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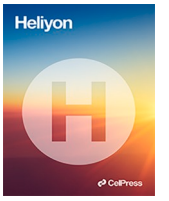

Research article

Analysis of IDH and EGFR as biomarkers in glioblastoma multiforme: A case-control study

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A B S T R A C T

Background: Glioblastoma multiforme (GBM) is a very aggressive primary central nervous system (CNS) tumor with limited therapeutic options and poor prognosis. This study aimed to analyze the association between single nucleotide polymorphisms (SNPs), including IDH1 rs121913500C > T, IDH2 rs11540478G > A, and EGFR rs1468727C > T, and their association on the risk and overall survival of GBM patients in Jordan.

Methods: Using a case-control study design involving 63 GBM patients and 226 healthy controls was conducted at King Abdullah University Hospital in Jordan. DNA extraction was performed using formalin-fixed and paraffin-embedded tissue for GBM samples and blood samples for controls. SNPs analysis was performed using the Sequenom iPLEX assay sequencing technique. Survival outcomes were assessed using Cox models and hazard ratios (HR), and single-cell RNA (scRNA) analysis was performed from GSE70630.

Results: The study showed a significant association between genotype frequency in GBM cases and controls for specific SNPs, including IDH1 rs121913500C > T, and EGFR rs1468727C > T. The G/G genotype of rs11540478 (IDH2) was associated with better prognostic outcomes in GBM patients. The scRNA analysis demonstrated the differential expression of IDH1, IDH2, and EGFR in GBM, with enrichment in central carbon metabolism in cancer.

Conclusion: Our findings suggest that SNPs, particularly in IDH1 and IDH2 genes and EGFR, may serve as diagnostic and prognostic biomarkers for GBM. While the study underscores the clinical relevance of these genetic variants, further investigations with larger and more diverse populations are essential to validate and extend these associations.

1. Introduction

Glioblastoma multiforme (GBM) is the most common malignancy affecting the brain and central nervous system (CNS), constituting 45.2 % of malignant primary brain and CNS tumors, 54 % of all gliomas, and 16 % of all primary brain and CNS tumors. GBMs are classified into primary and secondary subtypes [1,2]. The average annual-age-adjusted incidence rate (IR) of GBM is 3.19 per 100,000 population, making it the highest incidence rate among brain and CNS tumors with malignant behavior, followed by grade II diffuse astrocytoma, with an incidence rate of 0.56 per 100,000 according to Central Brain Tumor Registry of the United States (CBTRUS) [3]. Of all cancer-related deaths, primary CNS tumors are responsible for almost 3 % [4]. In Jordan, there has been an increase in GBM incidence rate change between 1990 and 2019 accounting for 105.9 % with an age-standardized incidence rate of 4.4

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per 100,000 and prevalence rate of 15.8 per 100,000 [5].

GBM demonstrates a relatively low 5-year survival rate of 8.1(%) in both sexes, with higher rates in females of 9.9 (%) compared to 6.9 (%) in males. Among other brain and CNS tumors, GBM shows the lowest 1-, 3-, and 5-year relative survival rates [6]. When comparing the survival outcomes of patients with GBM and high-grade astrocytoma, the SEER research data from 2005 to 2006 revealed that individuals with glioblastoma who underwent surgical resection and postoperative radiation had a median survival of 15 months and a 2-year relative survival rate of 26 % [7]. In contrast, a meta-analysis of studies indicated that patients with high-grade astrocytoma had a mean overall survival of 31.9 months with a 2-year survival rate of 38 % and a 5-year survival rate of 29 % [8].

Grading of diffuse gliomas, according to the World Health Organization (WHO), depends on the presence of nuclear atypia or pleomorphism, mitotic activity (count), vascular proliferation, and necrosis. Accordingly, grade II/IV diffuse gliomas include diffuse astrocytoma and oligodendroglioma, grade III/IV tumors include anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic oligo-astrocytoma and anaplastic ependymomas; and grade IV/IV tumors, such as glioblastoma multiforme. Grade III and Grade IV tumors are considered High-Grade Gliomas (HGGs) [9]. Grade IV gliomas, including Isocitrate dehydrogenase (IDH) wild-type GBM and IDH mutant astrocytoma, are among the most malignant primary CNS tumors. Among them, GBM is the most aggressive and currently untreatable brain tumor [10].

Over recent years, there has been an improved assessment of new molecular mechanisms and specific genes that play a role in the growth of GBM. Mutations in IDH1/IDH2 lead to the conversion of alpha-ketoglutarate to 2-hydroxyglutarate, an oncometabolite that plays a crucial role in driving gliomagenesis [11]. The mutational status of IDH works as a robust prognostic factor, influencing the course of CNS gliomas. This significance was explicitly emphasized in the 2021 World Health Organization (WHO) classification of CNS tumors [9,10]. EGFR is commonly overexpressed and/or hyper-activated in human malignancies, including glioblastoma, making EGFR-targeted therapeutic strategies a frequently employed treatment approach. EGFR overexpression and activation are known to profoundly influence hallmark traits of cancer cells, including enhanced survival, proliferation, and invasion [12,13]. The prognostic significance of EGFR gene and protein alterations is still under evaluation, particularly in GBMs. Among these, 40 % exhibit EGFR amplification, 60 % show overexpression, and 24%–67 % have a mutated gene [14].

Exploring new factors in the tumor's gene expression and epigenetic patterns, which contribute to its molecular diversity and aggressiveness, could potentially enhance clinical outcomes [15]. Investigations into the impact of synonymous polymorphisms on disease physiopathology have indicated that they may disrupt protein folding, impede mRNA stability, and hinder both constitutive and alternative splicing [16,17]. Our goal in this case-control study is to identify diagnostic and prognostic biomarkers using single-nucleotide polymorphisms of IDH and EGFR genes in a Jordanian population with GBM.

2. Materials and methods

The study received approval from the Institutional Review Board (IRB) of King Abdullah University Hospital (KAUH), Jordan (IRB code number 6/106/2017, dated June 8, 2017). All control subjects participated voluntarily and provided written informed consent. The need for a formal written informed consent from patients was waived by the IRB approval. The name of the subjects and cases were coded, blinded, and confidentially was ensured. All clinical investigations were conducted in accordance with the principles outlined in the Declaration of Helsinki.

2.1. Study design

A case-control study of 63 diagnosed GBM patients with sufficiently available clinical information were recruited from King Abdullah University Hospital (KAUH), the main tertiary hospital in north Jordan between the period 2015–2020. The diagnosis of all cases of GBM was done independently by a pathologist (SK) based on the 2016 WHO Classification of Tumors of the Central Nervous System [10]. A total of 226 healthy controls were included in the study. The names of both cases and control subjects were encoded and blinded to ensure confidentiality.

2.2. DNA extraction

Genomic DNA extraction for GBM patients was performed on formalin-fixed and paraffin-embedded (FFPE) tissue using the DNeasy Blood & Tissue Kit (Qiagen Ltd., West Sussex, UK), following the manufacturer's protocols. For control subjects, genomic DNA was extracted from blood samples using the QIAamp® DNA Mini Kit, also adhering to the manufacturer's instructions. The quality of the extracted DNA was assessed by agarose gel electrophoresis and ethidium bromide staining, while concentration and purity were measured using the NanoDrop 1000® spectrophotometer. The purified DNA samples, along with their concentrations, were sent to the

Table 1
The SNPs, SNPs positions and primers sequences for IDH1, IDH2, and EGFR genes.

SNP-ID	Gene	Chr^	bp[a]	Primer Forward	Primer Reverse
rs121913500	IDH1	2	209113112	ACGTTGGATGACATGACTTACTTGATCCCC	ACGTTGGATGAAAATATCCCCCGGCTTGTG
rs11540478	IDH2	15	90085305	ACGTTGGATGTTCTGGTGCTCCCGATAGTG	ACGTTGGATGTGATGGGAAGACGATTGAGG
rs1468727	EGFR	7	55162412	ACGTTGGATGTTTACTCTCTGGGCATGGAC	ACGTTGGATGGCCTATCAGCTAAAGGATTC

[a] bp: base pair (Genomic Position). ^ Chr: Chromosome.

Australian Genome Research Facility (AGRF), Melbourne Node, Melbourne, Australia, for genotyping. Genotyping with the Sequenom MassARRAY® system (iPLEX GOLD) (Sequenom, San Diego, CA, USA) was performed at the AGRF by following the manufacturer's protocols (Sequenom, San Diego, CA, USA). Table 1 shows SNPs' positions and primers' sequences for IDH1, IDH2, and EGFR genes.

2.3. Survival outcomes

The survival outcome in our study was overall survival (OS), defined as the time from surgery to the time of death or the last follow-up for those still alive at the time of final data collection and analysis. To examine the prognostic impact of the identified SNPs, as well as age and sex, the univariable Cox proportional hazards model was employed. Log-rank test and Kaplan-Meier curves were used to represent survival rates between groups. A significant association with survival was determined at p-value <0.05. Survival analyses were performed in the R software package (Version 4.3.1) using the *survminer*, *survival*, and *finalfit* packages.

2.4. Single-cell RNA analysis

Single-cell (scRNA) datasets of GBM patients were obtained from the Gene Expression Omnibus (GEO) under the following accession number: GSE70630 by Filbin et al. [18] comprising 3829 cells from six IDH1 and IDH2 mutant freshly resected human samples and sequenced using SmartSeq2. The *Seurat* package (v2.2) in R was used for scRNA analysis. The *CreateSeuratObject* function was used to generate a Seurat object for each dataset with the detection of genes that are expressed in at least 3 cells and cells expressing at least 200 genes. The *PercentageFeatureSet* function was used to calculate the percentage of mitochondrial gene content using the "^MT-" regex pattern to match the genes. After quality control (QC) filtering for cells with unique molecular identifiers (UMIs) $>$ 400, gene expression $>$100 and $<$ 8000, and mitochondrial gene content $<$5 %, a total 3829 cells were retained from GSE70630. Count data were normalized using the "*LogNormalize*" method and a scaling factor of 10,000. The top 2000 variable genes were obtained using the Variance Stabilizing Transformation (vst) method which transforms the count matrix into a constant variance along the values with respect to library size. Genes were then centered using the *ScaleData* function, and linear dimensionality reduction using principal component analysis (PCA) was used to summarize the variable genes. The number of principal components that capture the highest variability was determined using the *ElbowPlot* function. The *FindNeighbors* and *FindClusters* functions were used to calculate the shared nearest neighbor (SNN) between every cell and identify cell clusters by the SNN. Subsequently, non-linear dimensionality reduction was performed using the Uniform Manifold Approximation and Projection (UMAP) method from the *RunUMAP* function and visualized using the dimensionality reduction plot *DimPlot*. The *FeaturePlot* function was used to visualize gene expression across each cell in the *DimPlot*, and *VlnPlot* was used to visualize the distribution of gene expression across cell clusters. Cell clusters were annotated using the *SingleR* package and a reference dataset from the *HumanPrimaryCellAtlasData*, and matched with the original annotation from each dataset.

2.5. Statistical analysis

For continuous variables, the mean ± standard deviation (SD) was used when data followed a normal distribution, as determined by the Shapiro-Wilk test. When data deviated from normality, the median (Q1, Q3) was reported. Categorical variables were presented

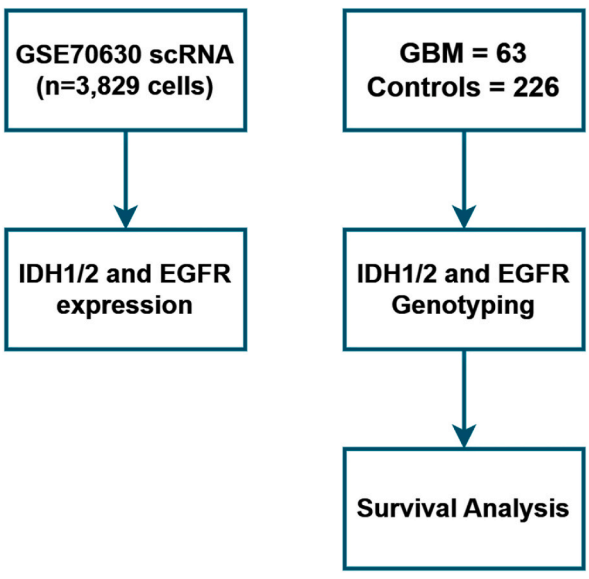

Fig. 1. Flow diagram of the study.

as frequencies (percentages). The correlation between demographic, clinical, and genetic variables and study groups was assessed using the Wilcoxon (Mann-Whitney U) test for continuous variables, and the chi-squared (X^2) or Fisher's exact test for categorical variables when the category count was less than 5. A logistic regression model was applied to the identified SNPs to analyze the association between genotypes and study groups. A significance level was set at p-value <0.05. All analyses were conducted using the R software package (Version 4.3.1) with the glm and gtsummary packages.

3. Results

3.1. Baseline characteristics

Fig. 1 shows the flow diagram of the study. A total of 63 GBM cases were included in our study, of which 37 (58.7 %) were males, and 26 (41.3 %) were females. The mean age at diagnosis was 50.1 (18.4), median overall survival was 2.8 (0.5, 9.9) months, and 30 (47.6 %) patients were dead. Mean tumor size was 126.7 (96.9) cm, with 31 (49.2 %) patients having right-sided tumors. The majority of patients 80 % (n = 48) had liquefactive necrosis, with 21 (35.6 %) having necrosis spanning the whole tumor. A total of 226 healthy control subjects were retrieved, with a mean age of 30.6 (11.8) and 147 (65.3 %) of them were females. Table 2 shows the baseline characteristics of included GBM cases and controls.

3.2. Genotype frequency of the identified SNPs

Of the identified four SNPs, a statistically significant difference in genotype frequency between GBM cases and controls was seen in rs121913500 (*IDH1*) in which 100 % of healthy controls had C/C genotype, while 5 (7.9 %) of GBM cases had C/T genotype (p-value<0.001). The C/C genotype of rs1468727 (*EGFR*) was predominant in 69 % of GBM cases, while C/T genotype was predominant in 39 % of healthy controls (p-value = 0.040). Table 3 shows the single allele and genotype frequency of the identified SNPs between GBM cases and controls.

3.3. Inheritance models

Four inheritance models were constructed: codominant, overdominant, dominant, and recessive. Logistic regression model showed significant association of the codominant model of rs1468727 (EGFR) with higher likelihood of C/T genotype in healthy controls compared to GBM cases (OR: 0.41, 95 % CI: 0.20–0.83, p-value = 0.013) and no significant difference in the T/T genotype. While the overdominant model of rs1468727 (EGFR) showed that C/C-T/T genotype was associated with higher prevalence in GBM cases compared to controls. The dominant model of rs1468727 (EGFR) showed that the C/C genotype was significantly associated with GBM

Table 2
Baseline characteristics of GBM cases included in our study.

Variable	GBM (n = 63)
Age at diagnosis, Mean (SD)	50.1 (18.4)
Sex, n (%)	
Females	26 (41.3 %)
Males	37.0 (58.7 %)
Survival Status, n (%)	
Alive	33 (52.4 %)
Dead	30 (47.6 %)
Overall survival (months), Median (Q1, Q3)	2.8 (0.5, 9.9)
Serum LDH, Mean (SD)	34.0 (179.0)
Total protein, Mean (SD)	47.3 (33.1)
Monocytes, Mean (SD)	3.7 (4.0)
Lymphocytes, Mean (SD)	9.2 (10.0)
Platelets, Mean (SD)	281.9 (96.4)
Tumor size, Mean (SD)	126.7 (96.9)
Tumor laterality, n (%)	
Right	31 (49.2 %)
Left	29 (46.0 %)
Bilateral	3 (4.8 %)
Necrosis, n (%)	
Coagulative	7 (11.7 %)
Geographic	1 (1.7 %)
Liquefactive	48 (80.0 %)
None	4 (6.7 %)
Degree of necrosis, n (%)	
Foci of palisading necrosis	34 (57.6 %)
Whole tumor	21 (35.6 %)
None	4 (6.8 %)
Radiotherapy, n (%)	9 (14.3 %)
Chemotherapy, n (%)	6 (19.4 %)

Table 3
Allele frequency of identified variants between GBM cases and control subjects.

SNP ID	GBM (n = 63)	Controls (n = 226)	P-value
rs11540478			
Allele G	125	445	
Allele A	1	5	
NA	0	1	
Genotype G/G	62 (98 %)	220 (98 %)	>0.9
Genotype A/G	1 (1.6 %)	5 (2.2 %)	
NA	0	1	
rs121913500			
Allele C	121	450	
Allele T	5	0	
NA	0	1	
Genotype C/C	58 (92 %)	225 (100 %)	**<0.001**
Genotype C/T	5 (7.9 %)	0 (0 %)	
NA	0	1	
rs1468727			
Allele C	88	305	
Allele T	22	127	
NA	8	10	
Genotype C/C	38 (69 %)	110 (51 %)	**0.040**
Genotype C/T	12 (22 %)	85 (39 %)	
Genotype T/T	5 (9 %)	21 (10 %)	
NA	8	10	

cases compared to higher association of C/T-T/T genotypes in healthy controls. Table 4 showed the odds ratio of the four models of inheritance of the identified SNPs.

3.4. Survival analysis

The univariable Cox proportional hazard model revealed that the G/G genotype of the codominant model of rs11540478 (*IDH2*) is associated with a significantly better prognosis in GBM patients compared to the A/G genotype (HR: 0.02, 95 % CI: 0.0–0.29, p-value = 0.005) as shown in Fig. 2. Table 5 shows the univariable cox proportional hazard model analysis of the identified SNPs.

3.5. Immune and functional landscape of IDH1, IDH2, and EGFR

We investigated the expression of IDH1, IDH2, and EGFR the GSE102130 scRNA dataset with a total of 3829 cells were retrieved. IDH1 was mainly expressed by malignant cells, followed by patient-derived xenograft (PDX) and oligodendrocytes. While IDH2 was mainly expressed by immune cells and malignant cells. EGFR expression was predominantly by tumor cells. Fig. 3 shows the expressions of IDH1, IDH2, and EGFR in scRNA dataset. Protein-protein interaction analysis showed significant enrichment in the following KEGG pathways between IDH1, IDH2, and EGFR: "2-Oxocarboxylic acid metabolism", "TCA Cycle", "central carbon metabolism", "glutathione metabolism", "Biosynthesis of amino acids", and "peroxisome".

4. Discussion

Glioblastoma multiforme (GBM) is a highly aggressive and malignant type of brain cancer that originates in the brain's glial cells, specifically the astrocytes. It is the most prevalent and lethal primary brain tumor in adults [19]. Despite extensive research efforts, the survival of glioblastoma patients frequently remains constrained, and there is still a lack of curative therapy [20]. Following the release of the 2021 WHO classification of tumors of the CNS, it was recommended that a unified diagnosis and a comprehensive report, incorporating both morphology and genetic findings, be integrated into the classification of gliomas [9,21]. Therefore, our goal is to

Table 4
Logistic regression models on four modes of inheritance of the identified SNPs between GBM cases and controls.

SNP	Model	Genotype	Controls, N = 226	GBM, N = 63	OR (95 % CI)	P-value
rs1468727 (*EGFR*)	**Codominant**	C/C	110 (51 %)	38 (69 %)	1	
		C/T	85 (39 %)	12 (22 %)	0.41 (0.20–0.83)	**0.013**
		T/T	21 (9.7 %)	5 (9.1 %)	0.69 (0.24–1.96)	0.484
	Overdominant	C/C-T/T	131 (61 %)	43 (78 %)	1	
		C/T	85 (39 %)	12 (22 %)	0.43 (0.22–0.86)	**0.017**
	Dominant	C/C	110 (51 %)	38 (69 %)	1	
		C/T-T/T	106 (49 %)	17 (31 %)	0.46 (0.25–0.87)	**0.017**
	Recessive	C/C–C/T	195 (90 %)	50 (91 %)	1	
		T/T	21 (9.7 %)	5 (9.1 %)	0.93 (0.33–2.58)	0.887

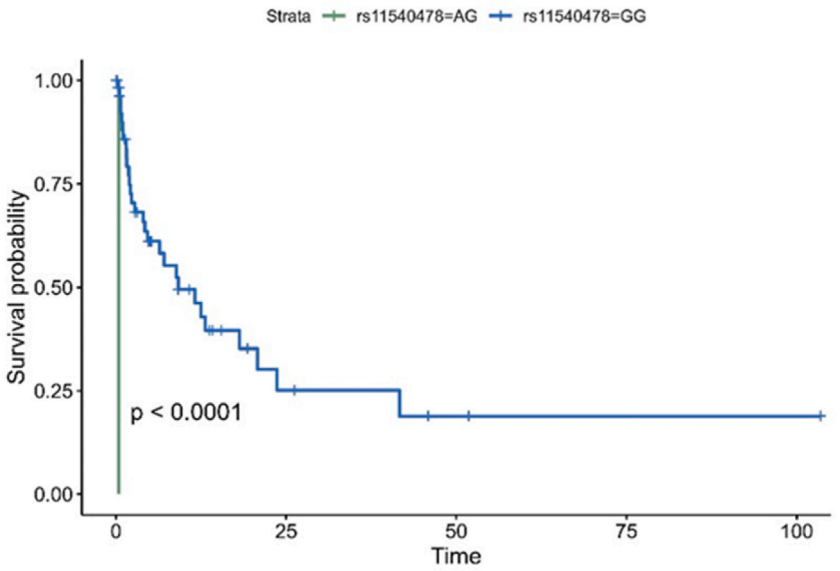

Fig. 2. Kaplan-Meier curves of significant survival-associated SNPs of codominant mode of rs11540478 (IDH2) showing better prognosis in the G/G genotype compared to A/G genotype.

Table 5
Univariable Cox proportional hazard model for overall survival of four modes of inheritance of identified SNPs in GBM cases.

SNP ID	Model	Genotype	HR (95 % CI, P-value)
rs1468727 (*EGFR*)	**Codominant**	**C/C**	–
		C/T	0.70 (0.24–2.05, p = 0.511)
		T/T	2.27 (0.74–6.98, p = 0.152)
	Overdominant	**C/C-T/T**	–
		C/T	0.63 (0.22–1.82, p = 0.388)
	Dominant	**C/C**	–
		C/T-T/T	1.05 (0.46–2.43, p = 0.905)
	Recessive	**C/C–C/T**	–
		T/T	2.46 (0.81–7.41, p = 0.111)
rs11540478 (*IDH2*)	**Codominant**	**A/G**	–
		G/G	0.02 (0.0–0.29, **p=0.005**)
rs121913500 (IDH1)	**Codominant**	**C/C**	–
		C/T	0.98 (0.23–4.19, p = 0.973)

investigate the association between specific single nucleotide polymorphisms (SNPs), including IDH1 rs121913500C > T, IDH2 rs11540478G > A, and EGFR rs1468727C > T and their potential influence on the risk and overall survival of patients with GBM within the Jordanian Arab population.

A total of 63 GBM patients were included in our study, with male predominance accounting for 58.7 %. This finding was concordant to another study in Jordan in 2021, of 800 GBM cases of which 63 % were males [22]. Previous literature extensively documents gender-based variations in the occurrence and outcome of CNS tumors [23]. In a study by Yu et al., it was discovered that androgen receptor signaling might contribute to the development of GBM in males by suppressing transforming growth factor β (TGF-β) receptor signaling [24]. Another study by Gilbert et al., on 833 GBM patients also showed male predominance with 58 % rate which is in line with our results [25,26].

Out of the four identified SNPs, a significant difference in genotype frequency between GBM cases and controls was observed in IDH1 rs121913500C > T, in which healthy controls exhibited the C/C genotype, whereas 7.9 % of GBM cases possessed the C/T genotype. Isocitrate dehydrogenase (IDH) comprises a set of enzymes responsible for catalyzing the oxidative decarboxylation of isocitrate to alpha-ketoglutarate within the Krebs cycle. These IDH enzymes exist in three isoforms: IDH1, IDH2, and IDH3 [27,28]. IDH1, located on chromosome 2, facilitates the NADP (+)-dependent oxidative decarboxylation of isocitrate into 2-ketoglutarat. IDH2, located on chromosome 15, participates in intermediary metabolism and the generation of energy [29]. IDH1 and IDH2, which depend on NADP(+), operate at a crucial juncture in cellular metabolism, influencing lipogenesis, oxidative stress, and oxygen-sensing signal transduction [30,31]. Our findings showed a favorable prognostic effect of IDH2 rs11540478G > A in GBM, with no significant association of IDH1 rs121913500C > T. The prognostic relevance of the IDH1 SNP was initially evaluated in acute myeloid leukemia (AML), revealing its independent association with a poorer prognosis in myeloid leukemia with a normal karyotype (AML-NK) [32,33].

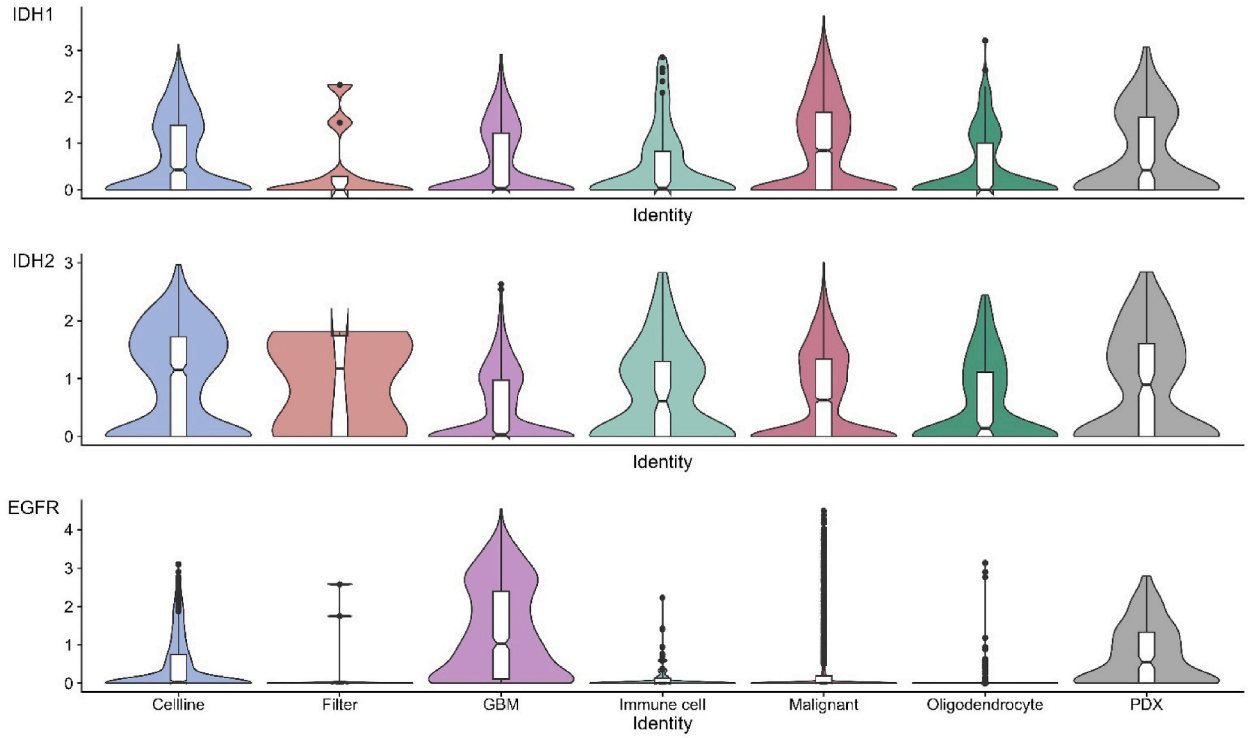

Fig. 3. Single-cell analysis of IDH1, IDH2, and EGFR Violin plots showing genes' expression across cell types.

Vorasidenib, an orally administered brain-penetrant inhibitor targeting mutant IDH1 and IDH2 enzymes, exhibited initial efficacy in gliomas with IDH mutations. Among individuals diagnosed with grade 2 IDH-mutant glioma, Vorasidenib demonstrated a notable enhancement in progression-free survival and prolonged the duration before the next intervention was required [34]. However, it is still not tested in high-grade gliomas and glioblastoma. Ivosidenib an IDH1 inhibitor approved in hematological malignancies such as IDH-mutant AML showing favorable outcomes in high-grade patients [35]. A phase I trial on advanced IDH-mutant gliomas, Ivosidenib also exhibited a favorable safety profile, prolonged disease control, and mitigated the growth of non-enhancing tumors [36].

Epidermal growth factor receptor (EGFR) gene situated on chromosome 7, plays a functional role in regulating the development and homeostasis of epithelial tissue. In pathological contexts, particularly in lung and breast cancer, as well as glioblastoma, EGFR serves as a driver of tumorigenesis [37]. Genetic alterations in EGFR dictate aberrant EGFR trafficking, leading to heightened signaling and the development of tumors [38]. Numerous alterations in the EGFR gene have been detected in gliomas, particularly glioblastomas. These alterations encompass amplifications, deletions, and SNPs. Several trials have been unsuccessful in establishing definitive evidence linking various changes in the EGFR gene and protein to survival outcomes. Additionally, investigations into targeted anti-EGFR therapy have produced inconsistent results [39].

A study by Wang et al. exploring the prognostic role of EGFR SNPs showed that EGFR rs1468727C > T has been connected to treatment response in breast cancer patients [40]. Our findings were concordant with the results of Yan et al. study the genotype C/C was significantly associated with GBM cases in the codominant model of rs1468727C > T. These findings suggest that mutants situated in the EGFR gene might be factors associated with increased risk of GBM; however, larger and more diverse populations with EGFR polymorphisms are necessary to validate these associations [41]. Mellinghoff et al. showed that response to EGFR kinase inhibitors was associated with coexpression of EGFR deletion mutant variant III and Phosphatase and tensin homolog (PTEN) gene [42]. There is suggestive evidence indicating that molecular profiles might anticipate the response to EGFR inhibitors in certain patient subgroups [43]. In previous research, individuals with recurrent disease demonstrated a higher likelihood of responding to erlotinib or gefitinib when their tumors exhibited EGFR overexpression, amplification, or mutation, and were simultaneously found to have intact PTEN or lacked Akt phosphorylation [44].

EGFR amplification serves as a molecular target for tailoring individualized patient care. EGFR overexpression could be a promising focus in glioblastoma therapy. The inhibition of EGFR using monoclonal antibodies or small-molecule tyrosine kinase inhibitors (TKIs) has received approval for treating various tumor types [45]. The occurrence of EGFR amplification is primarily observed in IDH wild type gliomas. Additionally, Bale et al. discovered a higher prevalence of EGFR gene amplification and overexpression in IDH wild type gliomas compared to IDH mutated gliomas [46,47].

Our study presents a comprehensive examination on Glioblastoma Multiforme (GBM), within the Jordanian Arab population. With several strengths presented in our study, we implemented a case-control design which facilitates the examination of rare diseases and enables the simultaneous exploration of multiple risk factors [48]. Additionally, our study adopts a molecular classification approach

based on IDH genetic variants, aligning with the latest WHO guidelines, and highlighting the pivotal role of molecular markers in diagnostic processes [9].

Despite its strengths, our study faces some constraints, such as a relatively small sample size and a case-control design relying on data from a single tertiary hospital which may limit the generalizability of the findings to a broader population. Given their often-retrospective nature, case-control studies can identify correlation between exposures and outcomes; however, they fall short of establishing causation due to the absence of a follow-up period. Another limitation of our study is the absence of sample size calculations, as we included all GBM patients who underwent DNA sequencing within the specified period at our hospital, which may affect the generalizability and statistical power of our findings. Future prospective studies could involve larger, multi-center studies, incorporation of advanced technologies like next-generation sequencing, functional analyses of genetic markers, and exploration of GBM in diverse populations are warranted to better understand the disease. Our findings hold clinical promise for the development of personalized diagnostic and treatment strategies, emphasizing the evolving landscape of cancer research and the potential for targeted interventions to improve overall survival outcomes for GBM patients.

Data availability

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35323.

CRediT authorship contribution statement

Sohaib M. Al-khatib: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ayah N. Al-Bzour:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Mohammad N. Almajali:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Tariq A. Jarrad:** Writing – original draft, Software, Formal analysis, Data curation. **Laith N. AL-Eitan:** Writing – original draft, Methodology, Investigation, Data curation. **Nour Abdo:** Writing – original draft, Investigation, Formal analysis.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35323.

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