CASE REPORT OPEN (In Check for updates) Metabolic reprogramming in glioblastoma: a rare case of recurrence to scalp metastasis

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BACKGROUND: Glioblastoma (GB), an aggressive brain malignancy with a poor prognosis of 1.5–2 years, rarely exhibits extracranial metastasis (ECM). However, metabolic reprogramming has emerged as a key driver of GB progression and invasiveness. This study presents a rare case of recurrent GB with scalp metastasis, exploring how metabolic shifts enable GB cells to evade treatment and adapt to hostile environments, offering insights for developing innovative therapies.

METHODS: Tandem mass spectrometry (MS/MS) was employed to analyze amino acid profiles in both the recurrent and metastatic stages of GB. Systems biology approaches were used to uncover genetic alterations and metabolic reprogramming associated with the progression from recurrence to metastasis.

RESULTS: Our analysis revealed distinct amino acid utilization patterns in a patient with a molecular phenotype of wild-type IDH-1&2, TERT mutation, non-mutated BRAF and EGFR, and non-methylated MGMT. During recurrence and metastasis, significant differences in amino acid profiles were observed between blood and cerebrospinal fluid (CSF) samples. Additionally, protein-protein interaction (PPI) analysis identified key genomic drivers potentially responsible for the transition from recurrent to metastatic GB.

CONCLUSIONS: Beyond established risk factors such as craniotomy, biopsies, ventricular shunting, and radiation therapy, our findings suggest that metabolic reprogramming plays a crucial role in the transition from recurrent to metastatic GB. Targeting these metabolic shifts could provide new avenues for managing and preventing extracranial metastasis in GB, making this an important focus for future research.

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INTRODUCTION

Glioblastoma (GB) is adults' most common malignant tumour of the central nervous system (CNS), with a median survival rate of approximately 14.6 months [1, 2]. The majority of patients with glioblastoma face progression of the disease locally, while the occurrence of extracranial metastasis remains exceptionally uncommon, affecting only about 0.4–0.5% of cases [3]. Among this rare subset, the prognosis is notably grim, with a median survival of just 1.5 months following the onset of metastasis [4]. The most commonly affected sites include the lungs, pleura, lymph nodes, bone marrow, bones, and liver [5]. Although there has been an increase in reported cases of extracranial metastasis in recent years, the underlying causes and mechanisms remain unclear and are not yet fully understood.

Nearly a century ago, Otto Warburg's groundbreaking work identified aerobic glycolysis as a hallmark of cancer, laying the foundation for studying cancer metabolism [6]. While early research focused heavily on central carbon metabolism pathways such as the citric acid cycle and pentose phosphate pathway, recent advances have highlighted the critical role of non-carbon metabolism in supporting cancer cell growth and survival. Among these, amino acid metabolism has emerged as a key area of interest, given its profound influence on cellular processes [7, 8].

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In cancer cells, amino acid metabolism is reprogrammed to support critical functions: protein synthesis, nucleic acid biosynthesis, energy production, redox balance, ammonia detoxification, epigenetic regulation, and activation of pathways like mTORC and autophagy, highlighting its role in sustaining tumor growth and survival [9, 10]. These multifaceted roles underscore the significance of disrupted amino acid metabolism in cancer and highlight its potential as a target for therapeutic interventions, particularly in the evolving context of the tumor microenvironment [11–13].

Cell transformation, or tumorigenesis, involves metabolic reprogramming, enabling cancer cells, including GB, to adapt energy production for enhanced survival, invasiveness, and therapy resistance [10, 14, 15]. Extensive evidence shows that multiple energy pathways are altered during cellular transformation, enabling cancer cells to meet their high demands for growth and repair [14]. Among the critical molecules involved in this process are amino acids (AAs), which play diverse and essential roles in cellular function. Beyond serving as the "building blocks" of proteins, amino acids contribute to tumor progression in several ways: (a) certain AAs are converted into α-keto acids, which are further transformed into glucose, fats, or ketone bodies to fuel energy production [15]; (b) some AAs act as neurotransmitters or hormone precursors, influencing cellular communication and behavior [16] ; (c) glycine, serine, methionine, and histidine provide one-carbon units essential for DNA synthesis and antioxidant defenses, protecting cancer cells from oxidative damage [16]; and (d) AAs serve as epigenetic modifiers, influencing which genes are turned on or off and, consequently, affecting cell phenotypes [14]. These metabolic shifts in amino acid utilization are particularly prominent in GB, where they promote tumor survival and invasion and contribute to resistance against standard treatments such as chemotherapy and radiation [17]. This highlights the critical role of amino acid metabolism in the progression of GB, particularly in the transition from recurrence to metastasis.

In this study, we report a case of extracranial GB metastasis and identify a shift in amino acid consumption preferences during the progression from recurrence to metastasis. Additionally, we apply systems biology to analyze the metabolite-related pathways involved in this transition.

METHOD

Genetic profile

The genetic profiling of the selected genes (IDH-1&2, TERT, BRAF, EGFR, and MGMT) was conducted following the methodology detailed in our previous work [1], which thoroughly describes the techniques utilized.

Cerebrospinal fluid (CSF) sample

Informed consent was obtained, and the patient was positioned in the left lateral decubitus position. The L4-L5 interspinous space was identified, anesthetized with 10 mL of lidocaine, and prepared with antiseptic. A sterile drape was applied, and a 21-gauge spinal needle was inserted, bevel aligned parallel to the Dural fibers, and angled cephalad. After feeling the characteristic "pop" indicating Dural penetration, CSF was confirmed via intermittent withdrawal. CSF was collected, the stylet reinserted, the needle removed, and the site dressed with a sterile bandage. The patient was positioned supine for recovery.

Amino acid analysis in CSF was conducted using tandem mass spectrometry (Shimadzu CLAM2040, MS/MS) with CHROMSYSTEM MS/ MS kits. A 10 μ L CSF aliquot was mixed with 200 μ L of internal standard solution, vortexed, and centrifuged at 6000 rpm for 10 minutes at 4 °C. The 150 μ L supernatant was evaporated at 45 °C under nitrogen gas for 20 minutes, treated with 50 μ L each of acetyl chloride and 1-butanol, shaken, and incubated at 65 °C for 15 minutes. After drying under nitrogen at 45 °C, 100 μ L of 75% acetonitrile was added, shaken, and 10 μ L was injected into the mass spectrometer. The analysis ran at a 150 μ L/min flow rate for 2.074 minutes using multiple reaction monitoring (MRM) [18].

Dried blood spot (DBS) sample

DBS samples were collected via heel prick during recurrent and metastatic phases, dried on Whatman 903 cards, and stored at -20 °C. For analysis, blood was extracted from the cards and processed. The MS/MS system was verified using electrospray ionization (ESI) and multiple reaction monitoring (MRM) with commercially available standards. A 3.2 mm blood disc was punched out, treated with 100 µl of internal standard, shaken for 30 minutes at 7000 rpm, and evaporated. After adding Reagent B and incubating at 60 °C for 20 minutes, the sample was dried, treated with Reagent C, shaken, and 20 µl of the supernatant was injected into the MS/MS system (Supplementary Fig. S1).

Metabolite set enrichment analysis (MSEA)

To explore the biological significance of key metabolites, we performed a metabolite set enrichment analysis (MSEA) using MetaboAnalyst 5.0, considering SMPDB and KEGG databases for Recurrent DBS, Metastasis DBS, and Metastasis CSF samples. We then compared metabolite patterns across these sample types to identify those involved in the progression from recurrence to metastasis. We conducted an integrative pathway analysis of significant metabolites and proteins associated with glioblastoma development and metastasis to gain further insight, focusing on essential proteins involved in GB.

Glioblastoma-related key protein identification

Pivotal genes involved in GB were identified through two strategies. First, a literature review highlighted genes influencing key pathways like homeostasis, cellular metabolism, telomere maintenance, chemoresistance, and epigenetic regulation. Six genes were selected for further analysis (Supplementary Fig. S2 A). The second strategy involved searching the KEGG database for pathways related to glioblastoma, including cholesterol signaling, cytoskeleton remodeling, metabolism, fatty acid metabolism, and autophagy. A list of 1943 genes was compiled (Supplementary Fig. S2 B) and used to reconstruct a protein-protein interaction network using GeneMANIA and Cytoscape. An additional six genes were identified through topological analysis and databases like ONcoDB and the Human Protein Atlas (Supplementary Figure S2 C&D). Combining both strategies, 11 final genes were selected, with EGFR common to both.

Joint pathway analysis

After identifying key glioblastoma-related proteins, we conducted an integrative pathway analysis of significant metabolites and proteins using MetaboAnalyst 5.0 for Recurrent DBS, Metastasis DBS, and Metastasis CSF samples. The analysis was based on three criteria: degree, betweenness, and closeness, and followed a three-stage process. First, we selected the top 20 pathways for each criterion. Second, we integrated the results for each sample, and third, we removed non-significant pathways using the false discovery rate (FDR). Ultimately, we identified 31 pathways for Recurrent DBS, 33 for Metastasis DBS, and 31 for Metastasis CSF, with several common and exclusive pathways in each sample.

Statement of translational relevance

This study uncovers novel metabolic shifts and genetic alterations during the transition from recurrent to metastatic glioblastoma, particularly in a rare case with scalp metastasis. By identifying key drivers of this aggressive behavior, our findings offer potential new targets for therapeutic intervention, providing a critical advancement in managing extracranial metastasis in glioblastoma.

RESULTS

Case description

A 33-year-old woman, previously healthy with no significant family or psychosocial issues, presented at Sina Hospital's emergency department four years ago with severe headaches and sleepiness. A non-contrast CT scan revealed a left temporoparietal hemorrhagic lesion (Wernicke's area; speech perception and processing area), causing a pressure effect on cerebral tissues and significant brain swelling. Her initial Karnofsky Performance Scale (KPS) score was 70. Emergency surgery was performed to maximize safe tumor removal, achieving complete resection. Following surgery, she underwent combined treatment for GB with concurrent temozolomide (TMZ) and radiation therapy (RT), followed by maintenance TMZ cycles, as per the Stupp protocol [19, 20]. Her KPS improved to 90 shortly after surgery and reached 100 during the follow-up.

After eighteen months, she developed new neurological symptoms, including right-sided weakness, memory problems, and difficulty speaking. MRI revealed multifocal recurrence in the left frontal and temporal lobes, including near the surgical site. Due to the involvement of critical brain areas, she underwent awake craniotomy and complete resection of all lesions, including an extra-neural GB metastasis in the scalp. The pathological analysis confirmed wild-type IDH-1&2, mutated TERT, nonmutated BRAF, EGFR, and non-methylated MGMT (Supplementary S3-S7). The pathological diagnosis was a malignant tumor (Fig. 1), and immunohistochemical (IHC) examination (Fig. 2) confirmed that the diagnosis was consistent with the recurrence and metastasis of GB; the examination also demonstrated the following: β-catenin (negative), Desmin (negative), glial fibrillary acidic protein (GFAP) (positive), Myogein (negative), p53 (negative), SMA (Alpha Smooth Muscle Actin) (negative), and Vimentin (positive). In our IHC analysis, GB samples exhibited positive staining for GFAP and vimentin, which are associated with the mesenchymal transition. These markers' expression indicates cellular plasticity and has been linked to increased invasive potential and metastatic behavior in glioblastoma. The positivity for GFAP and vimentin in our samples supports the hypothesis that these cells are undergoing a metastatic transition, consistent with the aggressive nature of GB progression [21-24].

Following surgery, she received further treatment, including radiation therapy and concurrent TMZ with bevacizumab, which initially improved her speech and mobility. However, four months later, the tumor recurred, necessitating another craniotomy for complete mass removal. Despite initial improvement, her KPS declined to 40 within a month, and she passed away approximately two years after her initial diagnosis Table 1.

Analysis of amino acid profiles in blood and CSF of a patient with recurrent and metastatic glioblastoma

In this study, the DBS sample from the recurrent stage showed decreased concentrations of Arginine, Methionine, Phenylalanine, Tyrosine, and Valine compared to the reference range, while Glutamic acid levels were elevated. The concentrations of the remaining amino acids were within normal limits [25] (Supplementary S8). In the metastasis stage, DBS samples showed decreased concentrations of Alanine and Glycine. In contrast, Glutamic acid remained elevated, with other amino acids within the normal range. In the metastasis CSF sample, Alanine, Glutamic acid, Methionine, Phenylalanine, Valine, Glycine, Leucine, Isoleucine, Histidine, Lysine, Tryptophan, and Threonine were all at the upper limits of the reference range (Table 2).

Metabolic profile and exclusive metabolite-related pathways for each sample

A simultaneous review of the metabolite set enrichment analysis results (Table 3) and the joint pathway analysis of metabolites and proteins (Table 4) reveals that aminoacyl-tRNA biosynthesis plays a pivotal role across all three samples. Notably, phenylalanine, tyrosine, tryptophan, arginine, and their respective biosynthetic pathways were exclusively observed in the recurrent DBS samples. In contrast, aspartate and glutamate were found only in the metastasis DBS samples. The metastasis CSF samples exclusively contained valine, leucine, isoleucine, and their biosynthesis, along with glutathione and its metabolism, were common to both the metastasis DBS and metastasis CSF samples. These results are crucial for improving diagnostic methods and deepening our understanding of the mechanical processes contributing to disease progression.

DISCUSSION

Various studies have demonstrated the significance of metabolic reprogramming at different stages of glioblastoma (GB) creation, progression, and development. Research in this field has led to the classification of metabolic pathways into five primary groups: Glycolysis (Warburg Effect) and the Pentose Phosphate Pathway (PPP) [26–30], Amino Acid Metabolism [31–33], Lipid Metabolism [34–36], Nucleotide Metabolism [37, 38], and the TCA Cycle and Oxidative Phosphorylation [39–43]. Additionally, several biological processes contribute to the driving forces behind metabolic reprogramming. These include hypoxia [44–47], the tumor microenvironment and extracellular matrix [48–50], interactions with the immune system [51–55], the presence of stem cells and tunneling nanotubes (TNTs) [56–59], effects of therapeutic drugs [60, 61], and various epigenetic factors [62, 63].

Glioblastoma (GB) metastasis beyond the central nervous system (CNS) is exceptionally rare, mainly due to the protective function of the blood-brain barrier (BBB), the lack of lymphatic drainage, and the typically short overall survival (OS) of this aggressive malignancy [64]. Previous studies have reported that the average interval from initial diagnosis to extracranial tumor dissemination is around 11 months [65, 66]. Metastasis to vertebral sites, however, tends to occur later, with a median time of around 26 months [65, 66].

The mechanisms behind GB metastasis outside the CNS remain unclear but are believed to involve a mix of iatrogenic, genetic, and molecular factors, requiring further research [64]. Identified risk factors for extracranial metastasis include craniotomy, stereotactic biopsy, ventricular shunting, younger age, radiation therapy, prolonged survival, tumor recurrence, and sarcomatous components [64]. Over 90% of patients with extracranial metastases have had craniotomy, suggesting that glial cell dissemination through the bloodstream during surgery is a likely pathway for spread [67, 68].

GB cells may also metastasize via cerebrospinal fluid through peritoneal shunts or by seeding soft tissues through craniotomy defects. Chronic wound infections and tumor resection may increase the risk of extracranial metastasis due to direct surgical seeding [69, 70]. The mechanisms driving the osteolytic metastasis of GB are hypothesized to involve complex, bidirectional interactions between brain tumor cells and bone tissue [71].

Despite extensive research on molecular variants associated with GB and its subtypes, a critical gap persists in identifying genomic drivers enabling GB metastasis. Through protein-protein interaction network reconstruction, we identified six key genes— Ubiquitin C (UBC), Fibronectin 1 (FN1), Epidermal Growth Factor Receptor (EGFR), Catenin Beta 1 (CTNNB1), Jun Proto-Oncogene (JUN), and Mitogen-Activated Protein Kinase 1 (MAPK1)—that may play pivotal roles in GB invasion (Supplementary S9-S11).

FN1, in particular, has been suggested as a diagnostic marker to differentiate GB from low-grade astrocytoma, with its expression playing a functional role in the progression of malignant gliomas through the TGF-β-induced epithelialmesenchymal transition (EMT) pathway [72]. Hendrych et al. identified genetic alterations in NF1, NOTCH3, AIRDA1, and MTOR in cases of glioblastoma metastasizing to the spine. Interestingly, while the BRAF mutation is commonly found in primary tumors, it is absent in metastatic lesions, where NF1 mutations are detected, indicating that tumor cells lacking the BRAF mutation may acquire metastatic potential [73]. Moreover, the Epidermal Growth Factor (EGF) has been shown to promote glioblastoma metastasis by inducing matrix metalloproteinase-9 (MMP-9) through an EGFR-dependent mechanism [74]. In the context of metastasis, the aberrant activation of MAPK signaling in glioblastoma cells is believed to enhance their invasive capacity, potentially leading to the formation of secondary tumors at distant sites, although extracranial metastasis remains a rare occurrence [75, 76].



Fig. 1 Imaging and histopathology of GB and Its metastasis to the scalp. Axial (a), sagittal (b), and coronal (c) views of brain MRI before the second surgery, along with the postoperative counterparts (d-f). In the preoperative imaging, multicentric GB involving left posterior frontal and left temporoparietal regions concomitant with metastasis to the scalp of the left frontal area is seen. Compared to postoperative imaging, gross total removal (GTR) of the lesions has been obtained. g Hypercellular neoplastic glial tissue (blue arrow) with palisading necrosis (black arrow) and vascular proliferation (yellow arrow) (hematoxylin-eosin, x200). h Hypercellular neoplastic glial tissue (blue arrow) with vascular proliferation (yellow arrow) (hematoxylin-eosin, x200). i Neoplastic glial tissue (blue arrow) with invasion to muscle bundles (black arrow) (hematoxylin-eosin, x200). j Neoplastic glial tissue (blue arrow) invades muscle bundles (black arrow) (hematoxylin-eosin, x200). j Neoplastic glial tissue (blue arrow) invades muscle bundles (black arrow) (hematoxylin-eosin, x200).



Fig. 2 Immunohistochemical (IHC) staining in the patient's recurrent and metastatic tumor. IHC staining of the patient's recurrent and metastatic glioblastoma (GB) tumor samples highlights the expression of markers associated with mesenchymal transition (EMT), including GFAP and vimentin. These markers indicate cellular plasticity, correlated with enhanced invasive potential and metastatic behavior in GB. Panels (**a**, **h**) show beta-catenin with negative (0) staining, indicating no detectable expression in the analyzed tumor cells, while panels (**b**, **i**) demonstrate desmin with negative (0) staining, reflecting its absence in the tumor. Panels (**c**, **j**) display strong positive (+3) staining for GFAP, suggesting potential involvement in tumor invasiveness. Panels (**d**, **k**) show myogenin with negative (0) staining, indicating no overexpression in the analyzed samples. Panels (**f**, **m**) highlight SMA with negative (0) staining, reflecting no significant expression. In contrast, panels (**g**, **n**) show vimentin with strong positive (+3) staining, supporting its role as a marker of mesenchymal transition in glioblastoma. Based on standardized scoring, each marker's staining intensity is categorized as Negative (0) or Positive (+3). The combined positive staining for GFAP and vimentin provides evidence supporting mesenchymal transition and enhanced invasive and metastatic potential in GB. GFAP Glial Fibrillary Acidic Protein, SMA Spinal Muscular Atrophy, IDH1&2 Isocitrate dehydrogenase 1, WT wild-type, TERT Telomerase reverse transcriptase, MUT Mutated, EGFR Epidermal growth factor receptor.

In recurrent GB, metabolism shifts toward increased glycolysis, altered lipid and amino acid metabolism, and enhanced hypoxiadriven pathways. Metastatic GB adapts further, utilizing diverse energy sources, boosting antioxidant defenses, and remodeling the extracellular matrix, demonstrating distinct metabolic strategies at each stage [77]. In other words, transitioning from recurrence to metastasis is a journey from mere survival to profound adaptation.

Our results revealed that phenylalanine, tyrosine, and arginine pathways were unique to the recurrent DBS samples, while

Table 1. Sum	mary of in	portant	patient data.									
Admission	Date	KPS	Surgery	Post-op KPS after 2 weeks	Chemoradiotherapy	Extent of Resection (EOR%)	IDH-1	IDH-2	TERT mutation	EGFR	BRAF- V600E	MGMT
1st	Sep, 2020	70	Emergent	100	Yes (standard) ^a	06	Wildtype	Wildtype	mutated	Neg	Neg	<10%
2nd	Mar, 2022	80	Elective (Awake Craniotomy)	100	Yes (considering previous Treatments) ^b	100	Wildtype	Wildtype	mutated	neg	neg	<10%
3rd	Jul, 2022	80	Routine	85	Chemotherapy ^c	100	No check	No check	No check	No check	No check	No check
4th	Aug, 2022	40	Died after a week	AN	NA	NA	NA	AN	NA	NA	NA	NA
^a Concurrent te	mozolomid tunn et al	le (TMZ) (7 regimen	75 mg/m2/day for 6 w	veeks) and con	ventional RT (60 Gy in 33 fracti	ons) and then six m	laintenance cyc	cles of TMZ (150)-200 mg/m2/da	iy for the first !	5 days of a 28	day cycle)

according to supplet an regument

Six maintenance cycles of TMZ (150–200 mg/m2/day for the first 5 days of a 28-day cycle) plus bevacizumab (10 mg/kg q 2 weeks) the first 5 days of a 28-day cycle) plus bevacizumab (10 mg/kg q 2 weeks)

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aspartate and glutamate were exclusive to metastasis DBS. Notably, many tumours rely on arginine, as they lack the ability to synthesize it [78, 79]. Arginine deprivation causes elongated cell morphology and loss of intracellular lamellipodia, inhibiting cell motility, adhesion, and invasion. In GB, arginylation is crucial for actin assembly, and arginine deprivation reduces N-terminal arginylation of β -actin, impairing these processes [80] (Supplementary S12).

Glutamine is the most depleted amino acid in tumor cells. It serves as a crucial metabolic fuel, meeting the extensive energy demands of metastatic cells for ATP, biosynthetic precursors, and reductants [77]. Enhanced glutamine availability bolsters cancer cell invasiveness, facilitating distant metastasis. Brain metastatic tumor cells, exhibiting metabolic plasticity, can derive energy from non-glucose sources, notably utilizing glutamine and branched-chain amino acids (BCAAs) as alternative fuels [81, 82]. Brain metastases show a significant reliance on glutamine metabolism, with overexpression of xCT promoting cystine uptake and glutamine oxidation, thus aiding metabolic adaptation and redox balance [83]. Given the brain's high demand for glutamate, cells require an amino group donor, primarily sourced from BCAAs, to sustain glutamate anabolism. Consequently, cerebral vascular endothelial cells express numerous neutral amino acid transporters to support substantial BCAA uptake [84, 85]. Additionally, aspartate metabolism has been linked to tumor metastasis, where metastatic cancer cells consume substantial glutamine to compensate for TCA cycle deficiencies, increasing their dependence on asparagine. Inhibiting asparagine synthetase (ASNS) can induce rapid apoptosis in these metastatic cells [86].

Serine and glycine are critical amino acids required substantially during metastasis to support biosynthetic processes such as glycolysis, glutathione synthesis, and nucleotide production [87]. In the microenvironment of brain metastases, the levels of serine and glycine are notably lower than in plasma [88], likely due to the high demand of metastatic tissues for these amino acids [89]. Tryptophan metabolism is also crucial in glioblastoma progression, exhibiting distinct alterations between recurrent and metastatic stages. In recurrent GB, Indoleamine 2,3dioxygenase (IDO) upregulation shifts tryptophan metabolism toward kynurenine production, activating the hydrocarbon receptor (AhR) pathway to enhance immune suppression and treatment resistance. During metastasis, the kynurenine pathway demonstrates metabolic flexibility, adapting tryptophan degradation to different microenvironments, thereby further promoting immune evasion, tumor invasion, and colonization [90, 91].

In summary, this case-based report explores and compares the shifting amino acid profiles observed in recurrent and metastatic phases of GB. Our findings suggest a preference for specific amino acids associated with recurrence and metastasis. Notably, phenylalanine, tyrosine, tryptophan, arginine, and their respective biosynthetic pathways were uniquely detected in recurrent samples. Conversely, aspartate and glutamate were exclusive to metastasis samples. Moreover, valine, leucine, isoleucine, and their biosynthetic pathways were uniquely identified in metastasis CSF samples. Glycine, serine, and alanine and their biosynthesis, glutathione, and metabolism were shared between metastasis DBS and CSF samples. These metabolic preferences might extend to other cancer types, warranting further investigation. While large-scale evaluations of these metabolic shifts are resource-intensive, small-scale case studies like this can offer valuable insights to inform and guide future research directions.

Emerging research highlights that modulating specific amino acid availability can significantly enhance cancer therapies' efficacy. This positions amino acid metabolism as a promising avenue for therapeutic intervention in oncology. However, the

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ladie 2.	Analyzing amino	acids in pa	atients with	recurrent and	metastatic	GRINI USING	tandem mass	spectrometry	(1015/1015).

	Amino acids		Measurement	Reference values	
Recurrent DBS sampling	Arg	Arginine	27.1	35–125	*
	Glu	Glutamic Acid	171	15–130	*
	Met	Methionine	12.8	15–40	*
	Phe	Phenylalanine	27.2	30–82	*
	Tyr	Tyrosine	25.5	35–110	*
	Val	Valine	73.6	120–320	*
Metastasis DBS sampling	Ala	Alanine	153.29400405723<	160–530	*
	Glu	Glutamic Acid	142.496969	15–130	*
	Gly	Glycine	97.82302141	140–420	*
Metastasis CSF sampling	Ala	Alanine	85.5992458	16_46	*
	Glu	Glutamic Acid	94.48296003	<=5	*
	Leu	Leucine	35.3955945	5_22	*
	Isoleu	Isoleucine	13.80910619	<=12	*
	Met	Methionine	9.292019355	2_7	*
	Phe	Phenylalanine	24.51995232	6_20	*
	Val	Valine	55.89986622	8_30	*
	Gly	Glycine	23.4087393	5_20	*
	Thr	Threonine	80.90422618	14_59	*
	His	Histidine	71.53312551	7_24	*
	Lys	Lysine	336.1633393	10_36	*
	Trp	Tryptophan	5.32824915	<=5	*

Asterisks (*) indicate amino acid levels that deviate from the reference range provided in the Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases (Second Edition, pages 42 and 44). These deviations were identified by comparing the patient's data to the reference values, as this is a single-patient study, and statistical significance cannot be determined.

Table 3. The results of metabolites enrichment analysis based on significant metabolites in three different samples are obtained through two databases (SMPDB and KEGG) (Tables 3–1).

A					
	SMPDB (FDR)	KEGG (FDR)			
Recurrent DBS sampling	Phenylalanine and Tyrosine Metabolism (0.034)	Aminoacyl-tRNA biosynthesis (5.71E-08)			
		Phenylalanine, tyrosine, and tryptophan biosynthesis (0.0032)			
		Phenylalanine metabolism (0.0158)			
		Arginine biosynthesis (0.0238)			
Metastasis DBS sampling	Alanine Metabolism (3.65E-04)	Aminoacyl-tRNA biosynthesis (0.00241)			
	Glutathione Metabolism (3.65E-04)	Alanine, aspartate and glutamate metabolism (0.0209)			
	Glutamate Metabolism (0.00337)	Glutathione metabolism (0.0209)			
	Glycine and Serine Metabolism (0.00446)	Porphyrin and chlorophyll metabolism (0.0209)			
	Glucose-Alanine Cycle (0.00869)	Glyoxylate and dicarboxylate metabolism (0.0209)			
	Urea Cycle (0.0373)				
	Ammonia Recycling (0.039)				
Metastasis CSF sampling	Glycine and Serine Metabolism (0.0305)	Aminoacyl-tRNA biosynthesis (1.70E-17)			
	Alanine Metabolism (0.0374)	Valine, leucine, and isoleucine biosynthesis (6.19E-06)			
	Glutathione Metabolism (0.0475)				
В					
Recurrent DBS sampling	Phenylalanine, tyrosine, and tryptophan, Aminoacyl-tRNA biosynthesis, Arginine				
Metastasis DBS sampling	Alanine, aspartate and glutamate, Glutathione, Amin Alanine Cycle, Urea Cycle, Ammonia Recycling, Porph	oacyl-tRNA biosynthesis, Glycine and Serine, Glucose- nyrin and chlorophyll, Glyoxylate and dicarboxylate			
Metastasis CSF sampling	Glycine and Serine, Alanine, Glutathione, Valine, leucine and isoleucine, Aminoacyl-tRNA biosynthesis				

Comparing the results obtained from these three samples revealed a specific amino acid pattern in each sample (Table 3–2). A: The results of Metabolites Enrichment Analysis. B: a specific amino acid pattern in each of the samples.

 Table 4.
 To identify specific pathways, the joint pathway analysis results of three types of samples were compared two by two (recurrent DBS vs. metastasis DBS, recurrent DBS vs. metastasis CSF, and metastasis DBS vs. metastasis CSF).

5 elements included exclusively in Recurrent DBS:	7 elements included exclusively in Metastasis DBS:	
Protein digestion and absorption	Proteoglycans in cancer	
Chagas disease (American trypanosomiasis)	Glutathione metabolism	
mTOR signaling pathway	Prostate cancer	
Phenylalanine, tyrosine, and tryptophan biosynthesis	T cell receptor signaling pathway	
Arginine biosynthesis	PI3K-Akt signaling pathway	
	Natural killer cell-mediated cytotoxicity	
	Wnt signaling pathway	
4 elements included exclusively in Recurrent DBS:	4 elements included exclusively in Metastasis CSF:	
Chagas disease (American trypanosomiasis)	Valine, leucine and isoleucine biosynthesis	
Phenylalanine, tyrosine and tryptophan biosynthesis	Glutathione metabolism	
Arginine biosynthesis	Prostate cancer	
Circadian entrainment	Glycine, serine and threonine metabolism	
6 elements included exclusively in Metastasis DBS:	4 elements included exclusively in Metastasis CSF:	
Proteoglycans in cancer	Protein digestion and absorption	
Circadian entrainment	Valine, leucine, and isoleucine biosynthesis	
T cell receptor signaling pathway	Glycine, serine and threonine metabolism	
PI3K-Akt signaling pathway	mTOR signaling pathway	
Natural killer cell-mediated cytotoxicity		
Wnt signaling pathway		

The joint pathway analysis results were obtained by comparing Recurrent DBS vs Metastasis DBS.

metabolic intricacies of GB surpass those of many other cancers, driven by its pronounced intratumoral heterogeneity. Often described as a "tumor within a tumor," GB harbors diverse cellular subpopulations, each contributing distinctively to disease progression, therapeutic resistance, and recurrence [92]. The GB tumor microenvironment is a complex and dynamic ecosystem comprising a wide array of elements such as bulk tumor cells, proliferative cell populations, brain tumor-initiating cells (BTICs), rare clones, resident microglia, and infiltrating immune cells derived from the bone marrow. Despite significant advances, the metabolic distinctions between these GB subtypes remain largely unexplored. Unraveling the metabolic dependencies unique to these subgroups can revolutionize therapeutic strategies, paving the way for more targeted and individualized treatment approaches for this highly aggressive and multifaceted disease [92].

Several critical considerations and unanswered questions surround targeting amino acid metabolism in GB and other cancers. It is well-established that amino acids play central roles in tumor biology, regulating numerous processes such as signaling pathways, interactions within the tumor microenvironment, and epigenetic modifications. Moreover, clinical evidence supports those restricting specific amino acids may enhance cancer treatment outcomes [93, 94]. However, significant gaps in understanding remain. For instance, among the diverse effects mediated by individual amino acids, which specific role is most influential in driving tumor progression or suppression? Furthermore, is the altered amino acid metabolism observed across different cancers causally linked to their initiation and progression, or is it merely a secondary consequence of tumor development? Additionally, how do genetic variations influence amino acid metabolism across primary, recurrent, and metastatic stages of cancer? Addressing these questions is essential to fully harness the therapeutic potential of targeting amino acid metabolism in oncology.

DATA AVAILABILITY

Data is provided within the manuscript or supplementary information files.

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AUTHOR CONTRIBUTIONS

ABB drafted and edited the manuscript with input from all authors. HL-N contributed to the systems biology analysis, data interpretation, manuscript drafting, and editing. NZ, SP, MB, and FJ assisted with data curation. MA and NA contributed to the pathological evaluation and immunohistochemistry (IHC) analysis. FM prepared the pathology slides and performed the IHC staining. SA also prepared pathology slides, conducted the IHC tests, and verified the results. AN and SS participated in manuscript review and editing. JL contributed to the MS/MS analysis, interpretation of results, preparation of pathology slides, and drafting of the MS/MS methods section. EN was involved in pathological analysis. AP-R supervised the project and contributed to writing, reviewing, and editing the manuscript. SG provided overall project supervision and finalized the manuscript draft. All authors reviewed the manuscript.

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The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

The patient involved in the study gave informed consent. The Ethics and Research Committee of Tehran University of Medical Science, Neurosurgical Department of Sina Hospital (IR.TUMS.SINAHOSPITAL.REC.1399.111) approved this study, and all methods were in compliance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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