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Review

Proteomics and metabolomics approach in adult and pediatric glioma diagnostics

Tomasz Pienkowski [a,*], Tomasz Kowalczyk [a,b], Noemi Garcia-Romero [c,d], Angel Ayuso-Sacido [c,d,e], Michal Ciborowski [a]

[a] Clinical Research Centre, Medical University of Bialystok, M. Sklodowskiej-Curie 24a, 15-276 Bialystok, Poland
[b] Department of Medical Microbiology and Nanobiomedical Engineering, Medical University of Bialystok, Mickiewicza 2C, 15-222 Bialystok, Poland
[c] Faculty of Experimental Sciences, Universidad Francisco de Vitoria, 28223 Madrid, Spain
[d] Brain Tumor Laboratory, Fundación Vithas, Grupo Hospitales Vithas, 28043 Madrid, Spain
[e] Faculty of Medicine, Universidad Francisco de Vitoria, 28223 Madrid, Spain

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ABSTRACT

The diagnosis of glioma is mainly based on imaging methods that do not distinguish between stage and subtype prior to histopathological analysis. Patients with gliomas are generally diagnosed in the symptomatic stage of the disease. Additionally, healing scar tissue may be mistakenly identified based on magnetic resonance imaging (MRI) as a false positive tumor recurrence in postoperative patients. Current knowledge of molecular alterations underlying gliomagenesis and identification of tumoral biomarkers allow for their use as discriminators of the state of the organism. Moreover, a multiomics approach provides the greatest spectrum and the ability to track physiological changes and can serve as a minimally invasive method for diagnosing asymptomatic gliomas, preceding surgery and allowing for the initiation of prophylactic treatment. It is important to create a vast biomarker library for adults and pediatric patients due to their metabolic differences. This review focuses on the most promising proteomic, metabolomic and lipidomic glioma biomarkers, their pathways, the interactions, and correlations that can be considered characteristic of tumor grade or specific subtype.

Abbreviations: WHO, world health organization; pHGG, pediatrics High Grade Glioma; pLGG, pediatrics Low Grade Glioma; HGG, High Grade Glioma; LGG, Low Grade Glioma; MRI, Magnetic Resonance Imaging; CT, Computed Tomography; CNS, Central Nervous System; CSF, Cerebrospinal Fluid; SWATH/SWATH-MS, Sequential Window Acquisition of All Theoretical Mass Spectra; TMT, Tandem Mass Tag; iTRAQ, isobaric Tag for Relative and Absolute Quantification; iodoTMT, iodoacetyl Tandem Mass Tag; iTRAQH, isobaric Tag for Relative and Absolute Quantification of Hydrazide; LF, Label Free; DDA, Data Dependent Acquisition; ESI, Electrospray Ionization; SRM, Selected Monitoring Reactions; MRM, Multiple Monitoring Reactions; NMR, Nuclear Magnetic Resonance; LC, Liquid Chromatography; GC, Gas Chromatography; CE, Capillary Electrophoresis; GC–MS, Gas Chromatography Mass Spectrometry; LC-MS, Liquid Chromatography Mass Spectrometry; GC–MS/MS, Gas Chromatography with Tandem Mass Spectrometry; LC-MS/MS, Liquid Chromatography with Tandem Mass Spectrometry; GC-TOF, Gas Chromatography Time-of-Flight; LSI, Lipidomic Standards Initiative; EI, Electron Ionization; CI, Chemical Ionization; H2, Deuterium; C13, Carbon Isotope 13; VEGF, Vascular Endothelial Growth Factor; SOCS3, Suppressor Of Cytokine Signaling 3; GBM, Glioblastoma; LDHA, Lactate Dehydrogenase A; EGFR, Epidermal Growth Factor Receptor; EGFRvIII, Epidermal Growth Factor Receptor variant III; IL-13Ra2, Interleukin-13 Receptor alpha 2; PDGFRA, Platelet-Derived Growth Factor Alpha Receptor; COF-1, Cofilin-1; PGK1, Phosphoglycate kinase 1; IDH, Isocitrate Dehydrogenase; MB, Medulloblastoma; MGMT, O^6-methylguanine-DNA-methyltransferase; mMGMT, Methylated MGMT; uMGMT, Unmethylated MGMT; MMP, Matrix Metalloproteinases; TIMP, Tissue Inhibitor Metalloproteinase; GFAP, Glial Fibrillary Acidic Protein; GSH, glutathione; TCA cycle, Tricarboxylic Acid Cycle; 2-KG, alpha-ketoglutarate; 2-HG, 2-hydroglutarate; NADPH/NADP+, Nicotinamide Adenine Dinucleotide Phosphate; ROS, Reactive Oxygen Species; HIF-1a, Hypoxia-Induced Factor 1-a; HSP90α, heat shock protein 90; SDH, Succinate Dehydrogenase; FH, Fumarate Hydratase; LDH, Lactate Dehydrogenase; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartyl-glutamic acid; GABA, 4-Aminobutyric acid; ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; AMP, Adenosine monophosphate; UTP, Uridine triphosphate; UDP, Uridine diphosphate; UMP, Uridine monophosphate; FA, Fatty Acids; ACL, ATP Citrate Lysae; ACC, Acetyl-CoA Carboxylase; FASN, Fatty Acid Synthase; NSC, Neural Stem Cells; BTSC, Brain Tumor Stem Cells; GDP1, Glycerol-3-phosphate Dehydrogenase 1; GS, Glutamine synthetase; ATRX, Alpha-Thalassemia/mental retardation, X-linked gene; DAXX, Death-domain Associated proteins gene; TAF-15, TATA-Box Binding Protein Associated Factor 15; S100B, S100 calcium-binding protein B; TMSB4, Thymosin Beta 4; PA, Pilocytic Astrocytoma; IPA, Ingenuity Pathway Analysis; FMNL1, Formin-like protein 1; RBPJ, recombination signal binding protein for immunoglobulin kappa J region; PAYZ1, POZ-, AT hook, and zinc finger-containing protein 1; YKL-40, Chitinase-like protein.

* Corresponding author.
E-mail address: tomasz.pienkowski@umb.edu.pl (T. Pienkowski).

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1. Introduction

Gliomas are the most common primary brain tumors in adults and children, which present histological characteristics of normal glial cells [1]. Although adult and pediatric gliomas may not differ histologically, they vary in biochemical and metabolic aspects [2,3]. Currently, the clinical classification of gliomas is based on histopathological and molecular features of solid biopsies [4]. Gliomas are divided into four grades based on the degree of proliferation, indicating their mitotic index, and the presence of necrosis during histopathological examination. According to the World Health Organization (WHO) guidelines, grades I and II are considered low-grade gliomas (LGG) and can be easily resected depending on their localization. Grades III and IV are considered high-grade gliomas (HGG); they have poor prognosis and are mostly undifferentiated and malignant [4]. The imaging techniques used for glioma diagnosis are magnetic resonance imaging (MRI) or computed tomography (CT) that use a combination of multiple X-rays. Both methods should be complemented with a needle or stereotactic biopsy to confirm the diagnosis, performed by a pathologist [4].

The main hallmark of human tumor development is genome instability, contributing to abnormal proliferation, invasiveness, metabolic reprogramming, angiogenesis, promotion of inflammation, immunoevasion, and avoiding cell death by over- or under-expression of proteins or metabolites [5]. In brain tumors field, biomarkers obtained by non-invasive or minimally invasive techniques are in high demand considering their potential use as an addition to preventive medicine or to reduce the period between first unspecific symptoms and final diagnosis. Importantly, tumor heterogeneity can occur within different areas of a patient's tumor, affecting the results of currently used methods that are based on tissue analysis [5]. In such cases, a possible approach is the use of multiomic biomarkers present in the systemic circulation and cerebrospinal fluid (CSF). In addition to glioma biomarkers present in the blood, urine, CSF, and extracellular vesicles (EVs) or as free particles, biomarkers may also be bound to cell surfaces that could be considered relevant in diagnostics and collected through biopsy or from cells prevalent in the circulating system [6]. A multiomics approach may be the most conclusive approach in biomarker research because of its multiple capabilities to distinguish disruptions in sample composition.

Currently, most articles related to glioma biomarkers are focused on transcriptomics. For example, a recent comprehensive review [7] covering the most promising approaches in transcriptomics makes unnecessary further efforts on this topic by further multiplying the same conclusions. The present review provides a deeper understanding of the glioma biochemical pathway alterations and their connections, which may help differentiate specific types and subtypes of gliomas, discover new therapeutic targets, and support diagnosis.

2. Overview of omics approaches

2.1. Proteomics

Proteomics as a dynamically developing field of science offers several different analytical strategies for the analysis of the proteome. The most frequently used proteomic approach in clinical analyses is bottom-up, especially its modified "shotgun" version [8]. As in the case of bottom-up, a mixture of proteins isolated from tumor tissues or plasma samples is digested, and the amino acid sequence of each peptide is determined by tandem mass spectrometry (MS) in combination with chromatography. The shotgun method, unlike the bottom-up method, allows for the analysis of hundreds of thousands of released peptide sequences derived from tissue or other biological samples. Until now, this shotgun approach has been widely used in clinical trials, especially in oncology [9]. Shotgun analysis is classified as a quantitative technique without the use of markers (label-free (LF) quantification in the data dependent analysis (DDA) mode). In the LF method, quantitative data is obtained by measuring the area of the chromatographic peak of a given peptide in the MS1 mode, while the identification takes place on the basis of the mass spectrum obtained in the MS2 mode. This approach provides quantitative data on proteome expression, resulting in a high identification rate similar to that of more precise methods using chemical tags [10]. LF methods performed in the DDA mode are not recommended for the analysis of large sample sets owing to variability in chromatography and peptide retention times, or a decrease in MS sensitivity due to the accumulation of contaminants in the source. Therefore, this approach is often used in clinical trials with small sample sets [11,12]. In clinical trials with a large number of samples, this leads to an incomplete data set, especially for proteins with low expression levels. In clinical proteomics, it is important to ensure the highest possible repeatability of determinations, uniform identification, and precise quantification for large set of samples.

To minimize the impact of stochastic changes affecting the obtained result, a number of methods can be used to increase the precision of determinations. These include methods based on chemical labelling (isobaric tag for relative and absolute quantification of hydrazide (iTRAQ), tandem mass tag (TMT), and derivatives) or approaches that increase proteome coverage and the precision of measurements by fragmentation of all peptides in the sample (data-independent analysis (DIA) and sequential window acquisition of all theoretical mass spectra (SWATH-MS)). Currently, the quantification of proteins in clinical samples, such as tissues or biological fluids, is largely carried out using both of the above mentioned techniques, owing to the low cost of sample preparation (DIA and SWATH) or the increased precision of measurements based on chemical labelling (TMT and iTRAQ) [13]. A more recent variation of the shotgun approach is SWATH-MS using DIA. SWATH-MS is based on the cyclic acquisition of precursor ions using means of isolation windows that cover the entire range of masses analyzed in spectral libraries. SWATH-MS combines the advantages of high reproducibility and sensitivity of targeted methods, such as selected reaction monitoring (SRM) or multiple reaction monitoring (MRM), with increased coverage of proteome identification, typical for DDA [14]. SWATH-MS is versatile and has a variety of applications, including quantitative protein determination, in personalized oncology [15]. In addition, targeted methods such as SRM or MRM are also widely used in clinical trials, for example to track the effectiveness of patients' treatment, especially after chemotherapy [14].

A different approach increasing the precision of measuring large-scale protein expression involves the use of markers based on compounds labelled with stable isotopes (carbon-13 (C13), deuterium (H2), nitrogen-15 (N15)). Protein extracts containing C13-labelled amino acids are widely used in basic research using cell cultures or (less commonly) laboratory animals. In the case of clinical trials, however, it is not often used owing to the high cost [16]. Isobaric markers, such as TMT, iTRAQ), and tandem iodoacetyl tag (iodoTMT), significantly increase the precision of proteomic quantification based on chemical marking. They enable chemical modification of the primary amines in the peptide molecule (N-terminus of the peptide and the side chain of the lysine) present in the digested protein mixture. The intensity of each reporter ion released as a result of fragmentation is proportional to the proportion of peptide from a given sample in the mixture. This allows for precise determination of quantitative relationships between individual samples [17]. However, the use of TMT or iTRAQ is not a fully optimal method for detecting differences in protein profiles in individual tumors or plasma samples because multiplexing methods also label low signal peptides at background levels [18]. The use of an optimal approach is a key aspect, not only in the search for potential biomarkers, but also for the identification of biochemical or biological disorders occurring in cancer patients.

2.2. Metabolomics

As in the case of proteomics, metabolomics studies are aimed at identifying and quantifying (or semi-quantifying) small molecule

metabolites present in the tested sample [12]. Two analytical techniques dominate in metabolomics research: nuclear magnetic resonance (NMR) spectroscopy or MS combined with various separation methods, such as liquid chromatography (LC), gas chromatography (GC), or capillary electrophoresis (CE). The combination of these analytical platforms is necessary for a high metabolome coverage, as they enable the detection, characterization, and quantification of low-molecular-weight metabolites from different classes. NMR can uniquely identify and quantify a wide range of organic compounds but is limited to metabolite concentrations in the micromolar range or higher. In contrast, LC-MS is better suited for the analysis of labile and non-volatile, non-polar (reverse phase chromatography), and polar (normal phase chromatography) compounds in their native form over the entire range of biological concentrations. GC–MS can be used for the analysis of several classes of compounds, including organic acids, most amino acids, sugars, sugar alcohols, aromatic amines, and fatty acids. In contrast to other MS-based techniques, the analysis of the metabolome using GC–MS requires prior chemical derivatization of the analyzed compounds in order to improve both the chromatographic separation and the possibility of ionization in the gas phase with a source using electron (EI) or chemical (CI) ionization. This limits the range of metabolites that can be efficiently analyzed by GC–MS to those having the appropriate functional groups to enable the formation of appropriate derivatives. CE-MS is an excellent tool for the study of polar and ionic metabolites, including inorganic ions, organic acids, amino acids, vitamins, thiols, carbohydrates, peptides, nucleotides, and nucleosides [19]. Considering the utility of a single analytical technique to measure metabolites, LC-MS provides the highest metabolome coverage (identification, qualitative assay), excellent sensitivity, and dynamic range [12]. Consequently, despite the enormous contribution of NMR spectroscopy to the study of the multicomponent metabolome, MS, especially LC-MS, has a higher sensitivity and is able to quickly separate and identify individual metabolites in complex mixtures. It is currently the best tool for precise metabolomics with high throughput of clinical samples. Moreover, the LC-MS system allows for the detection of thousands of features within one series, and when used in a targeted manner, it can be successfully used in large-scale clinical trials. Regardless of the separation and identification techniques used, metabolites can be measured by a variety of approaches, such as metabolic profiling or targeted assay analyses of a given set of metabolites. Metabolic profiling is aimed at detection and semi-quantitative determination of metabolites present in a biological sample in a manner similar to the shotgun method mentioned in the section 2.1 [20]. Given its clinical applications, this approach is often used in biomarker-oriented research as well as in interventional studies aiming to evaluate treatment efficacy [21]. However, untargeted research has its limitations. For MS-based studies, only a limited number of samples can be analyzed. The reason for this is the semi-quantitative nature of such studies, wherein, considering clinical applications that aim to use metabolite data for diagnostic purposes, this information should be quantified and presented as a metabolite concentration, not an instrument signal or a relative value [21]. Targeted metabolomics, on the other hand, allows the quantitative measurement of small molecules from most of the metabolite classes for which chemical standards and internal standards are available, ideally in the form of compounds labelled with deuterium or stable carbon isotopes. An additional advantage of the targeted approach is the exemplary treatment protocol that can be tailored to the specific class of metabolites to be measured. In this way, more selective analytical protocols can be applied, allowing more efficient extraction and elimination of molecules that may interfere with the ion source, thus significantly improving the detection limit.

As lipids constitute one third of all metabolites, a separate area of metabolomics, called lipidomics, has developed. Recently, the role of lipids in colorectal cancer [22], acute myeloid leukemia [23] and hepatocellular carcinoma [24] has been reviewed.

3. Proteomics in glioma research

Presently, several protein biomarkers are used as aids in diagnosing tumors other than gliomas [25]. However, it is important to note that some of these may be low-specific in brain tumor diagnostics, and may be observed in other homeostasis disruptions, not necessarily oncological, *e.g.*, vascular endothelial growth factor (VEGF) [26]. Tumor progression is correlated with elevated VEGF, which is also related to increased expression of suppressor of cytokine signaling 3 (SOCS3) in glioblastoma (GBM) (Fig. 1) [27]. Notably, the potential role of SOCS3 in GBM neovascularization is presumed owing to the inverse correlation between protein levels of Von Hippel–Lindau tumor suppressor and cullin5 [28]. Moreover, neovascularization is often observed in GBM with a poor prognosis [4]. Thus, this correlation may be facilitated in postsurgical prognostics of prescribed therapy based on the response toward angiogenesis inhibitors [28]. Another potential biomarker, which has been confirmed in other tumors [29], is the enzyme lactate dehydrogenase A (LDHA), responsible for increased lactate production and glucose uptake in malignant cells. Di et al. [29] showed that knockdown of LDHA in U87 and U251 cell lines leads to downregulation of VEGF expression and that LDHA expression levels in HGG were significantly elevated compared to those in LGG and normal brain cells. Thus, LDHA, SOCS3, and VEGF expression levels may be helpful in estimating glioma differentiation (Supplementary Table S1) (Fig. 1).

However, the most common overexpressed proteins in gliomas are epidermal growth factor receptors (EGFRs) [26]. EGFR is a transmembrane glycoprotein that when stimulated leads to PI3K signaling as well as activation of intracellular MAPK pathway, *src* kinase, and STAT transcription factor (Fig. 1), affecting cellular proliferation [26]. EGFR variant III (EGFRvIII) is considered a specific GBM mutation [30] that promotes HGG growth through paracrine mechanisms by secreting, among others, interleukin-6, which activates signaling pathway enhancing tumoral development (Fig. 1) [31]. Additionally, Newman et al. [32] reported that EGFRvIII is correlated with interleukin-13 receptor alpha 2 (IL-13Ra2) and patient survival outcome correlates significantly with the IL-13Ra2 expression (Supplementary Table S1) (Fig. 1). Furthermore, cells co-expressing EGFRvIII IL-13Ra2 seem to exhibit a higher growth rate and increased anchorage-independence, while migratory potential was not changed [32].

Another interesting overexpressed specific growth factor receptor is platelet-derived growth factor receptor alpha (PDGFRA), whose stimulation may contribute to uncontrolled cellular growth (Fig. 1) [26]. Although PDGFRA is present in astrocytomas and GBM, it is considered a prognostic biomarker mostly for the proneural GBM subtype (Supplementary Table S1) [26]. Recently, cofilin-1 (COF1) and phosphoglycerate kinase 1 (PGK1) proteins were correlated with poor prognosis in radioresistant diffuse astrocytoma (Fig. 1). Moreover, PGK1 upregulation is correlated with increased glucose metabolism, affecting tumor progression, while COF1 is involved in the regulation of cellular morphology and motility (Supplementary Table S1). However, both have been reported in other tumors, which allows us only to applicate them as radiosensitivity prognostic biomarkers [33]. In our search for correlations between proteins and metabolites we came across a historic paper by Philips et al. [34] that links PDGFRA malignant alteration with isocitrate dehydrogenase 1 (IDH1) mutation (Fig. 1). The same study also rejected the thesis that PDGFRA is related to the co-amplification of EGFR. Subsequently, Flavahan et al. [35] concluded that gain-of-function IDH mutations induce PDGFRA expression improving glioma fitness, which seems consistent with poor survival. Additionally, O^6-methylguanine-DNA-methyltransferase (MGMT) is also considered a predictive response biomarker in secondary GBM [36]. MGMT functions in GBM are based on DNA repair in response to damage caused by alkylating chemotherapy [36]. Kessler et al. [37] reported that unmethylated MGMT (uMGMT) tumors lose PDGFRA amplifications upon progression, whereas methylated MGMT (mMGMT) causes PDGFR amplification that seems to increase tumor vulnerability to

chemotherapy (Fig. 1). However, upregulation of TNF–NFkB pathway in mMGMT patients showed upregulation of MGMT and increased chemoresistance. In contrast, upregulation of INF-a pathway in uMGMT tumors increases sensitivity to chemotherapy [37]. In addition, most of the IDH mutants in GBM have methylated MGMT promoters. Moreover, patients with wildtype IDH and uMGMT show weak response to chemotherapy and a poor survival rate (Supplementary Table S1) [37].

As mentioned above, some proteins may be facilitated as radiosensitivity prognostic biomarkers. The most promising among these are matrix metalloproteinases (MMP), especially MMP-2 and MMP-9, whose overexpression is significantly higher in recurrent gliomas than in primary ones and are considered responsible for angiogenesis, neurodegeneration, blood–brain barrier degradation, and proteolysis control [38]. Additionally, MMP-2 is highly expressed in gliomas with large diameter and high malignancy, but its expression is lower in gliomas with small diameter and low malignancy [39]. Thus, co-expression of both MMPs indicates a poor prognosis in glioma recurrence [38]. Moreover, tissue inhibitor metalloproteinase 1 (TIMP-1) and TIMP-2 that are present in malignant cells and counteract MMP-2 and MMP-9 were reduced with tumor recurrence (Fig. 1) [38]. Zhou et al. [38] showed that tissue irradiation resulted in increased expression of MMP-9 both *in vitro* and *in vivo*, which stimulated invasion of glioma cells. Notably, MMP-2 is positively correlated with VEGF, which results in angiogenesis acceleration through multiple signal transduction pathways [39]. Additionally, Guo et al. [40] proposed a third hypothetical marker for overall survival, MMP-26, that is presumed to be expressed in higher grade astrocytomas and participates in tumor invasion and metastasis, indicating poor prognosis (Supplementary Table S1).

Another study indicates that the overexpression of glial fibrillary acidic protein (GFAP) may be a promising marker for GBM diagnosis, grade, and tumor-type differentiation [26]. GFAP is a cell-specific marker restricted to astrocytes, responsible for their structural maintenance and any dysregulation may indicate alterations in the brain (Fig. 1) [41]. However, GFAP overexpression is quite common after brain injury or surgery [41]. Additionally, in our recent study we reported that GFAP dysregulation is a part of alterations that are abundant in gliomas, but is not a singular discriminatory factor [26]. Brehar et al. [42] indicated that GFAP-δ isoform overexpression in HGG grades may be a reliable diagnostic marker. Moreover, many initial diagnosis pathways are activated during head trauma, which may present false positive GBM diagnosis when considering GFAP abundance measurements alone. Nevertheless, GFAP nor GFAP-δ isoform abundance cannot be considered as a singular biomarker, and despite seemingly promising results in research it may not meet its expectations in clinical use (Supplementary Table S1). Moreover, the expression level of YKL-40 glycoprotein, a ligand of IL-13Ra2 [32], is elevated in HGG cells (Fig. 1) [26]. Currently, we speculate its impact on tumorigenesis as it plays a role in the stimulation of angiogenesis, apoptosis evasion, and cell proliferation [26]. However, similar to GFAP, YKL-40 elevated levels may not be correlated with glioma manifestation and are also noted during many pathological conditions, including inflammatory diseases such as neuroinflammatory conditions of Alzheimer's Disease

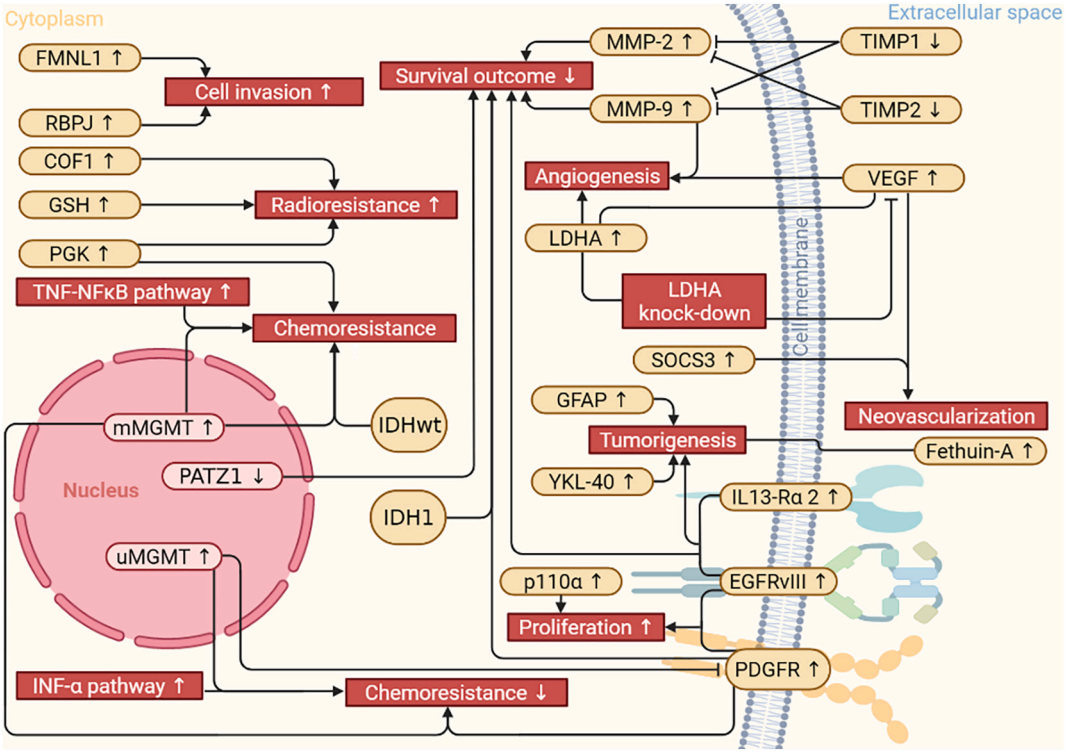

Fig. 1. Interactions between biochemical pathways during glioma development. Our current micromolecular understanding of gliomagenesis is based on multiple biochemical pathway alterations in tumor cells. Pathways correlate with each other as inductors, co-inductors, or inhibitors, resulting in alternative disease outcomes in parallel presence. The altered expression of proteins is represented by arrows (in the boxes with protein name) pointed up for overexpressed and down for underexpressed proteins. Red boxes with arrows represent the impact of altered protein on glioma cells. An arrow with its head up represents an increase of the outcome. An arrow with its head down represents a weakening of the outcome. Arrows or flat heads leading from altered protein to red boxes represent direct impact, while connecting lines represent the indirect or amplified impact on the outcome due to the synergistic effect of protein. COF-1 - Cofilin-1, EGFRvIII - Epidermal Growth Factor Receptor variant III, FMNL1 - Formin-like protein 1; GSH - glutathione, GFAP - Glial Fibrillary Acidic Protein, LDHA - Lactate Dehydrogenase A, IL-13Ra2 - Interleukin-13 Receptor alpha 2, mMGMT - Methylated MGMT, MMP - Matrix Metalloproteinases, PATZ1 - POZ-, AT hook-, and zinc finger-containing protein 1; PDGFRA - Platelet-Derived Growth Factor Alpha Receptor, PGK1 - Phosphoglycate kinase 1, RBPJ - recombination signal binding protein for immunoglobulin kappa J region; IDH - Isocitrate Dehydrogenase, uMGMT - Unmethylated MGMT, TIMP - Tissue Inhibitor Metalloproteinase. Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[43]. Nevertheless, both these biomarkers may not be sufficient in definitive glioma diagnosis; they may provide valuable information about survival prognostics after confirming the diagnosis (Supplementary Table S1) [44]. On the other hand, ectopic Fethuin A produced by astrocytomas, which is originally synthesized in the liver, appears to be responsible for tumor growth, motility, and invasion signaling through activated EVs (Fig. 1) [45]. Both the liver and astrocytes synthesize Fethuin A that is endocytosed and then binds to histones through sialic acid residues The same study also speculates, by referring to historical data, that GBM patients with normal serum fethuin-A levels show prolonged survival (Supplementary Table S1) [45]. Although these data seem promising, unfortunately it is the only paper on this topic in the last 10 years.

GMB can be divided into four molecular subgroups – proneural (PN), neural, classical, and mesenchymal (MES); that are also subject to proteomics analysis. . However, we will focus on the most prevalent PN and MES, the least and the most aggressive subtypes of GBM, respectively. Moreover, we are aware of different co-existing subtypes in the same tumor [46]. Also, following chemotherapy or radiation therapy, there is a possibility of a shift from PN to MES subtype [46]. A similar molecular transition was observed in carcinomas during the epithelial-mesenchymal transition, which enhanced the invasiveness of cancer cells and led to unfavorable prognoses [46]. Thus, there is a critical need for monitoring such molecular events during ongoing therapy.

Proteome changes during PN to MES transition (PMT) are hallmarks of glioma aggressiveness and poor prognosis. Expression differences of already known biomarkers of pathological changes may indicate the transition occurrence during routine medical checks. For instance, Phillips et al. [46] observed YKL40 increased expression in MES in comparison to PN. Also, Faried et al. [47] identified periostin as an MES subtype biomarker, which was not elevated in PN. However, this data was obtained through online available-glioma databases, and its relevance in glioma patients' serum is still being under research. Periostin was associated with direct interaction with MMP-9 and indirect promotion of HIF-1α expression by glioma cells, which contributes to glioma invasiveness phenotype [47]. Another difference in PN and MES abundance of the same protein was observed by Higa et al. [48]. Formin-like protein 1 (FMNL1), being a mediator in the assembly of filamentous actin networks, was proposed as an independent predictor of poor prognosis in GBM, which upregulation in MES subtype supports cell migration and invasion (Supplementary Table S1). In contrast to these findings, Eckerdt et al. [49] showed that expression of p110α is highest in the PN subtype. Increased abundance of p110α indicated upregulated PI3K/AKT pathway, which is a key mediator of PDGFRA signaling in GBM (Supplementary Table S1) [49]. Zhang et al. [50] investigated strictly PMT, not only differences in subtypes and pointed that recombination signal binding protein for immunoglobulin kappa J region (RBPJ), which is a crucial factor in the Notch signaling pathway, may be used as PMT occurrence biomarker. This study indicates that RBJP overexpression promotes cell proliferation and invasion through activation of IL-6-STAT3 pathway, also leading to PMT. Moreover, RBPJ levels were higher in glioma stem cells than in differentiated GBM cells and not observed or lower in WHO grade II and III, respectively (Supplementary Table S1) [50]. Interestingly, different levels of the same protein can be observed in the same subtype of GBM. Altered expression of PATZ1 protein can be used to divide patients with PN subtype into two groups with different overall and progression-free survival. Lower levels of PATZ1 protein seem to correlate with poor outcomes (Supplementary Table S1) [46].

Although glioma proteomics biomarkers are currently widely investigated, there is a lack of specific altered proteins that could be considered relevant for gliomas compared to other tumor biomarkers. Thus, multiomics research could correlate presently known protein biomarkers with possibly coexisting metabolic or lipid biomarkers, whose presence without such correlation may be irrelevant.

4. Metabolomics in glioma research

Maintenance of cellular structure and signal transduction *via* second messenger molecules is managed in cells through lipids synthesis. The main structural components of biological membranes include different classes of lipids that can modulate its fluidity, composition, and membrane-dependent cellular functions. Moreover, molecular signaling is affected by lipids acting independently or in conjugation with proteins, through structural–functional modulation. Cancer cells, through metabolic pathway reprograming, accelerate proliferation rate, which may correlate with alterations in lipid synthesis and signaling pathways, especially in gliomas that are rich in lipid content owing to their localization in the brain tissue [51]. The most common metabolic alteration is the "Warburg effect," wherein the affected cells exhibit elevated uptake and utilization of glucose for glycolysis. Moreover, recent studies state that aerobic glycolysis is the core cellular metabolism pathway that provides cancer cells with energy and is necessary for dysregulated macromolecules, such as lipids, carbohydrates, nucleic acids, or proteins, responsible for cell proliferation [52]. Lipid metabolism, similar to glucose metabolism, is regulated by the common oncogenic signaling pathways and is presumed to be important for the initiation and progression of tumors [51]. Recent metabolomics studies suggest that there are disease-specific alterations in metabolism, which may allow us to simplify and authenticate the diagnosis process, without unnecessary surgeries [53,54]. Altered low-molecular-weight metabolite composition in body fluids may be specific or at least correlated with a group of diseases and may serve as biomarkers in tissue, plasma, serum, or urine. Moreover, Zhao et al. [53] proposed that metabolites obtained from plasma may be used in the molecular classification of gliomas. They identified the 10 most promising metabolic biomarkers—uridine, uracil, arginine, agmatine, ornithine, biotin, lactate, cysteamine, glucosamine, and oxalic acid—as well as differences in metabolite composition according to the IDH mutation status, of which the most difference was observed in *N*-acetylputrescine abundance (Fig. 2). Easy accessibility makes body fluids more convenient for clinical use than biopsy or surgery and may harbor prognostic biomarkers before any identifiable manifestation in MRI or pathomorphological screening. Shen et al. [54] have reported the possibility of predicting survival in GBM based on metabolites present in plasma. Increased glycolysis is observed in cancer cells as compared to healthy cells, and the tricarboxylic acid (TCA) cycle, one of the major metabolic pathways in organisms, is altered (Fig. 2) [54]. Thus, we may focus on the alterations in these classical pathways, such as glycolysis and TCA cycle that are well studied in glioma to identify reliable biomarkers (Supplementary Table S1).

Mutations in IDH1 are common in gliomas and occur in over 70% of LGG and secondary GBM [55]. Currently, two other known isoforms, IDH2 and IDH3, are present in the mitochondria, while IDH1 is present in the cytosol and peroxisomes [55]. Wt-IDH is responsible for catalyzing the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (2-KG) (Fig. 2), along with the production of nicotinamide adenine dinucleotide phosphate (NADPH) [55], a cofactor for maintaining nominal levels of reduced glutathione and counter react on reactive oxygen species (ROS) [55]. Mutant IDH catalyzes the reduction of 2-KG into 2-hydroglutarate (2-HG), whose accumulation inhibits the function of enzymes dependent on 2-KG, thereby affecting histone and DNA hypermethylation [55]. Moreover, mutant IDH is known to inhibit the activity of wt-IDH, through binding of 2-KG-dependent dioxygenase by 2-HG, causing elevated expression of hypoxia-induced factor 1-a (HIF-1a) and thus leading to tumor formation (Fig. 2) (Supplementary Table S1) [55]. In addition to 2-HG presence, two somatic mutations in metabolic enzymes, succinate dehydrogenase (SDH) and fumarate hydratase (FH), have been found to correlate with tumorigenesis *via* metabolic reprograming through oncogenic signaling in IDH mutants (Fig. 2) [55]. In addition, Zhao et al. [53] reported *N*-acetylputrescine and methionine plasma level differences as discriminators of IDH mutation presence in glioma patients; plasma N- acetylputrescine

abundance was lower in wt-IDH and that of methionine was higher in mutant-IDH patients (Fig. 2) (Supplementary Table S1). Moreover, Branzoli et al. [56] reported elevated *in vivo* levels of cystathionine in IDH-mutated and 1p/19q-codeleted gliomas as compared to that in healthy brain tissue. However, the accumulation of cystathionine in tissue was not correlated with glioma grade. Interestingly, elevated levels of cystathionine were detected in human breast cancer tissue [56]. In addition to methionine, tryptophan is also abnormally metabolized in glioma cells compared to normal astrocytes. Increased tryptophan levels were correlated with immune evasion and promotion of tumor formation. However, methionine and tryptophan are not the only amino acids in the human body that are altered in gliomas. Hypotaurine, a product of taurine oxidation, was positively related to the occurrence, development, and malignancy of gliomas [56]. Moreover, hypotaurine is responsible for activation of inhibition of proline hydroxylase 2, which inhibits HIF-1α degradation, leading to oncogene activation (Fig. 2) (Supplementary Table S1) [57].

Due to the accelerated growth of malignant gliomas, compared to healthy brain cells, there is an increased demand for any available nutrients, which in the brain are glucose and acetate. The demand is fulfilled through elevated uptake of glucose from the environment and subjecting it to accelerated glycolysis and oxidative phosphorylation. While acetate undergoes oxidation in the TCA cycle, upregulation of acetyl-CoA synthase occurs (Fig. 2) (Supplementary Table S1), which is responsible for the conversion of acetate into acetyl-CoA [58]. In a recent preliminary study, Baranovicova et al. [59] analyzed 60 plasma samples from glioma patients and found significantly increased levels of the glycolytic metabolites—glucose, lactate, and pyruvate—and significantly decreased levels of glutamate and metabolites involved in the TCA cycle, such as citrate and succinate, compared to those in healthy people (Fig. 2) (Supplementary Table S1). This acidosis favors processes such as metastasis, angiogenesis, and, more importantly, immunosuppression that has been associated with a worse clinical prognosis. Thus, lactate should be considered an important oncometabolite in the metabolic reprogramming of cancer [59]. Moreover, various tumors manifest upregulated levels of lactate dehydrogenase (LDH) responsible for conversion of pyruvate into lactate [60]. Lactate is the primary metabolite in the TCA cycle and any variation in its circulating pool may affect tumor growth (Fig. 2) (Supplementary Table S1) [59]. However, the study by Baranovicova et al. [59] showed that elevated levels of pyruvate, glucose, and lactate did not differ between the examined gliomas. The same cycle is also affected by *N*-acetylaspartate (NAA), the second-most abundant molecule in the brain tissue that is synthesized in neuronal mitochondria and is considered a marker for neuronal health. It is presumed that NAA serves as an acetyl group storage molecule that is synthesized when there is minimal overabundance of glucose (Fig. 2). Moreover, stored acetate can then be transported between cell types in the nervous system and utilized in the TCA cycle in astrocytes and neurons [61]. NAA was significantly decreased in human and mouse model xenograft GBM areas (Supplementary Table S1) [62]. However, reduced NAA abundance is a non-invasive marker in estimation of tissue damage after brain injury. Thus, disturbances in NAA abundance may be considered one of the many markers indicating glioma occurrence but not a discriminatory marker. In addition, NAA alterations in gliomas can be correlated with decreased levels of *N*-acetylaspartyl-glutamic acid (NAAG) as expected (Supplementary Table S1); NAAG is a dipeptide released from synaptic vesicles as a cotransmitter that acts with several other neurotransmitters, such as l-glutamate (Fig. 3). A 50-fold and 8.3-fold decrease in NAAG levels was detected in IDH1 and IDH2 mutants, respectively [61].

Moren et al. [63] suggested that higher levels of phenylalanine and mannitol in GBM may differentiate it from oligodendroglioma, which has elevated levels of creatine, 2-hydroxyglutaric acid, 4- aminobutyric acid (GABA), ribitol, myo-inositol, glycerol-2-phosphate, and glycerol-3-phosphate in tissue (Fig. 2). Increased serum levels of lysine and 2-oxoisocaproic acid in oligodendroglioma and higher levels of cysteine in GBMs were detected (Supplementary Table S1). Moreover, mannitol concentration increases with tumoral grade [63]. However, previous studies on GABA levels were non-consistent, because while one study failed to detect GABA in high-grade gliomas [64], another found increased levels of GABA in GBM compared to that in healthy brain tissue [65]. Moren et al. [63] observed that low levels of myo-inositol, an activator of C kinase protein that may contribute to tumor proliferation and survival, may be correlated with higher aggressiveness of the glioma phenotype and that it differentiates GBM from astrocytoma grades II and III [64] as well as oligodendroglioma [63]. However, Wright et al. [66] state that myo-inositol levels in GBM and astrocytoma grade III may be similar, but may be used to differentiate it from astrocytoma grade II (Supplementary Table S1). GABA involvement in gliomas is more complex. It is a main inhibitory neurotransmitter that is metabolized in astrocytes and regulates neuronal activity by providing carbon source for the synthesis of glutamine in glutamine–glutamate/GABA cycle (Fig. 3) [66,67]. Both GABA and glutamate are derived from the TCA cycle intermediate 2-KG (Fig. 2). Interestingly, mutant IDH1 causes conversion of 2-KG to 2-HG while oxidizing NADPH and NADP+, whereas wt-IDH1 catalyzes isocitrate to 2-KG (Fig. 2) [66,68]. This alteration negatively affects GABA concentration in glioma, which is consistent with recent studies [68]. Moreover, mutations in IDH1 results in NADPH synthesis impairment through loss of function in enzyme of interest and functionally altering it to oxidize NADPH to NADP+; this affects GSH synthesis that requires NADPH as a cofactor for the reduction of glutathione disulfide [69]. Additionally, increased excitatory glutamatergic signaling and GABA signaling impairment correlated with development of an epileptic focus that is also involved in stimulating glioma growth, which then stimulates seizures. Seizures occur in 50%–60% of patients with HGGs and up to 90% of patients with LGGs [69]. Moreover, mutations in IDH1 result in conversion of isocitrate to D-2-HG rather than 2-KG that eventually accumulates in intracellular space and malignant cells, where it acts as receptor antagonist to glutamate [68]. However, additional cystine-glutamate transporter system impairment increases the abundance of extracellular glutamate, presenting worse tumor prognosis and stimulation of seizures [69]. Interestingly, two recent meta-analyses identified IDH1 mutation to be correlated with a higher risk of preoperative epileptic seizures in LGGs (Supplementary Table S1) [70,71]. Moreover, the presence of 1p19q deletion seems non-significant in terms of correlation with seizure risk in LGGs as opposed to oligodendrogliomas that have higher seizure frequency [72]. However, altered glucose and acetate metabolisms, which contribute to proliferation or invasiveness of malignant cells, are not only ones affected by gliomas. In a study by Marin-Valencia et al. [73] on human GBM xenografts in mice, the total pool of glutamate, as a rich source of nitrogen and carbon for the biosynthesis of amino acids and nucleotides, was increased compared to surrounding healthy tissue (Supplementary Table S1). In healthy neurons, glutamine is metabolized to glutamic acid, which is part of the glutamic acid–glutamine cycle. GBM cells upregulate the conversion of glutamate into glutamine through elevated levels of glutamine synthetase enzyme. It is important to maintain a constant supply of glutamate in gliomas altered cells due to cysteine/glutamate antiporter xCT, which enables the uptake of cysteine to buffer cellular redox stress by synthesizing glutathione [73]. While some studies suggests that the intracellular pool of glutamine supports oxidative metabolism in primary glioma cells [74], others suggest that human-derived GBM mouse xenografts and human GBM orthotopic-derived cell lines prefer glucose as substrate for the TCA cycle [73]. However, studies suggest that high glutamine and glutamate uptake in cancer cells is dependent on extracellular glutamine levels [75,76]. This is consistent with recent studies that correlate increased levels of glutamine and glutamate in GBM local microenvironment (Supplementary Table S1) [77].

Zhao et al. [53], performed metabolomic profiling of plasma samples from glioma patients and obtained five metabolites, arginine, uracil, lactate, cysteamine, and ornithine, whose levels significantly differed

between LGG and HGG patients (Supplementary Table S1). Additionally, uridine plasma levels were the most altered and significantly higher in HGG than in LGG, whereas arginine showed the opposite trend. This study also suggests that uridine and ornithine abundance may differentiate between GBM patients and malignant glioma patients [53]. These findings indicate significant alterations in pathways related to amino acid, nucleotide, and carbohydrate metabolism. A recent study by Kampa et al. [77], based on screening metabolic differences in peritumoral tissue and IDH-mutated GBM, indicates that elevated levels of purines and pyrimidines may be associated with IDH-mutated GBM biomarkers. Uridine, uridine monophosphate (UMP), and uridine diphosphate (UDP) seem to be elevated in tumor areas (Fig. 2) [77], which is consistent with previous studies on metabolic analyses of plasma from GBM and HGG patients who possess higher levels of uridine than LGG patients (Supplementary Table S1) [53]. Moreover, it is similar for ADP and AMP as well as UDP and UMP (Fig. 2) [77], which is in accordance with previous studies that report significant elevation of AMP and ADP in GBM tissues and decreased level of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) hydrolysis in various gliomas (Fig. 2) [77]. Another study suggests that extracellular adenosine accumulates in hypoxic tumors and acts as an immunosuppressive factor [78]. However, research on purine and pyrimidine metabolites in body fluids in gliomas is lacking, and therefore, it is difficult to obtain more information on this topic; nevertheless, all previous studies on cell lines or tissues seem to report consistent results (Supplementary Table S1).

Brain tissue, in comparison to other tissues, has a high ROS production rate along with lower capacity for its removal [79] that may contribute to intensified tumorigenesis through DNA damages, resulting in genomic instability. Ascorbic acid is an important factor in tumor proliferation and angiogenesis; its treatment was correlated with tumor protection from radiation damage in glioma mouse xenografts, and decreased survival of glioma patients was correlated with increased uptake of dietary ascorbic acid (Supplementary Table S1) [77]. Kampa et al. [77] were the first to observe an increased presence of ascorbic acid in tumor tissue, and it seemed to show a gradual shift from GBM to peritumoral areas, similar to taurine. Their findings were in accordance with the findings of Cubillos et al. [80] that taurine concentration is increased in gliomas and peritumoral tissue areas, suggesting a correlation between the increased extracellular as well as intracellular taurine levels and cell proliferation. Similar to taurine and ascorbic acid, increased levels of glutathione (GSH) were also observed in GBM (Supplementary Table S1). GSH, like ascorbic acid and taurine, is a highly abundant antioxidant in the human body that metabolically participates in the biosynthesis of leukotrienes and prostaglandins and plays a role in the storage of cysteine. In tumors, high abundance of GSH is mostly associated with tumorigenesis, increased resistance against therapies, as well as cell protection from free radical damage [77].

Another study screened pre-diagnostic serum samples to determine the association between tocopherols and GBM risk. Obtained results

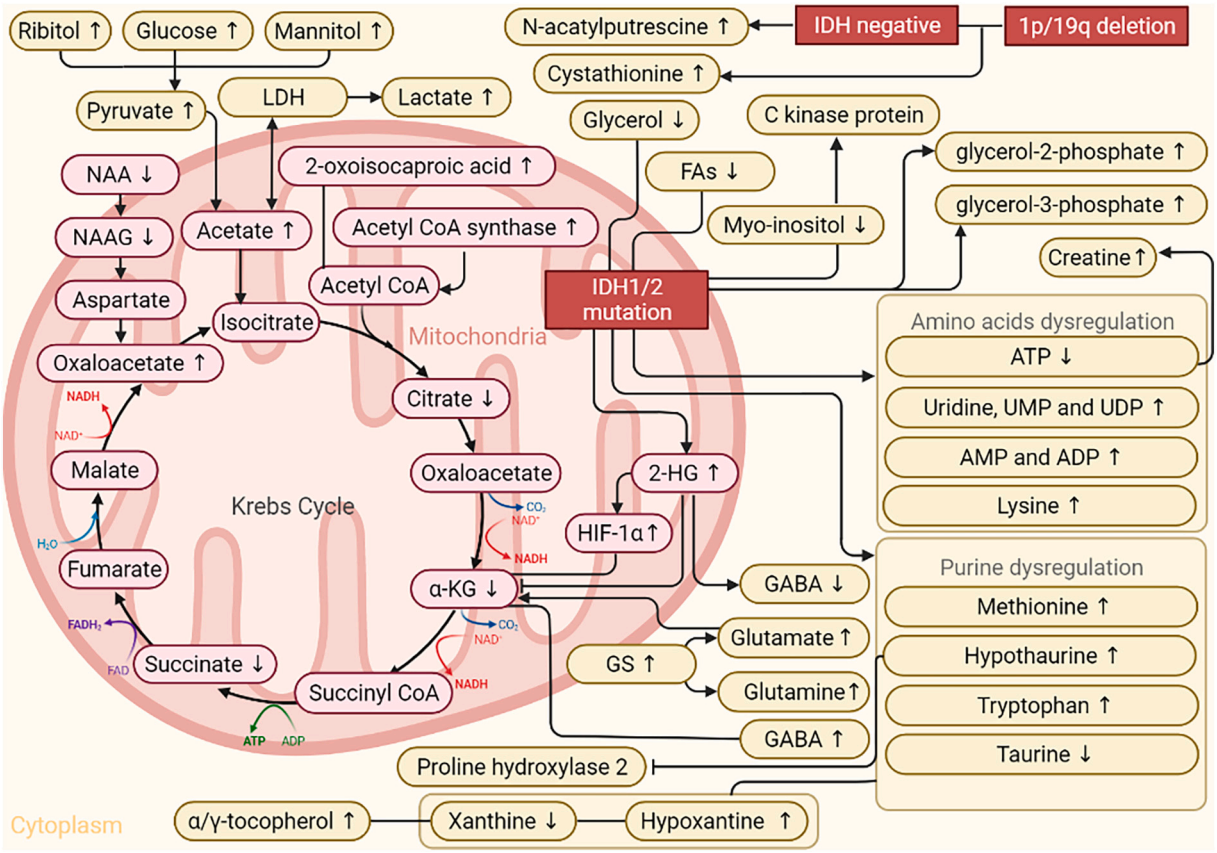

Fig. 2. Common metabolic alterations in gliomagenesis. Multiple metabolic alterations correlate with each other owing to their involvement in the Krebs cycle. The altered expression of proteins is represented by arrows (in the boxes with protein name) pointed up for overexpressed and down for under-expressed proteins. Red boxes with arrows represent the impact of altered proteins on glioma cells. An arrow with its head up represents an increase of the outcome. An arrow with its head down represents a weakening of the outcome. Arrows or flat heads leading from altered protein to red boxes represent direct impact, while connecting lines represent the indirect or amplified impact on the outcome due to the synergistic effect of protein. Adenosine triphosphate, ADP – Adenosine diphosphate, AMP – Adenosine monophosphate, ATP – Adenosine triphosphate, GABA – 4-Aminobutyric acid, GS – Glutamine synthetase; IDH – Isocitrate Dehydrogenase, LDH – Lactate Dehydrogenase, NAA – *N*-acetylaspartate; NAAG – *N*-acetylaspartyl-glutamic acid; FA – Fatty Acids, 2-KG – alpha-ketoglutarate, 2-HG – 2-hydroglutarate, UDP – Uridine diphosphate, UMP – Uridine monophosphate. Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indicated that elevated levels of α/γ-tocopherols as well as decreased levels of xanthine may be involved in the initiation of GBM (Supplementary Table S1). Moreover, elevated levels of α/γ-tocopherol were observed in non-small cell lung cancer in comparison to non-malignant tissue [81]. Interestingly, reduced levels of xanthine along with increased levels of hypoxanthine were detected in some cases, indicating deregulated purine metabolism and catalyzation of xanthine oxidoreductase. Moreover, xanthine oxidase, an enzyme that catalyzes the oxidation of hypoxanthine to xanthine, was reportedly elevated in tumoral brain tissues (Supplementary Table S1) [81].

5. Lipidomics in glioma research

Cancer development, in addition to proteomic and metabolomic alterations, is modulated by changes in the balance between fatty acid (FA) synthesis, uptake, and storage [82]. FAs are essential in cancer genesis because of their role in sustaining membrane biosynthesis during accelerated proliferation of tumor cells; they provide an important energy source during harsh conditions of metabolic stress and are second messengers in core molecular pathways, and thus, malignant transformation alters both biosynthetic and bioenergetic requirements for the development of cancer cells (Fig. 2). Moreover, the accumulation of lipid droplets in tumor tissue can serve as a cancer biomarker [82].

FAs are acquired by cells through two major sources, exogenous dietary and *de novo* endogenous synthesis. Proliferative embryogenic cells are dependent mostly on *de novo* synthesis of FAs, while most differentiated cells prefer exogenous dietary FAs [83]. A similar preference for *de novo* synthesis of FAs manifests in cancer cells, *e.g.*, breast cancer cells endogenously synthesize 95% of FAs. Endogenously synthesized FAs are esterified to phospholipids, which are considered essential structural lipids of the cell membrane that are required for signal transduction, polarization, intracellular trafficking, and migration of cancer cells. Moreover, lipid molecules, such as phosphatidic acid, lysophosphatidic acid, and diacylglycerol, are also responsible for the mediation of signal transduction in cancer cells due to alterations in their normal cellular functions, including proliferation, cell survival, and migration [83]. Thus, our current understanding indicates that cancer cells seem to be highly dependent on the *de novo* endogenous synthesis of lipids for survival and proliferation. Moreover, enzymes involved in FAs synthesis, such as ATP citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and FA synthase (FASN) are upregulated in cancer cells. ACL is responsible for the conversion of cytosolic citrate to acetyl-CoA and oxaloacetate. ACC then carboxylates acetyl-CoA to malonyl-CoA, the core intermediate in FA synthesis, which is converted by FASN into long-chain FAs. Increased expression and activity of FASN are common early symptoms of development and progression of lung cancer [84], proliferative index in prostate cancer [85], and are related to prognosis in melanoma [86]. Unfortunately, there are very few experimental studies demonstrating the direct connection between upregulated FA synthesis and FA conversion into phospholipids in cancer cells [83].

A correlation between low glycerol-3-phosphate dehydrogenase 1 (GDP1) expression and a better survival prognosis has been reported for GBM patients (Supplementary Table S1) [87]. Rusu et al. [88] through ribosome-profiling analysis of mouse neural stem cells (NSCs) and brain tumor stem cells (BTSCs) found GDP1 overexpression in BTSCs but not NSC. Similar high expression of GPD1 were observed in human GBM, which also correlated with a worse prognosis (Supplementary Table S1) [88]. Although GDP1 overexpression is not exclusive for GBM and has been observed in multiple cancer types, GBM and other gliomas possess

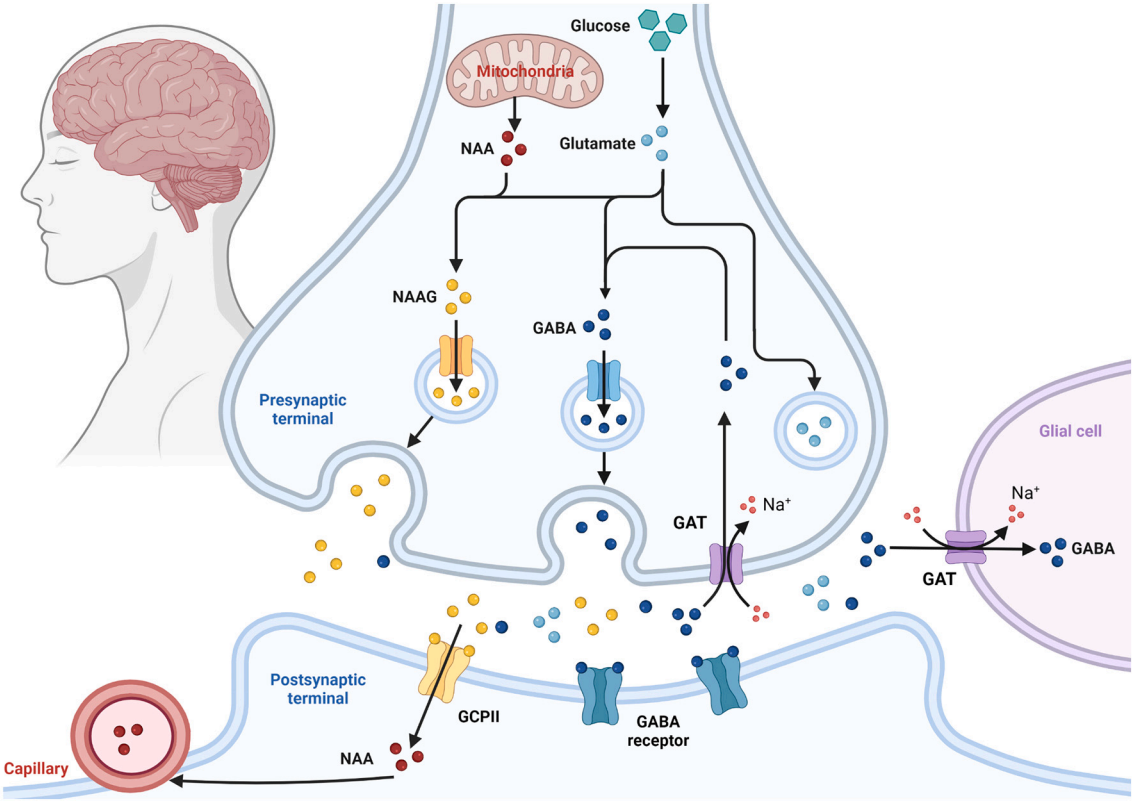

Fig. 3. Neurotransmitter pathways of hypothetical glioma biomarkers. Alterations in presented pathways may result in glioma development and be considered as small molecules biomarkers. Arrows represent pathways downstream. Each compound is represented by a colored dot (Red – NAA; Yellow – NAAG; Green – Glucose; Pale blue – Glutamate; Blue – GABA; Pink – Na^+ ion). GABA, 4-aminobutyric acid; GAT, gamma- aminobutyric acid transporters; GCPII, glutamate carboxypeptidase II; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartyl-glutamic acid. Adapted from "GABA synthesis and uptake," by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the highest expression of GDP1 after liver cancer and renal cell carcinoma [88].

In human gliomas with IDH1/2 mutations, discussed in depth in section 4, Zhou et al. [89] through metabolomics and lipidomics LC-MS-based analysis found significantly elevated levels of glycerol-3-phosphate, an amino acid and lipid synthesis precursor, in glioma tissue. However, glycerol, myo-inositol phosphate, or other total FAs and total phosphatidyl lipids were significantly decreased in the glioma tissue. Further, pathway analysis indicated a profound disruption in the TCA cycle (decreased acetyl CoA long chain synthases—ACSL1, ACSL4, and acyl-CoA synthase VL_3), glycolysis and gluconeogenesis, amino acid metabolism, lipid metabolism, as well as pantothenate and coenzyme A biosynthesis in glioma tissue with IDH1 mutation (Fig. 3). Moreover, while 2-HG was the most elevated metabolite, all detected triglycerides were markedly decreased in IDH1 mutants [89].

Ha et al. [90], based on GBM cell line analysis, obtained 500 significant lipids belonging to such classes as glycosphingolipids, glycerophosphoethanolamines, triradylglycerols, glycerophosphocholines, and glycerophosphoserines. Moreover, data showed that 90% of the significantly altered lipids were decreased compared to the control group, whereas majority of studies on lipidomics research in cancer tissues have reported increased synthesis of FAs in gliomas [90]. This study further suggests that the decreased lipid levels may be correlated with GBM dependency on FA as build material and energy source in addition to glucose derived from anaerobic glycolysis. Notably, other studies have shown upregulation of the lipolytic enzyme, monoacylglycerol lipase, in aggressive forms of cancer, which may contribute to the usage of FAs as an energy source in the tumor environment [90].

6. Pediatric glioma biomarkers

Despite the majority of pediatric-LGG (pLGG) occurring sporadically, the most common type is pilocytic astrocytoma (PA), which can be found in various locations in the central nervous system (CNS) [2]. Our current understanding is limited to the most common alteration of the BRAF gene, which is engaged in the MAPK signaling pathway, the KIAA1549-BRAF fusion, followed by the point mutation BRAFV600E (Fig. 4) [3]. Kurani et al. [3] performed Sanger sequencing and reverse transcriptase polymerase chain reaction and detected both oncogenic alterations in 41.1% and 8.9%, respectively, of 276 pediatric patients suffering from PA (Supplementary Table S1). Moreover, Kurani et al. [3] correlated the occurrence of these mutations with age, location, and gender. However, only KIAA1549–BRAF fusion genes correlated with the pediatric age group and cerebellar location was statistically significant, with the estimated overall frequency of BRAF fusion at 53.5% in pediatric PA cases (Supplementary Table S1) [3]. Lassaletta et al. [91] detected BRAF V600E mutation in 17% of 510 pLGG patients in a combined clinical and genetic institutional study with long-term follow-up. Patients with BRAF V600E mutation respond poorly to therapy as compared to those with wt-BRAF [91]. Thus, active diagnosis of BRAF V600E mutation and KIAA1549 BRAF fusion genes may guide physicians in selection of the effective treatment, which would limit the amount of time spent on adjusting standard therapies. However, it is important to note that BRAF mutations are not exclusive to PA, and have been detected in gangliogliomas, diffuse astrocytomas, and other low-grade astrocytomas [91].

However, the most aggressive and fast-growing pediatric gliomas belong to HGG (pHGG), which are associated with poor prognosis. The common alterations in pHGG, compared to other malignancies, are histone aberrations; recently, pediatric studies have focused on histone variants H3.1, H3.2, and H3.3 (Fig. 4) [92]. Both histone variants H3.1 (*H3F3A*) and H3.3 (*HIST1H3B* or *HIST1H3C*) can be detected in approximately 80% of diffuse intrinsic pontine gliomas, while H3.2 is less common (Supplementary Table S1). These alterations manifest due to a change in lysine to methionine at position 27 on the histone tail (K27M), resulting in tumor progression by interference with post-translational modifications of H3 [92]. GBM development was positively correlated with alterations in the transcription regulator ATRX [93], which is responsible for chromatin remodeling during incorporation of histone into pericentric heterochromatin or telomeres, and is associated with H3.3 histone (Fig. 4) [26]. Moreover, ATRX was positively correlated with mutations in IDH1, TP53, and death domain-associated proteins (DAXX) (Fig. 4), the latest creates with ATRX heterodimer which participates in H3.3 incorporation into DNA (Fig. 4) and is considered specific to pediatric GBM (Supplementary Table S1) [93].

Recently, Bruschi et al. [94] performed proteomics analysis of CSF from pediatric patients with different brain tumors, identifying six promising biomarkers for gliomas. Of these, TAF15 and S100B distinguished tumor from control (hemorrhagic conditions), which may present similar images on PET scans as tumor recurrence and may mislead physicians. TAF15 protein or TATA-binding protein-associated factor 2 N belongs to FET proteins that regulate lifespan and neuronal integrity and are considered proto-oncogenic owing to formation of oncogenic fusions [94]. However, TAF15 alterations are not exclusive for gliomas or even brain tumors and its overexpression is observed in lung cancer [95] and colorectal cancer [96]. Bruschi et al. [94] found lower TAF15 levels in tumors compared to control conditions, which is consistent with other studies [97], while S100B levels were upregulated (Fig. 4) [94]. Moreover, elevated S100B level is considered a biomarker for CNS infections and other brain-related pathologies, such as blood–brain barrier disruption or traumatic injury [98]. Bruschi et al. [94] concluded that serum levels of S100B can be considered a prognostic marker of survival in adult patients with recurrence of gliomas, but not at initial diagnosis, which may be useful in monitoring post-surgery patients [98]; however, there is a lack of evidence for this biomarker to be specific in pediatric brain tumors [94]. In conclusion, both markers may be adapted in the treatment monitoring of already diagnosed gliomas, but not for initial diagnostics (Supplementary Table S1).

Bruschi et al. [94] also identified upregulated biomarkers TMSB4 and CD109, capable of discriminating between LGG, glioneural tumors, and PA (Supplementary Table S1). TMSB4 protein is responsible for positive regulation of ATP biosynthesis and inhibition of actin polymerization, which may be tumorigenic and promote migration (Fig. 4) [94]. Moreover, TMSB4 was observed in NSCLC tumors, which indicates that it is non-specific for gliomas. On the other hand, CD109, a cell-surface antigen expressed by endothelial cells and T-cells, is considered a marker for glioma cells in perivascular tumors; it suppresses TGF-beta signaling *ipso facto*, contributing to tumor progression [99]. CD109 was also observed to be upregulated in GBM cell lines (Fig. 4) and stem cells [99]. Additionally, the most promising discriminative biomarkers presented by Bruschi et al. [94] that may differentiate medulloblastoma (MB) with embryonic origin from other tumors were 14.3.3 (YWHA-Z,G, E) and HSP90α. Both these proposed biomarkers may be considered discriminative factors of MB because of their presence in malignant pediatric brain tumors and their oncogenic properties as promoters of tumor survival and chemoresistance (Supplementary Table S1) [94].

It is important to state that biomarkers discovered in adult patients may not be applicablI in pediatric cases. Currently, there are very few studies on the differences in biomarkers between adult and pediatric patients suffering from the same malignancies. Thus, obtaining a wide collection of biomarkers discriminating between tumor presence and absence will be an invaluable advantage in diagnostics for both, adult and pediatric patients. Some adult LGGs can develop into HGGs, which is not common for pediatric gliomas [2]. Moreover, histone mutations are associated with pHGGs, whereas PTEN loss, IDH mutations, or EGFR amplifications, commonly observed in adults, are rare in pediatric patients [2]. However, it is important to state that some of the pediatrics biomarkers may coincide with biomarkers present in adults, but their abundance in testing material may differ. Thus, there is a critical need for research covering the differences between adult and pediatric biomarker abundance in the same medical condition. To summarize, a new approach in pediatric glioma diagnostics would be most desirable,

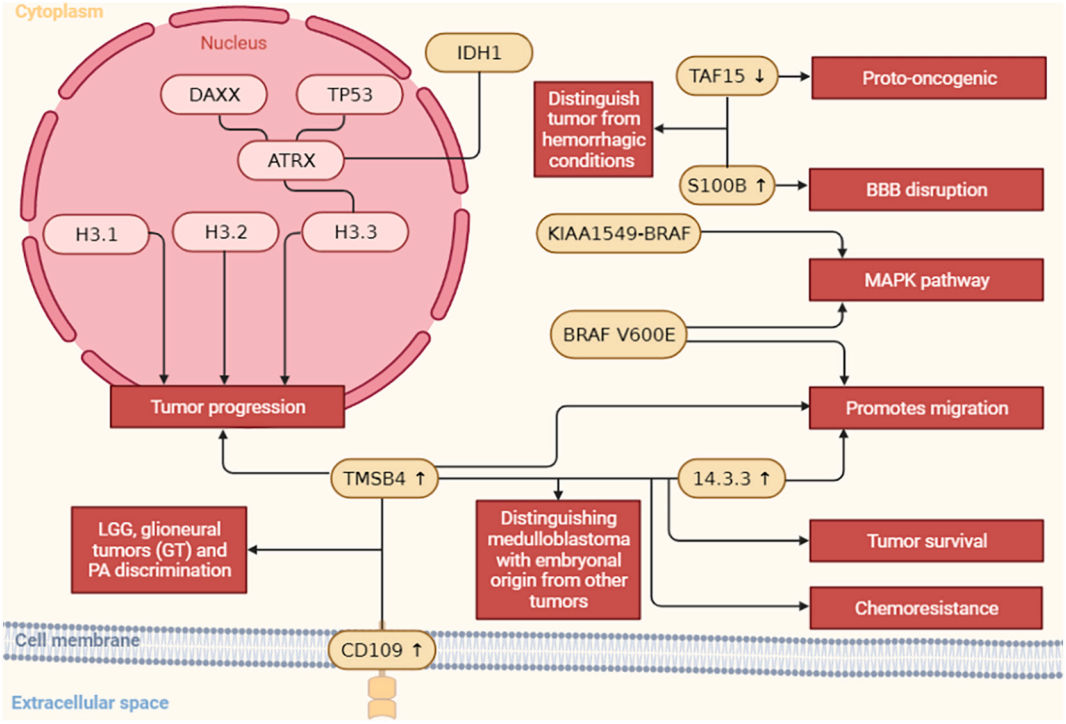

Fig. 4. The most common alterations present in pediatric gliomas. Altered expression of proteins is represented by arrows (in the boxes with protein name) pointed up for overexpressed and down for under-expressed proteins. Red boxes represent the impact of altered proteins on glioma cells. An arrow with its head up represents an increase of the outcome. Arrows leading from altered protein to red boxes represent direct impact, while connecting lines represent the indirect or amplified impact on the outcome due to the synergistic effect of protein. BBB – Blood Brain Barrier, S100B - S100 calcium-binding protein B, TMSB4 - Thymosin Beta 4, ATRX - Alpha-Thalassemia/mental retardation, X-linked gene, DAXX - Death-domain Associated proteins gene, IDH - Isocitrate Dehydrogenase. Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

considering its high mortality rates [99].

Experimental and bioinformatic analysis allows us to correlate the biomarkers through interconnections and interactions of disturbed biochemical and metabolic pathways (Fig. 5). However, there is a lack of research papers covering the multiomics approach in glioma biomarkers. Expanding our knowledge about correlations between altered biochemical and metabolic pathways may be an interesting avenue in future early-stage cancer diagnosis.

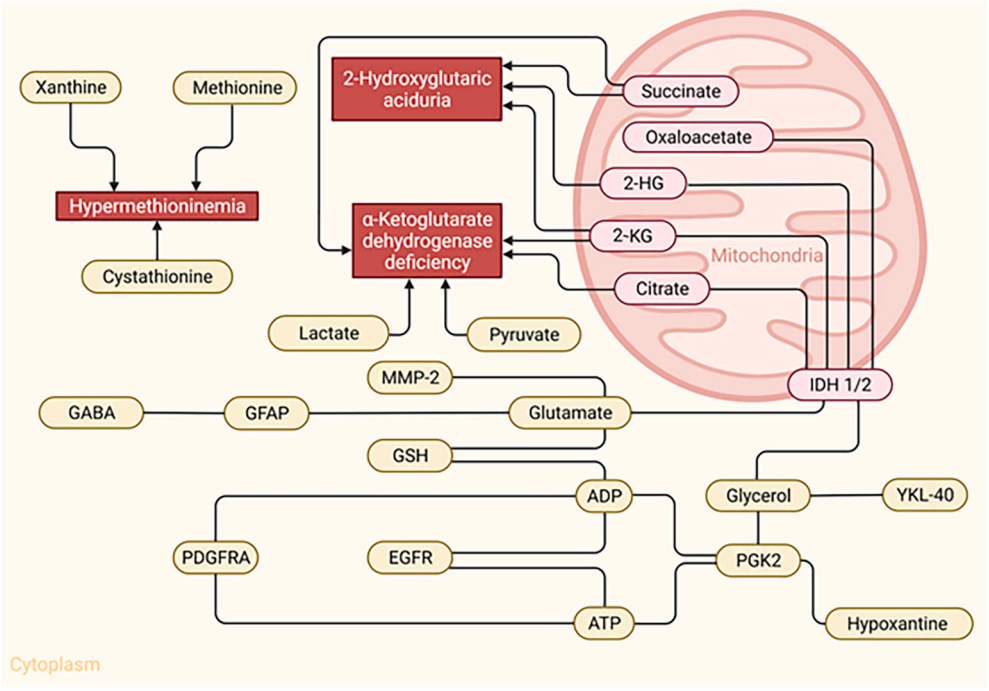

Fig. 5. Confirmed proteomic and metabolomic biomarker interactions. Red boxes present additional outcomes during disease. Arrows pointed to red boxes represent the direct impact of biomarker-altered abundance in the patient body. Linking lines have been confirmed through KEGG database interactions between biomarkers. Arrows leading from altered protein/metabolite box to red boxes represent direct impact, while connecting lines represent the indirect or amplified impact on the outcome due to the synergistic effect of proteins/metabolites. 2-HG – 2-hydroxyglutaric acid, 2-KG – 2-ketoglutarate dehydrogenase, ADP – adenosine diphosphate, ATP – adenosine triphosphate, EGFR – epidermal growth factor receptor, GABA – gamma-Aminobutyric acid, GFAP – Glial fibrillary acidic protein, GSH - glutathione, IDH – isocitrate dehydrogenase, MMP-2 – matrix metalloproteinase 2, PGK2 – Phosphoglycerate kinase 2, YKL-40 – Chitinase-like protein Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

7. Conclusions and perspectives

Biomarker diagnostics can be useful in an inconclusive state of disease where whether a patient is affected by a particular medical condition is uncertain; this would prevent unnecessary invasive treatments and medical personnel engagement in costly and risky procedures that may only worsen the patient's condition. Thus, elucidation of specific biomarkers that can distinguish between various subtypes of diseases or likelihood of recurrence after surgical tumor removal, for example, gliomas, is important. However, we cannot rely on a single biomarker to diagnose every type of glioma. Discovery and verification of disturbances in the abundance of multiple correlated biomarkers may simplify the diagnosis process in inconclusive situations; for example, distinguishing between postsurgical inflammation, detected by MRI, and recurrence of glioma or choosing a better surgical approach and proposing the best postoperative treatment. Moreover, predictive biomarkers present in body fluids may help establish the most effective preventive therapy in early tumor states that may eliminate the need for delicate neurosurgical resection if the tumor remains inaccessible. An interesting approach toward the research on circulating biomarkers seems to be the study of exosomes that are present in a number of body fluids. Exosomes, owing to their composition, can prevent degradation of biomarkers by circulating proteases and nucleases that may falsify the diagnostic value [25]. The limitations of a diagnostic biomarker test are related to its sensitivity and specificity as well as the analytical performance of the method used. Levels of any proposed biomarker should be evaluated by precise diagnostic methods that would assure reproducibility. However, it is important to characterize the expected performance of a diagnostic method used for biomarker evaluation under the most common conditions of use, such as the current state of the patient (*e.g.*, comorbidities, medications, or underlying disease state). Utilizing the knowledge on protein biomarkers along with metabolic and lipid biomarkers *via* a multiomics approach may bring us closer to the development of more specific and reliable diagnostic methods in patient diagnosis.

This paper aimed to collate potential minimally invasive diagnostic methods that would increase the level of comfort for patients and help avoid unnecessary and costly neurosurgical operations through the use of a multiomics approach. Thus, we presented the most promising biomarkers discovered over the last 10 years in multiple samples obtained by invasive or less-invasive methods and propose the use of multiomics approach for their detection in the CSF and blood. Moreover, generating a larger portfolio of different proteomics, metabolomics, and lipidomics biomarkers with functional relevance to a specific type or subtype of glioma is important for the further development of reliable diagnostic methods. Such a database with additional confirmed correlations between biomarkers would aid physicians in selecting the treatment protocol and researchers in the study of potential treatment methods based on biochemical pathways affected by disturbances caused by a disease. Presently, we can cross reference biochemical pathways of proteins and metabolites using Ingenuity Pathway Analysis (IPA), which is a bioinformatic tool based on comparative analysis. This approach has been used in other cancer studies for interpretation of specific signaling pathways contrasting with larger network analysis, using only identified proteins and metabolites during the course of the research [101]. Proximity Extension Assay (PEA) has recently gained researchers' interest in glioma biomarker proteomics [102]. PEA can be considered a validation method because of its high specificity and sensitivity. PEA technology is based on real-time PCR and overcomes specificity issues of multiplex immunoassays through subsequent extensions creating DNA reporter sequences, which through unique sequences reports only matched DNA–antibody pairs without cross-reactive events. The proteomics and metabolomics methods have been integrated for colon [103], colorectal [104], and ovarian cancer [101] research. Integrating both of these omics facilitates identification of alterations that could be missed using as single method. Moreover, integration of the two omics profiles through IPA may provide a systematic perspective of the characteristic changes occurring in specific types and subtypes of gliomas, and it may be a key aspect for discovering new biomarkers and drug strategies. Even the identification of non-specific biomarkers in glioma diagnosis may be sufficient for distinguishing differences between non-conclusive MRI scans in postoperative patients to prevent or perform reoperation. Importantly, we presented the association and impact of multiple human biomarkers on glioma progression, invasion, and survival, which may help more accurate estimation of poor outcomes in affected patients or facilitate personalized medicine as an aid in choosing the most suitable surgical approach or treatment for patients with less common biomarker combinations.

In the future, researchers should consider the multiomics approach proposed in this paper to identify biomarkers that interact with each other, causing unspecific disturbances, yet allowing to diagnose a specific state of an organism through correlations of proteome and metabolome. Additionally, co-existing non-correlated proteomics and metabolomics biomarkers may be considered distinguishing factors in gliomas, presenting us with another way to make diagnosis easier. However, many of the proposed biomarkers in literature stay invalidated owing to lack of material, small sample size, or basing whole research on gliomas xenografts alone. Thus, researchers should focus on validating already obtained results as well as search for new biomarkers.

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Author contributions

Tomasz Pienkowski - Writing - Original Draft; Funding Acquisition; Investigation; Methodology, Project Administration; Visualization; Tomasz Kowalczyk - Writing - Original Draft; Investigation; Methodology Software; Noemi Garcia-Romero - Writing – Review & Editing, Methodology;; Angel Ayuso-Sacido - Writing – Review & Editing; Supervision; Michal Ciborowski - Writing - Review & Editing Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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