion and controls, allocating 70% for training and the remaining 30% for testing. This partition was accomplished via the createDataPartition() function in 'caret', leading to distinct training and testing sample sets. The disease and control samples were uniformly integrated, ensuring the training set was exclusively used for model construction while the testing set served for evaluation purposes. Before model construction, we addressed missing data from quality control issues, employing the preProcess() function in 'caret' and leveraging the "knnImpute" method. Utilizing the train() function in 'caret', we built prediction models for disease based on the designated training sets. We adopted the random forest methodology as our classification algorithm, incorporating a fivefold cross-validation scheme alongside inherent parameter tuning. To discern the influence of each omics feature (be it protein or metabolite) on the model, we applied the varImp() function from the 'caret' package. Subsequent model performance was assessed using the predict() function on the testing set, ensuring no overlap with training data. The evaluation process involved ROC analysis, facilitating an assessment of model sensitivity and specificity, ultimately encapsulated as AUC scores. For binary classification tasks, we used the 'pROC' R package. Enrichment analysis has been performed using the Enrichr R package.

Results

Cohort description

Sixty-seven subjects were included in the study: 34 subjects as patients and 33 as controls. In the glioblastoma population (n = 34), median age at diagnosis was 63 years (age interquartile: 56–66) and sex ratio was 1.3. No significant age or sex differences were identified between patient and control group, Table 1. A summary of the clinical data is listed in Table 1 and detailed data are presented in Additional file 1: Tables S1 and S2. Regarding comedication 67.7% of the glioblastoma population were exposed to corticosteroid at baseline (median dose 35 mg daily [dose range: 0–40]). No significant changes in corticoid exposure were observed between the three times (p-value = 0.13). Among biological parameters, the most relevant and clinically pertinent changes were observed for platelet counts and albuminemia: a decrease of 34.9% and 9.1% between baseline and progression, respective p-value < 0.001 and 0.004, Table 2. Lymphocytes significantly changed during treatment in the glioblastoma population, median overall survival (OS) was 13 months (range: 10.0–17.0), and progression-free survival was 6 months (range: 5.0–6.8).

Table 1 Summary of the cohort characteristics at inclusion

Characteristics	Controls	Inclusion	p-value
Age[a]	59 (53–65)	63 (56–66)	> 0.9
Sex			
Female	14/33 (42%)	15/34 (44%)	> 0.9
Male	19/33 (58%)	19/34 (56%)	
Karnofksy score > 70		29/34 (85%)	
Obesity		7/34 (21%)	
Diabetes		7/34 (21%)	
Hypercholesterolemia		8/34 (24%)	
Corticoids (mg/j)		35 (0–40)	
Statin		5/34 (15%)	
Metformin		5/34 (15%)	
MGMT promoter methylation		6/34 (18%)	

[a] Median (IQR)

Differential expression analysis

Patients versus controls

We performed a differential expression analysis of metabolite and protein levels between controls and patients at each sampling point (Additional file 1: Tables S3, S4). A total of 371 omics features were measured. Regarding metabolites, 140 out of 188 were differentially expressed between patients and controls (74.5%). Among them, 75 Glycerophospholipids (83.3%), 26 acylcarnitines (65%) 13 biogenic amines (61.2%), 15 amino acids (71.4%), and 11 sphingomyelins (73.3%) were differentially expressed. Glutamate and Valine were more expressed in patients than in controls, the opposite was observed for Leucine, Isoleucine and Asparagine (Fig. 2). Pimeloylcarnitine was the most significantly and differentially expressed acylcarnitines. Spermidine was the most significantly differentially expressed biogenic amine. Lyso PC a C18:0, Lyso PC a C16:0, PC aa C36:5, PC ae C34:1, PC aa C34:1, were the most significantly differentially expressed glycerophospholipids. For proteins, 131 out of 183 were differently expressed (71.6%). The proteins NPY, KLK13, DCTPP1, CDKN1A, MANSC1, CXCL14, HMBS, SCLY, S100A4 and FCFBP1 had the highest fold change. The heatmap (Fig. 3) of the omics profiles highlights two groups. One group constituted of FGFPB1, S100A4, LysoPC a C16:0, LysoPC a C18:0, Pimeloylcarnitine and Spermidine is highly expressed in controls. In contrast, the second group composed of PC aa C36:5, PC aa C34:1, PC ae C34:1, Isoleucine, Leucine, Asparagine, NPY, HMBS, DCTPP1, MANSC1, SCLY, KLK13, CDKN1A, CXCL14 is highly expressed in patients. Notably, the differences of expression between Patients and Controls did not change under treatment or at tumor relapse. Detailed results are presented in Additional file 1: Table S5.

Longitudinal variation analysis

In the patient population, a subset of metabolites and proteins were differentially expressed between the three timepoints. Among them, 34 proteins (18.6%) and 12 metabolites (6.7%) significantly changed between inclusion, W6 and progression. Proteins and metabolites involved in carcinogenesis, tumor necrosis or immune reaction were particularly represented; in particular CD22, CXCL13, EGF, IL6, GZMH, KLK4 and TNFRSP6B. Notably the EGF protein significantly decreased between inclusion and progression (Fold change < —1). The most differentially expressed metabolites and proteins and their variation through time are detailed in Fig. 4. Detailed results are presented in Additional file 1: Table S5.

Predictive model

The measured concentrations and expressions of the 265 differentially expressed proteins and metabolites between patients at inclusion and control samples were analyzed. We explored the predictive performance of each lipid and proteinomics feature and all their combinations using

Table 2 Longitudinal variations of the cohort

Characteristics	Inclusion[a] n = 34	Week 6[a] n = 34	Progression[a] n = 34	p-value[a]
Overall Survival (months)	13 (10–17)			
Progression free survival (months)	6.0 (5.0–6.8)			
Corticoid treatment (mg/j)	35 (0–40)	40 (20–75)	40 (20–80)	
Bactrim	0/34 (0%)	23/34 (68%)	0/34 (0%)	
Hemoglobin (g/dl)	14.4 (13.8–15.3)	13.20 (12.5–14.6)	13.10 (11.5–14.4)	
Leucocytes (G/L)	9.0 (7.6–12.3)	7.4 (5.8–10.0)	6.2 (5.6–8.7)	
Platelets (G/L)	271 (230–320)	214 (149–239)	177 (148–214)	< 0.001
Albumin	44.0 (42.0–46.0)	42.0 (40.0–44.0)	40.0 (37.5–44.0)	0.004
Prothrombin time	1.12 (1.03–1.18)	1.09 (0.96–1.20)	1.10 (1.04–1.23)	
Creatinine (µmol/L)	66 (60–75)	72 (62–82)	64 (52–72)	

[a] Median (IQR)

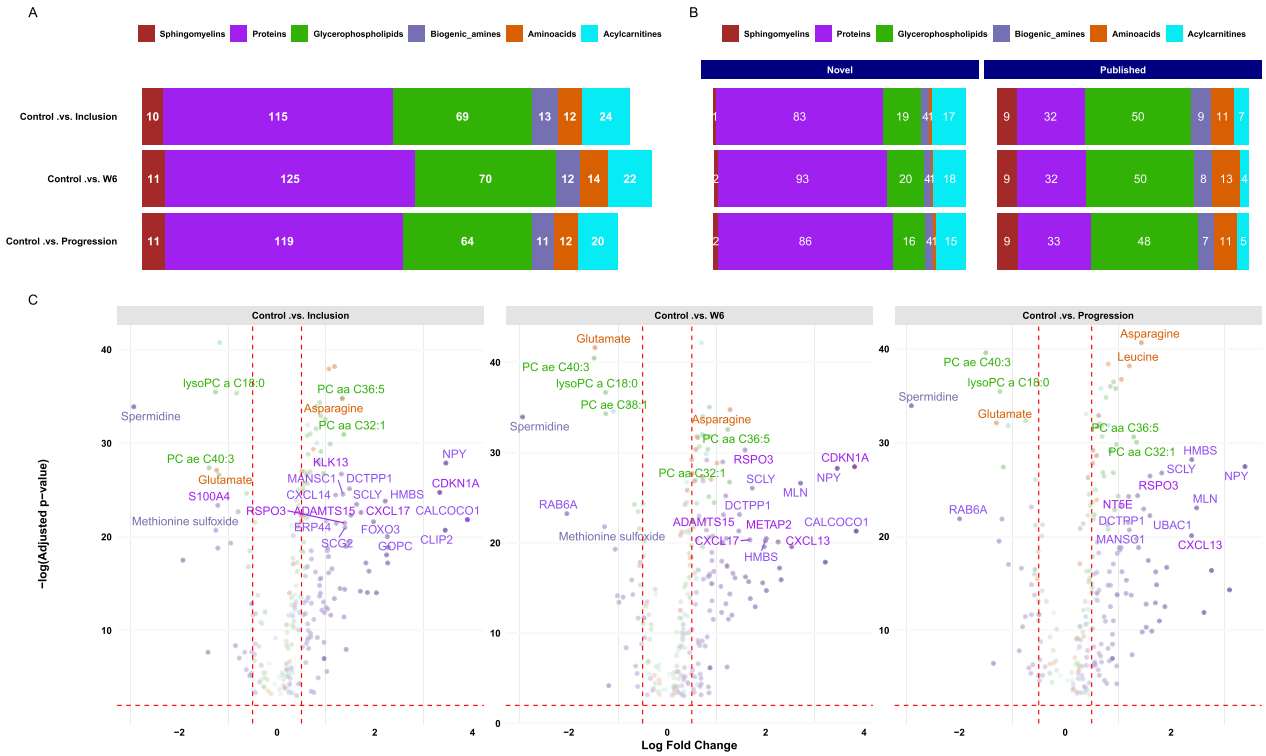

Fig. 2 Omics-based differential analysis. **A** Overview of the differential expression analysis of proteins and metabolites between patients *versus* controls at the three studied time (inclusion, W6 and progression). **B** Barplot showing the proportions of proteins and metabolites and their novelty compared with literature. **C** Volcano plot of the most differentially expressed proteins and metabolites between Patients and Controls at baseline (left), at progression (middle) and at W6 (right)

predictive machine learning. The generated data were separated into a training set (80%), and a test set (20%). The area under the curve and ROC curves were used as performance metrics. Out of the whole metabolomic and proteomic dataset, 5 metabolites: Pimeloylcarnitine, Leucine, Asparagine, LysoPC a C18:0, PC ae C40:3, and 5 proteins: NPY, KLK13, SCLY, S100A4, CXCL17 had and AUC above 0.74. When combined, these 10 omics features had an AUC equal to one and almost always accurately classified patients as patients and controls as controls. Interestingly, the protein NPY taken individually had a similar predictive performance. Regarding metabolites, pimeloylcarnitine had an AUC = 0.95 with one control sample being misclassified. The test set with the performance of each omics profile as well as their ROC curve and variation between controls, patients and timepoints, is described in Fig. 5. Detailed results are presented in Additional file 1: Tables S7 and S8.

Survival analysis

The association analysis of each of the 371 omics features with OS has been performed. To do so, we used an iterative Cox modeling at each time point. The median level in the patient samples at inclusion is used for each feature as a threshold defining the high and low expression groups. Unbalanced groups, albeit with a significatively different median OS, were excluded. The results yielded a total of 77 omics features that were significantly associated with OS at different time points (Fig. 6). Among them, proteins were the most represented (n = 51, 66.2%) followed by 26 metabolites (33.8%), including 8 acylcarnitines (10.4%), 11 glycerophospholipids (14.3%), 4 amino acids (5.2%), 2 biogenic amines (2.6%) and 1 sphingomyelin (1.3%). At inclusion, 28 proteins and 12 metabolites have been identified. Aside from PC aa C36:4, Hydroxyhexadecenoylcarnitine and Hydroxyhexadecadienylcarnitine, the high expression group were associated with the worst OS. Notably, a high expression of NPY, the protein with the best predictive performance of glioblastoma diagnosis in our cohort, was significantly associated with a poorer prognosis.

At Week 6, after treatment by RCT, 22 omics features were associated with OS, including 16 proteins and 6 metabolites. At progression, 35 omics profiles, including 25 proteins and 10 metabolites were associated with OS. Interestingly, omics features with similar function had a different influence on the prognosis. For instance, a high plasma concentration of glycerophospholipids

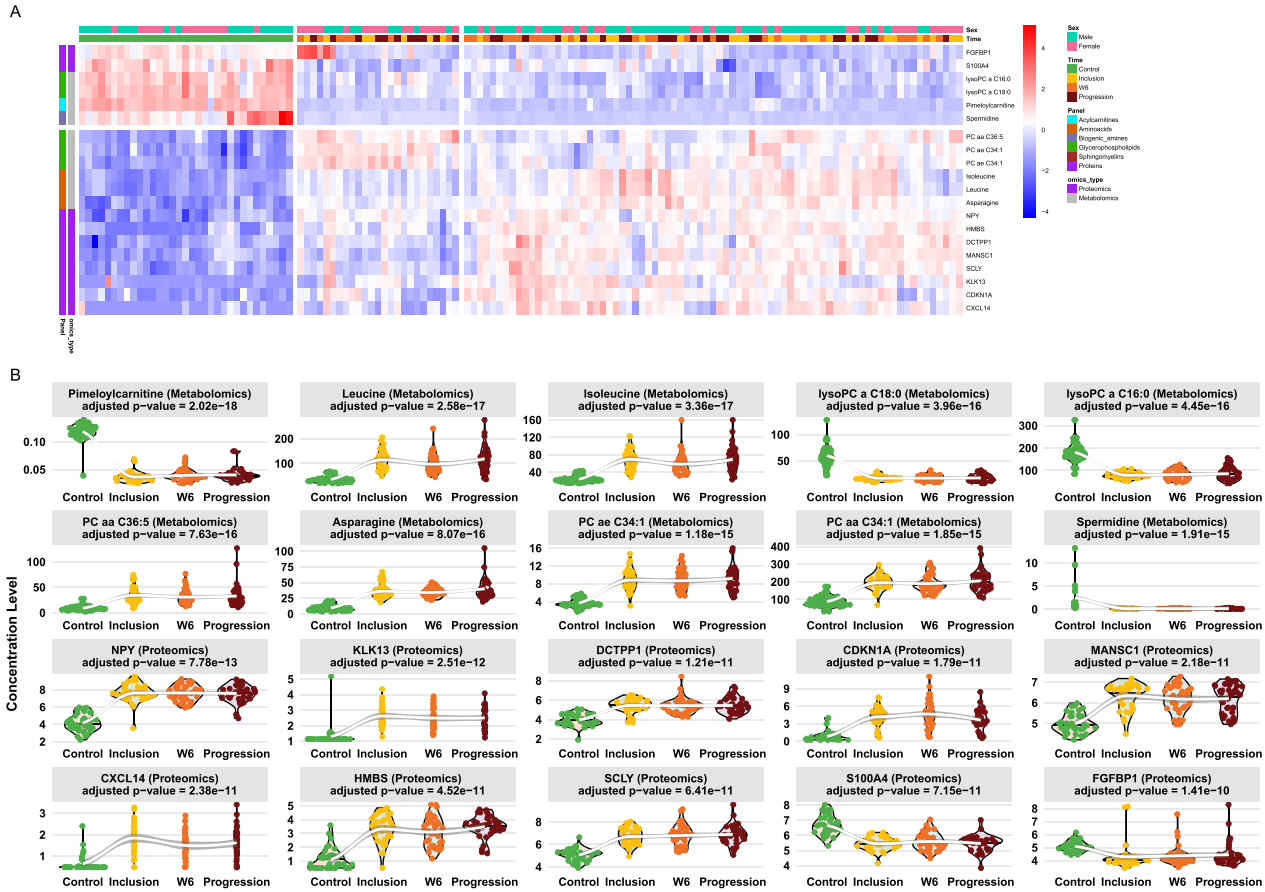

Fig. 3 Proteins and metabolites expression levels across samples. **A** Levels of omics features with two distinct profiles of between glioblastoma samples and controls. **B** Violin plots showing the omics feature levels across the different groups: controls, inclusion, week 6 (W6) and at progression

(GPC) was associated with a worst overall survival at inclusion, whereas a high expression of GPC at progression was predictive of a better OS. Moreover, 15 omics features including 13 proteins and 2 metabolites were associated with overall survival at different timepoints. Notably, the proteins ERBB2 (HER2) and ITGAV (Integrin subunit alpha V) were associated with OS at each timepoints. Hazard ratios of each omics profiles are detailed in Fig. 6. Detailed results are presented in Additional file 1: Supplementary data 9. We performed a pathway enrichment analysis and observed that the oncogenic pathways involved differed at each timepoint. At inclusion, Transmembrane Receptor Protein Tyrosine Kinase Activity, TGF Beta and TRAIL pathways were mainly involved, whereas GRB7 events in ERBB2 signaling were the main pathway at Week 6 and Progression. VEGF and pyrimidine catabolism were also among the involved pathways. Detailed data is presented in Additional file 1: Supplementary data 10.

Discussion

This longitudinal study included 34 biopsy-only patients with glioblastoma. The median OS for our population was 13 months, with a range of 10.0 to 17.0 months. The differential expression analysis of metabolite and protein levels between controls and patients at each sampling point (before radiochemotherapy, after treatment, and at disease relapse) yielded differential patterns. Regarding metabolites, 74.5% (140 out of 188) have changed and this included all assessed groups: 75 Glycerophospholipids, 26 acylcarnitines, 13 biogenic amines, 15 amino acids, and 11 sphingomyelins. For proteins, 131 out of 183 were differentially expressed with NPY, KLK13, DCTPP1, CDKN1A, MANSC1, CXCL14, HMBS, SCLY, S100A4, and FCFBP1 as the most expressed proteins. We compared our results with previously reported studies [15, 18, 19]. Most of these studies assessed patients' plasma proteome or metabolome before treatment. Out of 265 the reported differentially expressed omics features

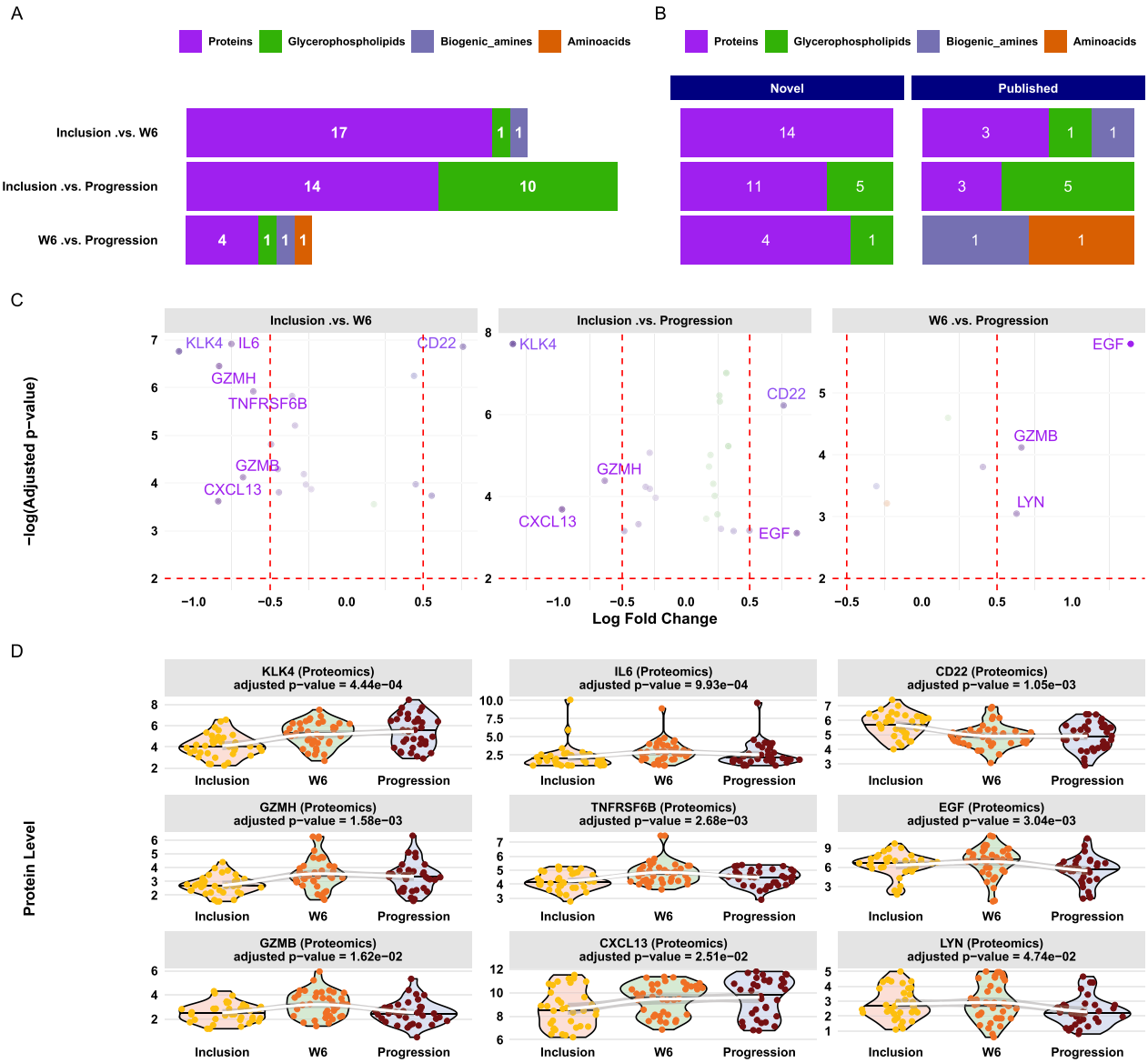

Fig. 4 **A** Overview of the differential expression analysis of proteins and metabolites between patients. **B** Barplot showing the proportions of proteins and metabolites and their novelty status regarding the literature. **C** Volcano plot highlighting the most differentially expressed proteins and metabolites between timepoints. **D** Violin plots of the most differentially expressed proteins between timepoints

reported in this study, 168 (63.3%) were not previously reported, including 128 proteins (76.2%) and 40 metabolites (23.8%). The novelty status of the differentially expressed proteins and metabolites is shown in Fig. 2B. Furthermore, through iterative selection, a predictive diagnostic model was derived, including 5 metabolites: Pimeloylcarnitine, Leucine, Asparagine, LysoPC a C18:0, PC ae C40:3, and 5 proteins: NPY, KLK13, SCLY, S100A4, CXCL17. The predictive analysis revealed the high diagnostic prediction of NPY. The NPY is a neurotransmitter highly expressed in the hypothalamus involved in many physiological functions. Overexpression of NPY has been described in glioblastoma preclinical models but, to our knowledge, never reported on glioblastoma patients' plasma [13]. However, NPY is known to be elevated in other conditions, such as myocardial infarction [20]. Other omics features had also shown a high predictive performance, such as pimeloylcarnitine, an acylcarnitine not known to be associated with glioblastoma. Further studies with a larger cohort are needed to confirm the reproducibility of these observations. The longitudinal design of our study allowed us to observe the time

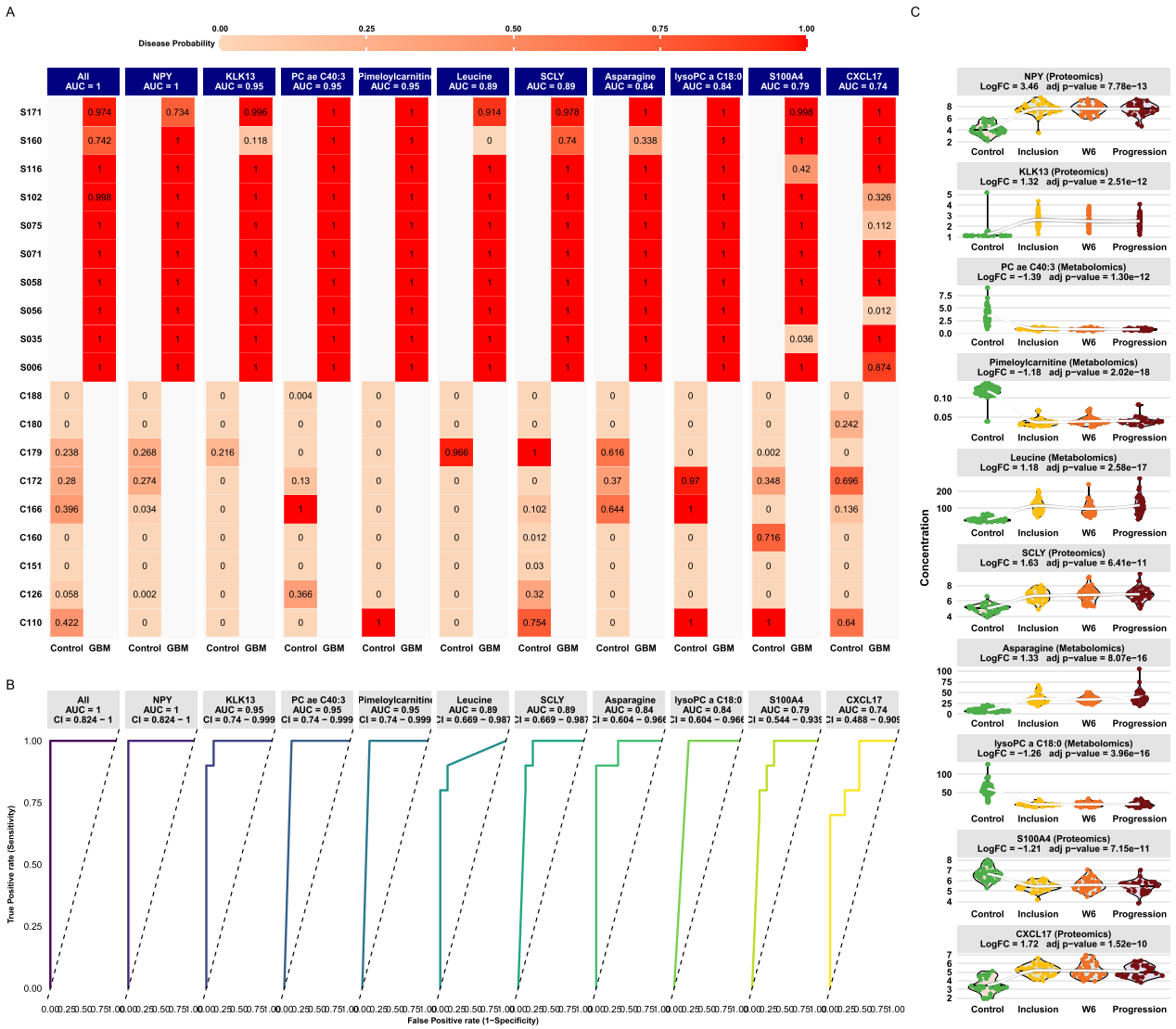

Fig. 5 Machine learning analysis. **A** Overview of the diagnostic performance of the predictive model. Each tile shows the probability of accurately classifying patients and controls from the test set. **B** ROC curves of each omics feature and their combined model. **C** Violin plots of the most significative omics features included in the model

variation of omics features after treatment and at relapse. Few proteins and metabolites varied under treatment and at progression. These included 34 proteins and 13 metabolites with CD22, CXCL13, EGF, IL6, GZMH, KLK4 and TNFRSP6B as the most varying between time points. A comparison with the literature was performed for omics profiles related to longitudinal variation (Fig. 3B). Among the proteins and metabolites differentially expressed over time, 28 were novel. When comparing inclusion versus progression, 5 glycerophospholipids and 11 proteins were not previously reported. Regarding inclusion *versus* W6, 14 proteins are novel. When comparing W6 versus Progression, 4 proteins and 1 glycerophospholipid were not previously reported. It could be hypothesized that under radiochemotherapeutic pressure, only core pathophysiological processes are triggered. For instance, we observed a significant variation of the EGF protein between inclusion and progression. Indeed, *EGFR* gene amplification is a cornerstone of glioblastoma molecular diagnosis [21]. However, targeted therapy using EGFR inhibitors such as erlotinib/gefitinib or, more recently osimertinib has no significant effect [22, 23]. Glioblastoma heterogeneity, drug delivery issues, and the use of alternative pathways are the main drivers behind this failure. However, most studies have been conducted as a second-line treatment after the conventional RCT and TMZ regimen.

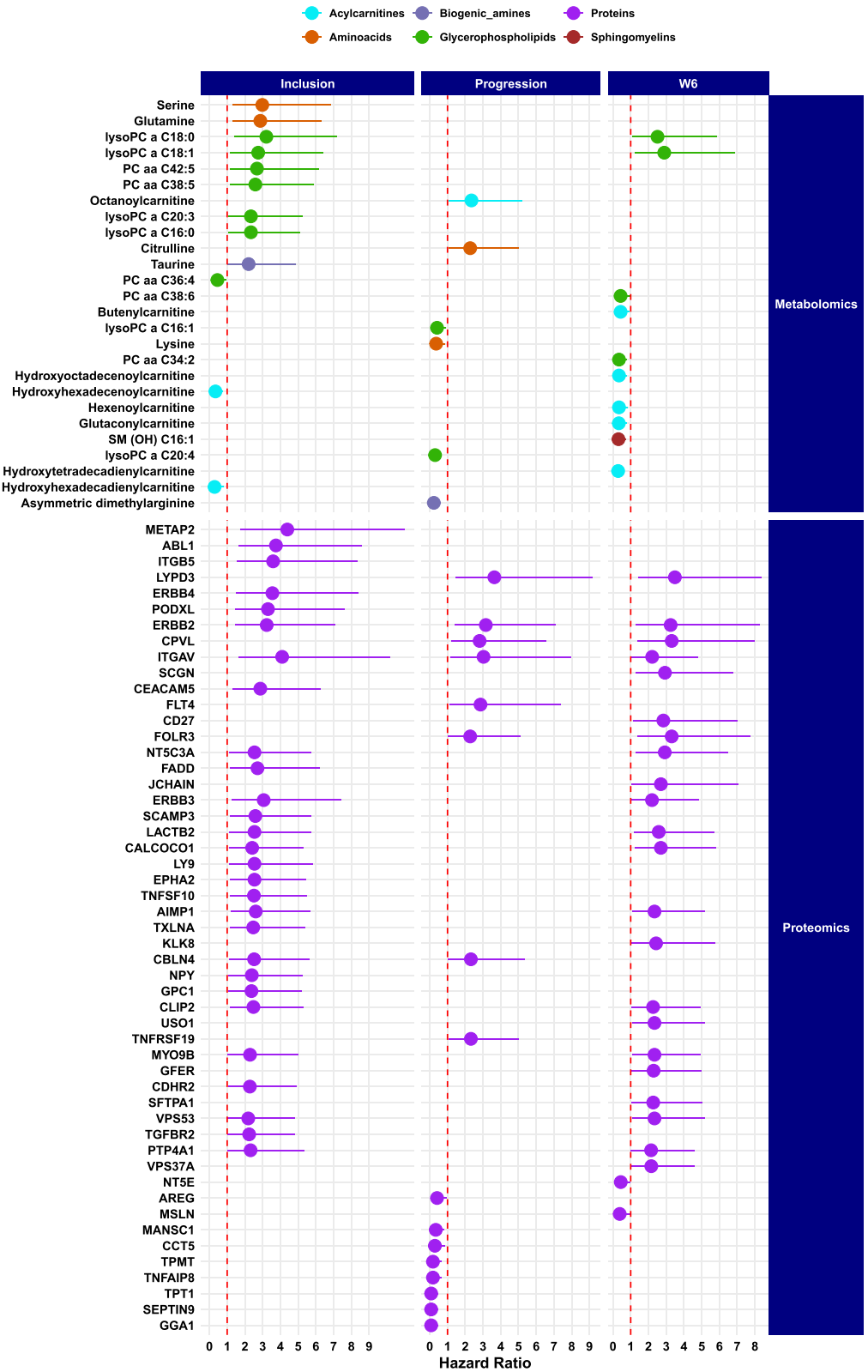

Fig. 6 Forest plot of the omics features associated with overall survival

As highlighted here in patient plasma, the loss of EGF expression at relapse might explain part of this failure and could help patients' selection in future anti-EGFR trials. Another protein that significantly varies under treatment is the Kallikrein 4 (KLK4). We observed that its expression increases under treatment and at progression. KLK proteins are hormone-regulated genes expressed in various human tissues, playing a role in various physiological functions. These functions include regulating blood pressure, tissue remodeling, processing of peptide hormones, and inflammation. For instance, KLK6, 7, and 8 are well-described in intracranial tumors. Drucker et al. reported an association of KLK6 expression with poorer overall survival in a cohort of 23 glioblastomas [24]. KLK4 is upregulated in prostate cancer, and its function in glioblastoma remains unknown. Regarding metabolites, the differential analysis between timepoints revealed important metabolic remodeling. The glycerophospholipids PC ae C42:4, PC ae C42:5, PC ae C 44:5, PC ae C44:6 significantly decreased after treatment and at progression. These observations shed light on glioblastoma metabolic remodeling under treatment. Indeed, glycerophospholipids are involved in cellular signaling processes and play key roles in plasma membrane composition and integrity. Wildburger et al. observed that glioma cells had elevated phosphatidic acid (PA) levels, specifically PC a 36:2, PC a 44:5, PC a 42:5, and PC a 42:7 [25]. PA is primarily utilized along with endogenous fatty acids (FAs) to supply glycerophospholipids for membrane synthesis. The increased accumulation of PA is associated with various alterations in cancer cell metabolism. Notably, PA stimulates the upregulation of several kinases involved in intracellular stress signaling pathways [26]. Our results show that 4 phosphatidylcholines significantly decrease during treatment and tumor progression. This observation could be explained by a metabolic shift directly due to RCT or the selection of RCT-resistant glioblastoma cells with different energy metabolism. The survival analysis showed that 77 omics features (51 proteins and 26 metabolites) were significantly associated with OS at different time points. Interestingly, the proteins ERBB2 (HER2) and ITGAV (Integrin subunit alpha V) were associated with OS at each timepoint. The set threshold to test the association of each protein and metabolite was their respective median in patients at inclusion. Out of the 26 metabolites (12 glycerophospholipids, 8 acylcarnitines, 4 amino acids, 1 biogenic amin, and 1 sphingomyelin) were associated with OS. Interestingly, glycerophospholipids or acylcarnitines were not constantly associated with the worst prognosis. Furthermore, their association with OS changed according to timepoints. This highlights the importance of metabolic remodeling due to oncogenic drivers switching under treatment pressure. For proteins, 51 were associated with OS, 27 at inclusion, 16 at W6, and 23 at progression. Only 2 proteins, ErBB2 and ITGAV were associated with OS at all timepoints. ITGAV is an important subtype of the integrin α chain family and is involved in angiogenesis. Wan et al. demonstrated that the overexpression of *ITGAV* had the best predictive survival performance among a set of 31 proangiogenic genes in glioblastoma [27]. The enrichment analysis unveiled different oncogenic pathways over time. It can be speculated that RCT treatment triggers core pathways involved in tumor survival. The exploratory nature of these analyses is the main limitation alongside the sample size and the omics coverage. Further large-scale studies keeping the same dynamic approach are needed to investigate the metabolic remodeling and pathway changes of glioblastoma under treatment. A potential limitation of this study is the risk of statistical overfitting. Despite efforts to mitigate these risks through cross-validation and cautious interpretation of results, these limitations should be considered. Furthermore, the study's findings would benefit from validation on larger cohorts to enhance generalizability and statistical power, ensuring that the results are applicable to a broader population and achieve clinical actionability.

In conclusion, the deep metabolic and signaling remodeling of glioblastoma can be probed in plasma samples to capture glioblastoma in situ status. Notably, NPY shows a strong predictive performance of glioblastoma. Secondly, few significant protein expression changes are detected in plasma before treatment, after treatment, and at relapse. This highlights the core resilience of glioblastoma under treatment that could allow a better selection of key oncogenic drivers after treatment. The EGF protein expression significantly changed under treatment and at relapse. Lastly, omics features were associated with OS, emphasizing the changes in the key pathways involved in glioblastoma prognosis. This could be promising for dissecting these changes in a larger cohort.

Conclusion

Proteomic and metabolomic analysis of plasma from unresected glioblastoma patients receiving radiotherapy and temozolomide reveals a singular circulating omic profile distinct from healthy subjects. The omics profile remains stable overall during the first line of treatment, with the exception of a few protein markers involved in tumor biology, such as EGF. Some omics features have prognostic or diagnostic potential in glioblastoma patients. This pilot study opens up new insights for the identification of circulating biomarkers in glioblastoma patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-024-01861-5.

Additional file 1.

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Author contributions
Conception and design: AT, SB, JDH and MF. Collection of study materials and/or patients' inclusion and data: AD, LFP, FDF, CA, IT, OL, FM and MF. Assembly of data and analysis: JDH, MF, FD, CP, TP, DM, CL, SB and AT. Statistical analysis: JHD, MF, SB and AT. Manuscript writing and editing: JHD, MF, SB and AT. Final approval of manuscript: all authors.

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Availability of data and materials
Deidentified data are available in Supplementary Data files.

Declarations

Ethics approval and consent to participate
This study is ancillary to the prospective GLIOPLAK trial (ClinicalTrial.gov NCT02617745, registered 11/30/2015). GLIOPLAK has been approved by the French National Committee for the Protection of Persons (RCB ID 2015-A00377-42).

Consent for publication
All the patients gave their written and informed consent. All De-identified and signed forms are available upon request.

Competing interests
MF declares income received for research purposes from Servier Foundation, benefits for interventions from Seagen and Novocure, and payment of congress fees from Gilead and Pfizer. The authors declare no conflict of interest.

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