European Review for Medical and Pharmacological Sciences 2023; 27: 8110-8118

Concurrent presence of diabetes affects the *GLUT3* programming of glucose metabolism in glioblastoma

A.A. KOCAELI[1], C. TEKIN[2], M. ERCELIK[2], G. TEZCAN[3], S.A. AKSOY[4], H. KOCAELI[5], A. BEKAR[5], M.O. TASKAPILIOGLU[5], S. TOLUNAY[6], B. TUNCA[2]

[1]Department of Endocrinology, Bursa State Hospital, Bursa, Turkey
[2]Department of Medical Biology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[3]Department of Fundamental Sciences, Faculty of Dentistry, Bursa Uludağ University, Bursa, Turkey
[4]Inegol Vocation School, Bursa Uludag University, Bursa, Turkey
[5]Department of Neurosurgery, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey
[6]Department of Pathology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

Abstract. – OBJECTIVE: Diabetes mellitus (DM)-mediated impaired glucose metabolism increase in the glioblastoma (GB) risk by inducing hyperglycemia and hyperinsulinemia. An integral membrane transport protein, glucose transporter 3 (*GLUT3*) facilitates glucose transport into GB tumor cells. We aimed to explore the regulation of *GLUT3* in GB tumors of patients who were concurrently diagnosed with DM.

PATIENTS AND METHODS: Formalin-fixed paraffin-embedded (FFPE) tumor samples were collected from 93 GB patients and retrospectively analyzed. Of the total, 15 patients were concurrently diagnosed with DM (GB-DM). The role of *GLUT3* in tumor aggressiveness was evaluated by analyzing its correlation with Ki67, P53 expression, *MALAT1* expression, and peripheral blood hemoglobin A1C (HbA1c) level. T98G cells were treated with empagliflozin and metformin to modulate *GLUT3*. The RNA expression of *GLUT3*, SOX2, and *MALAT1* was analyzed by real-time qPCR. The lactate levels of T98G cells were measured by Cobas c502 analyzer. A scratch wound assay was performed to investigate the migration rate of T98G cells.

RESULTS: *GLUT3* expression was lower in GB-DM tumors than in GB-only tumors. In GB-DM, the expression of tumoral *GLUT3* and peripheral blood glycated hemoglobin (HbA1c) levels were negatively correlated with P53 and Ki67. A decreased *GLUT3* shortened the disease-free survival duration in GB-DM patients. Empagliflozin reduced *GLUT3*, while metformin-induced *GLUT3* in T98G cells. The empagliflozin-mediated *GLUT3* suppression induced SOX2 and *MALAT1* expressions and influenced the migration capacity of T98G cells.

CONCLUSIONS: Our findings suggest that the low *GLUT3* expression of the tumors of GB-DM patients may induce the production of adenosine triphosphate (ATP) from cellular energy sources other than glucose metabolism. However, further studies are warranted to confirm these results.

Key Words:
Diabetes mellitus, Glioblastoma, HbA1c, Glucose metabolism, *GLUT3*.

Introduction

Diabetes mellitus (DM) increases the cancer risk and affects prognosis1. Evidence suggests several interlinks between DM and cancer[1-5]. In addition, hyperglycemia and hyperinsulinemia caused by DM have been reported to provoke the transformation into cancer cells[6]. Glioblastoma (GB), the most common malignant primary glioma of the central nervous system[7], is a cancer type wherein comorbidity such as DM commonly affects the patient's survival[6].

The adenosine triphosphate (ATP) production of cells is vital to several essential cellular processes and requires glucose metabolization[8]. In DM, glucose metabolism is commonly impaired[9]. In addition, excessive glucose concentrations due to an imbalance in glucose metabolism could provoke invasiveness and chemoresistance of GB tumors[10]. A family of integral membrane transport proteins, the glucose transporter (GLUT) s, facilitates glucose transport into the cells[11]. *GLUT3* is expressed explicitly by neurons and recognized as the neuronal GLUT among these proteins[12], playing a role in brain glucose homeostasis and function[13]. In GB, aggressive features of tumors have been linked to the overexpression of

Corresponding Authors: B. Tunca, Ph.D; e-mail: btunca@uludag.edu.tr; G. Tezcan, Ph.D; e-mail: gulcintezcan@uludag.edu.tr

GLUT3[10,14]. In contrast, it has been reported to be downregulated in DM[15]. Therefore, the expression and function of *GLUT3* in tumors of GB patients who also suffer from DM remain controversial.

In this study, we have described the regulation of *GLUT3* in GB tumors in patients who also have DM and its potential effect on tumor progression. In addition, we uncovered the role of *GLUT3* on GB cell aggressiveness by pharmacologically modulating its expression.

Patients and Methods

Patients

This study retrospectively assessed the formalin-fixed paraffin-embedded (FFPE) tumor tissue samples of 93 GB patients (56 male, 37 female). Fifteen of these patients (10 male, 5 female) were also diagnosed with type 2 DM (GB-DM) during the GB treatment. The diagnosis of DM was made in accordance with the standards of the American Diabetes Association for medical care in diabetes[16]. The Ki67 and P53 expressions were sourced from the pathology records to classify the tumor samples based on their aggressiveness. Long non-coding RNA (lncRNA) Metastasis Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) expression of the samples was determined as described in our previous study[17].

In this study, we did not include patients who received neoadjuvant chemoradiotherapy, those previously treated for other cancers, or those with a family history of cancer. The 85-month follow-up data of the patients were subjected to survival analyses. This study was approved by the Local Ethics Committee of Bursa Uludag University (approval number 2017-13/98).

Cell Lines and Reagents

T98G, a GB cell line, was obtained from the American Type Culture Collection (Rockville, MD, USA). Cells (passage 15) were grown in Dulbecco's Modified Eagle's Medium-F12 (DMEM/F12; HyClone, Logan, UT, USA), supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), L-glutamine (2 mM, BIOCHROME, Rahel-Hirsch, Berlin, Germany), and sodium pyruvate (1 mM, BIOCHROME, Rahel-Hirsch, Berlin, Germany) in a 5% CO2 incubator at 37°C.

Empagliflozin was purchased from Boehringer Ingelheim (500 nM, Biberach, Baden-Württemberg, Germany), while metformin was obtained from Bilim Pharmaceuticals (6 mM, Beyoglu, Istanbul, Turkey).

Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

A commercial RNA Isolation Kit was used to extract the total RNA from FFPE tumor samples (Zymo Research, Orange, CA, USA), and the total RNA of cells was extracted with a trizol reagent (Invitrogen, Carlsbad, CA, USA) as previously described[18]. A DU 730 spectrophotometer measured the RNA concentrations and quality (260 nm:280 nm; Beckman Coulter, Inc., Brea, CA, USA). Total RNA (500 ng) was reverse transcribed to cDNA according to the instructions of ProtoScript M-MuLV First Strand cDNA Synthesis Kit (New England Biolabs; Ipswich, MA, USA). The RNA expression level of *GLUT3, SOX2*, and *MALAT1* was determined according to the protocol of TaqMan™ Gene Expression Assays by using the ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The expression of GAPDH was used for normalization, and the relative quantification of *GLUT3* RNA was calculated by using the $2^{-\Delta\Delta Ct}$ method.

Lactic Acid Measurement

The continued medium of empagliflozin- and metformin-treated T98G cells were collected, and the lactic acid level was measured on the Cobas c502 instrument by using an enzymatic assay according to the manufacturer's instruction (Lact2; Roche Diagnostics, IN, USA).

Scratch Wound-Healing Assay

A confluent monolayer of T98G cells was scratched by dragging a 100-µL pipette tip across the thin membrane. The wounded monolayers were cultured for 24 h in empagliflozin and metformin. The image of the injured area of the monolayers was captured at 0 h and 24 h. The size of the wounded area was measured with the ImageJ v1.53s (National Institutes of Health, Bethesda, MD, USA). Each experiment was performed in duplicates.

Statistical Analysis

The difference in the RNA expression of *GLUT3* between GB-only and GB-DM was performed using an independent sample *t*-test. Pearson's correlation analysis was performed to determine the association of *GLUT3* RNA expression between the RNA expression of lncRNA *MALAT1*, the protein expression of Ki67 and P53, and peripheral blood hemoglobin A1C (HbA1c) levels. A Kaplan-Meier analysis and a log-rank test were performed to identify the effect of *GLUT3* expression on the survival of GB-only and GB-DM patients.

One-way analysis of variance with Tukey's post-hoc analysis was performed to analyze the RNA expression of *GLUT3, SOX2*, and *MALAT1*, the lactic acid production, and the scratch wound-healing assays after empagliflozin and metformin treatments of T98G cells. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 28.0; IBM Corp., Armonk, NY, USA). Statistical significance was defined in a 95% confidence interval, and $p < 0.05$ was considered to indicate statistical significance.

Results

Patients' Characteristics

The age of patients at the time of GB diagnosis was similar between the GB-only and GB-DM patients ($t = -1.73$; $p = 0.087$). The mean age of these patients was 58.17 ± 1.30 and 63.26 ± 1.74 years, respectively. In 10 GB-only and 4 GB-DM patients, the primary tumors were localized in the parietal region of the brain. In addition, the tumor was detected in the frontal region in 25 GB-only and 5 GB-DM patients; in the temporal region in 23 GB-only and 3 GB-DM patients; in the occipital region in 19 GB-only and one GB-DM patients; the insular region in one GB-only and one GB-DM patients; in the cerebellum in one GB-DM patient.

GLUT3 Expression was Elevated in Tumors of GB-Only Patients, while it was Weakly Induced in GB-DM

The RNA expression of *GLUT3* was lower in the tumors of GB-DM patients than in those of GB-only patients ($t = 2.360$; $p = 0.023$; Figure 1A). In addition, in GB-DM patients, the reduced *GLUT3* expression was correlated with elevated HbA1c levels ($r = -0.886$; $p < 0.001$; Figure 1B).

DM-Reduced GLUT3 RNA Promotes GB Tumor Aggressiveness

In GB-only tumors, the induced *GLUT3* RNA expression was not correlated with the *MALAT1* lncRNA ($p = 0.142$), Ki67 ($p = 0.759$), or P53 ($p = 0.246$) protein expressions. In addition, no association was detected between the *GLUT3* RNA expression and tumor size in GB-only tumors ($p = 0.948$). In contrast, the reduced RNA expression of *GLUT3* was correlated with induced *MALAT1* lncRNA ($r = -0.620$; $p = 0.014$; Figure 2A) and Ki67 ($r = -0.902$; $p < 0.001$; Figure 2B) protein expressions of GB-DM tumors.

In addition, the mean disease-free survival of GB-only patients was shortened depending on the high *GLUT3* RNA expressions (GB-only-*GLUT3*^{high}: 11.64 ± 1.44 months; GB-only-*GLUT3*^{low}: 36.16 ± 15.31 months; Figure 2C). Conversely, low *GLUT3* RNA expression lessened the mean disease-free survival in GB-DM patients (GB-DM-*GLUT3*^{high}: 18.14 ± 4.13 months; GB-DM-*GLUT3*^{low}: 5.62 ± 1.59 month; Figure 2D).

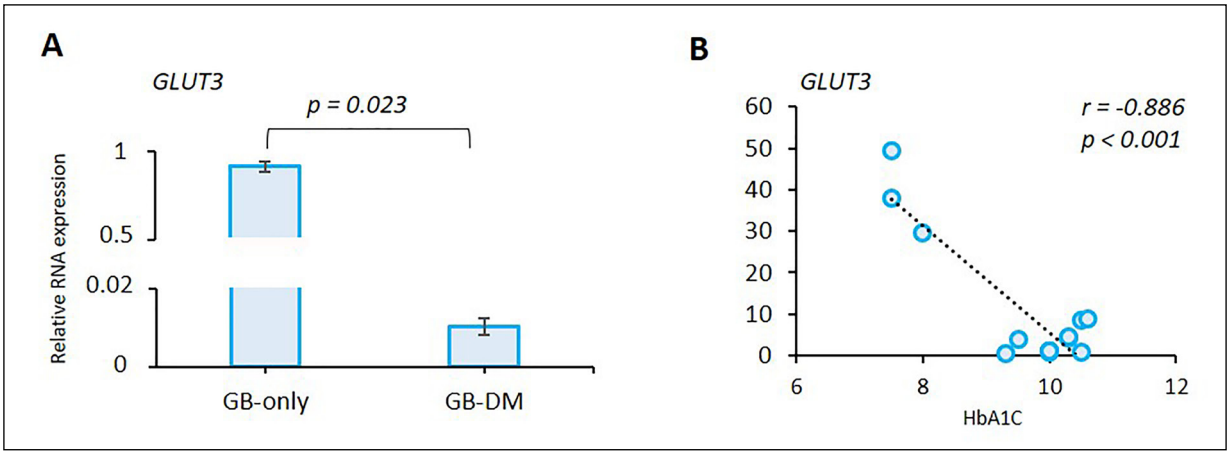

Figure 1. *GLUT3* RNA expression in GB-only and GB-DM patients. **A**, The comparison of *GLUT3* RNA between the tumors of GB-only and GB-DM patients. **B**, The correlation between *GLUT3* RNA and blood HbA1C levels in GB-DM patients. Seven DM-GB patients were on intensive insulin therapy; one was on oral antidiabetic medication. Strict glucose control was achieved (HbA1c <7.5) in two of these patients. In addition, in 5 of those patients, diabetic polyneuropathy and peripheral vascular disease were detected, and blood sugar control was poor (HbA1c >9). The three GB-DM patients were newly diagnosed with DM and had not received antidiabetic therapy before GB diagnosis. GB: Glioblastoma; GB-only: Tumor tissue of patients diagnosed with GB but not DM. GB-DM: Glioblastoma together with diabetes mellitus. Statistical significance was calculated using an independent sample *t*-test for A and B and Pearson's correlation analyses for C. *p*-values less than 0.05 were considered significant.

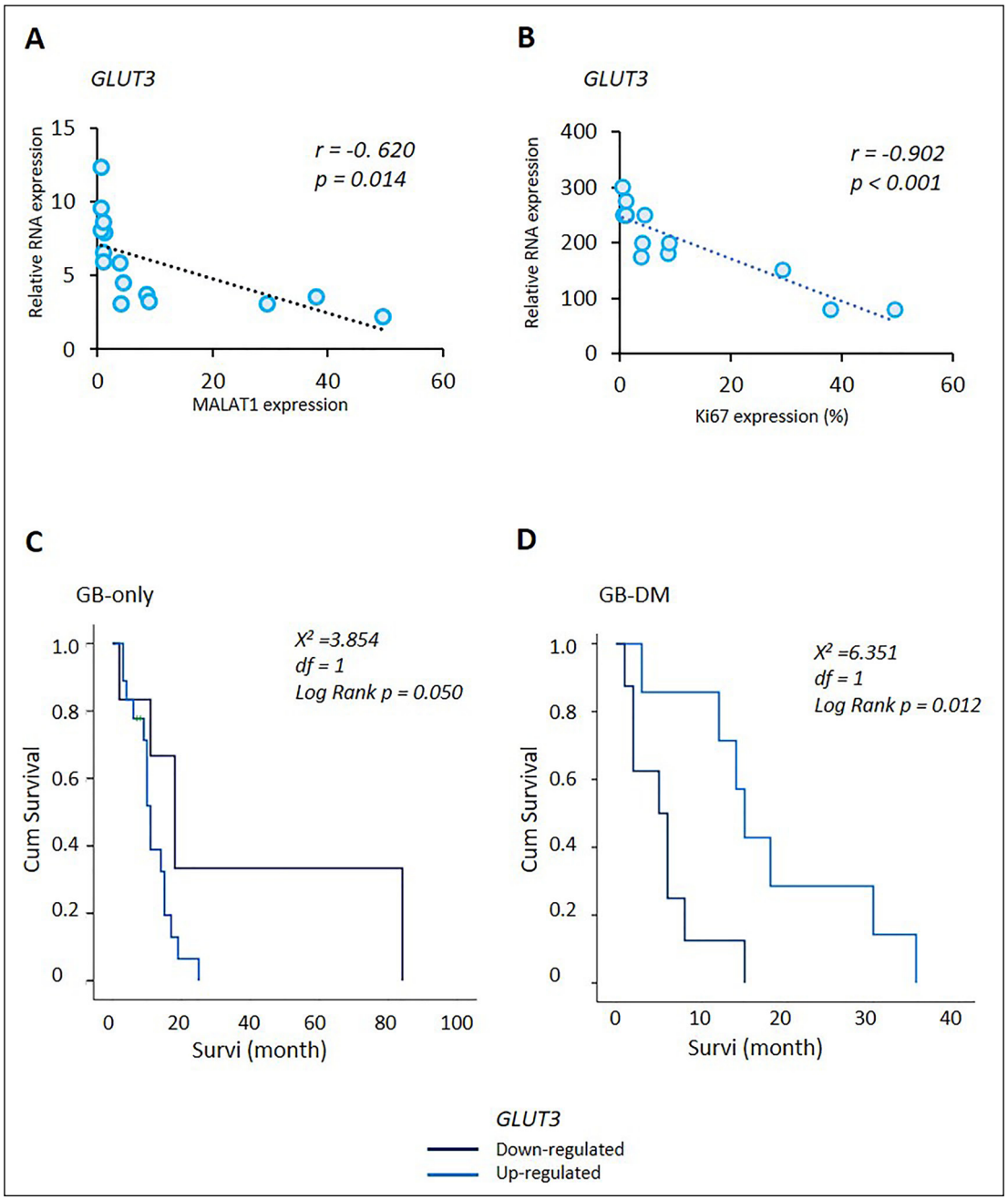

Figure 2. DM-reduced *GLUT3* induced GB tumor aggressiveness. The correlation of *GLUT3* RNA with *MALAT1* LncRNA (**A**) and Ki67 protein expression (**B**). **C-D**, The effect of *GLUT3* on disease-free survival of GB-only and GB-DM patients. GB-only: Tumor tissue of patients diagnosed with GB but not DM. GB-DM: Glioblastoma together with diabetes mellitus. *p*-values were calculated using Pearson's correlation analyses for (**A**) and (**B**) and Kaplan-Meier log-rank test for (**C**) and (**D**). *p*-values lower than 0.05 were considered significant.

The Pharmacological RNA Suppression of GLUT3 Enhances Stem-Like GB Cell Phenotype In Vitro

A GB cell line, T98G, was treated with a sodium-glucose cotransporter 2 (SGLT2) inhibitor, empagliflozin, and an insulin regulator, metformin, to confirm the role of glucose transport and metabolism in GB cells. Our findings revealed that, while *GLUT3* RNA expression was reduced by empagliflozin ($p < 0.001$), it was induced by

metformin ($p < 0.001$) in T98G cells when compared to that in the untreated cells (Figure 3A). The RNA expression of a stem-like cancer cell gene *SOX2* and lncRNA, *MALAT1*, was higher in *GLUT3*-suppressed cells by empagliflozin than in the untreated cells ($p < 0.001$, Figure 3B-C). In contrast, in T98G cells with metformin-induced *GLUT3*, the *SOX2* RNA was reduced ($p < 0.001$), and *MALAT1* was unaffected when compared to that in untreated cells (Figure 3B-E). In line with this, empagliflozin induced lactic acid production ($p = 0.005$). However, metformin did not affect the level of lactic acid compared to that in the untreated T98G cells. A wound-healing assay revealed the effect of empagliflozin suppression and metformin induction of *GLUT3* on the T98G cell migration rate. These findings suggest that the migration rate of empagliflozin-treated cells was almost similar to those in the untreated cells in 24 h ($p > 0.05$), while metformin reduced the migration capacity ($p = 0.049$). These findings suggest that suppressing *GLUT3* can induce lactate metabolism and aggressive tumor features, including stem-like cell phenotype and the migration ability of T98G cells.

Discussion

GB cells undergo aerobic glycolysis to produce ATP to support their growth, which paves the way for a microenvironment that promotes metastasis due to the conversion of glucose to lactate, as explained by the Warburg effect – a phenomenal transformation of glucose to lactate in an anaerobic microenvironment of tumor cells[19]. Therefore, elevated blood glucose level is recognized as one of the risk factors for GB[20]. *GLUT3* supports aerobic glycolysis by glucose uptake in astrocytes and its transport across the

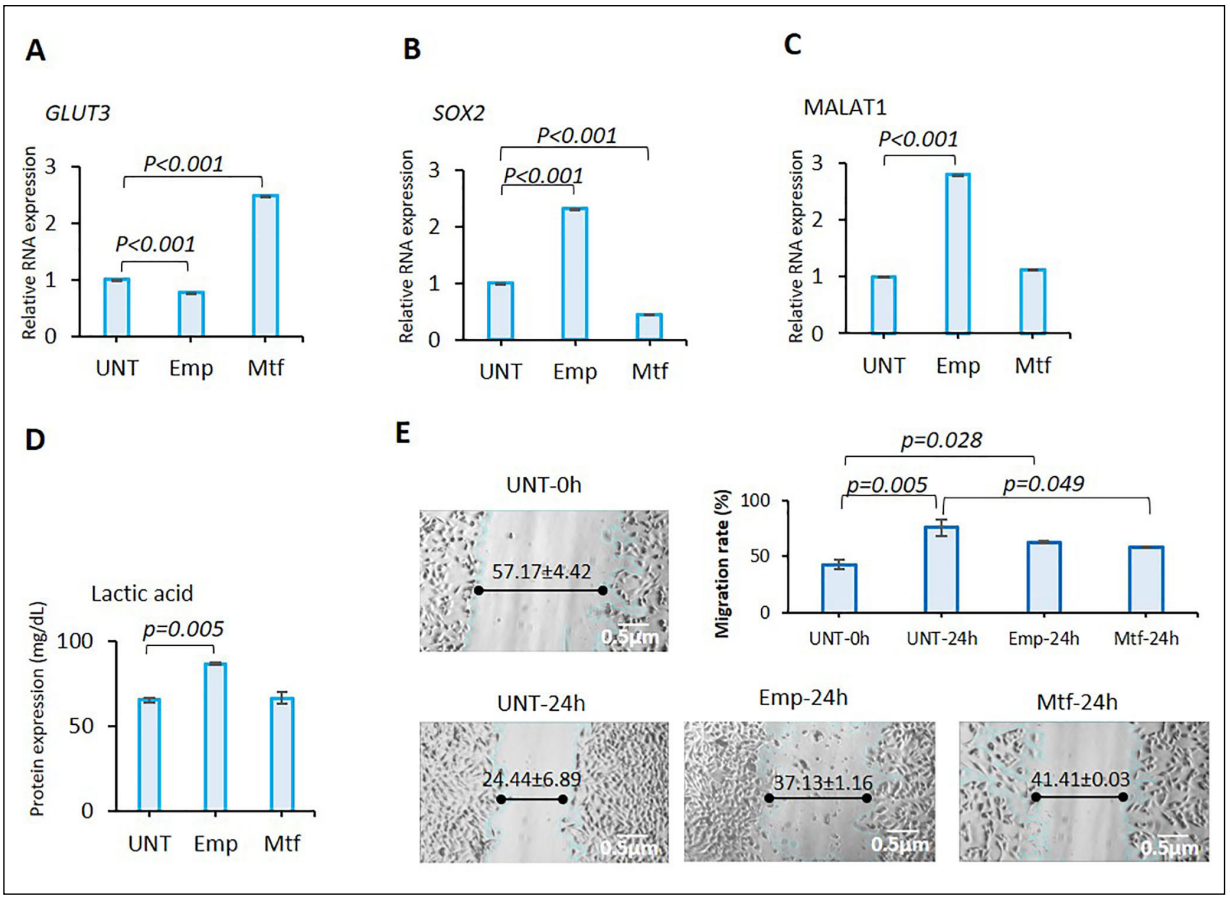

Figure 3. The *in-vitro* effect of *GLUT3* induction and suppression on T98G cells. The RNA expression of (**A**) *GLUT3*, (**B**) SOX2 and (**C**) *MALAT1* upon empagliflozin (500 nM) and metformin (6 mM) treatments for 24 h. **D**, The effect of empagliflozin and metformin on lactic acid production and (**E**) cell migration rate of T98G cells. All analyses were performed in three technical repeats. *p*-values were calculated using One-Way ANOVA and Tukey's test. *p*-values lower than 0.05 were considered significant. UNT: Untreated, Emp: empagliflozin, Mtf: Metformin.

blood – brain barrier[21,22]. *GLUT3* has been shown to be highly expressed in high-grade gliomas, suggesting its predominant role in glucose transport in GB tumors[14]. Furthermore, our findings showed an increased RNA expression of *GLUT3* in GB tumors relative to that in undiagnosed tissues at their surgical margins. High glucose concentrations have been shown to promote the expression of the aggressive features of GB cells, including enhanced proliferation and colony formation *in vitro*[10]. This effect was suggested by inducing the function of the G-protein-coupled chemoattractant receptor and epidermal growth factor[23]. Similarly, our data showed reduced lifespan in GB patients with high *GLUT3*-expressing tumors. Although these tumors had high Ki-67 index and elevated P53 expressions, indicating tumor growth[24,25], our findings did not determine a linear correlation between them. A previous study suggested that the role of *GLUT3* in aerobic glycolysis could be independent of P53[26]. In addition, while *GLUT3* RNA expression was analyzed in an equal amount of RNA for each sample, the sectioned FFPE tissues' heterogeneity in tumor cell density may have hindered a possible linear correlation between *GLUT3* and Ki67 or P53 in this study.

DM contributes to GB progression by impairing energy metabolism through the expression of changing glucose transporters[27,28]. The glucose transporters, including *GLUT3*, were downregulated in DM patients' blood leukocytes[15]. Supporting this observation, our findings indicated a negative correlation between *GLUT3* and HbA1c levels in GB-DM patients. In addition, we detected a lower *GLUT3* expression of GB tumors in GB-DM patients, suggesting the adverse effect of DM in brain cells resulting from impaired glucose transport mechanisms. A loss of *GLUT3* was meant to lead to a glucose deficit, which may cause the halting of ATP-dependent cellular processes in the brain cells[29].

In contrast, tumor cells metabolize lactate, glutamine, and fatty acids as alternative nutrients to produce energy in glucose-deprivation conditions[30,31] and cause lactic acidosis, which, in turn, generates a novel way for cancer survival[32]. To support this hypothesis, we reduced the expression of *GLUT3* by using a SGLT2 inhibitor empagliflozin[33], and induced it *via* a modulator of glucose uptake, metformin[34,35]. Our findings implicate that the empagliflozin mediated a reduction of *GLUT3* induced the lactic acid production of T98G cells, while metformin did not. These findings suggest that a decreased *GLUT3*-mediated glucose starvation could result in a similar scenario caused by the Warburg effect through the production of lactic acidosis[30]. A past study[36] showed that insufficient nutrition could promote the Warburg effect by producing reactive oxygen species and inducing the phosphorylation of AMP-activated protein kinase. Lactic acidosis leads to the development of an acidified microenvironment that provides an environment conducive to invasion and metastasis[37]. Indeed, in our study, the loss of *GLUT3* in tumors of GB-DM patients was correlated with enhanced tumor Ki67 index and a shortened disease-free life expectancy. In addition, empagliflozin suppression of *GLUT3* failed to attenuate the migration rate of T98G cells, although it was reduced by metformin, suggesting that although the tumors of GB-DM patients have a low capacity for cellular glucose uptake, they could maintain their energy metabolism to survive and continue growing.

P38 mitogen-activated protein kinase (P38-MAPK) signaling participates in DM pathogenesis as a result of an excessive caloric intake[38]. Therefore, P38-MAPK signaling inhibitors are used to target the Warburg effect[39]. P38-MAPK has been shown to be regulated by non-coding RNA[40], for instance, an abnormal expression of a MAPK targeting microRNA (miRNA), miR-362, promotes DM[41]. Moreover, the replacement of miR-362 inhibited cell growth and metastasis in GB[40]. Similar to miRNA, lncRNA plays a role in the maintenance of glucose metabolism by directly or indirectly affecting the Warburg effect[42]. Glucose has been reported to induce MAPK targeting lncRNA *MALAT1* in the serum of DM patients[43,44]. In addition, high *MALAT1* expression has been linked to augmented production of inflammatory cytokines in diabetic retinopathy[45]. Moreover, *MALAT1* was demonstrated to regulate cancer glucose metabolism by enhancing glycolysis[46], aggravating glucose deprivation-induced neuronal endoplasmic reticulum stress[47], as well as inducing severe metabolic acidosis[48]. Our previous study[49] emphasized the overexpression of lncRNA *MALAT1* in GB tumors of GB-DM patients than in those of GB-only patients. In addition, our current findings indicated a correlation between reduced *GLUT3* expression and a high level of *MALAT1* in GB-DM patients. Furthermore, the *in vitro* suppression of *GLUT3* by empagliflozin induced the expression of *MALAT1*. Past studies[50,51] have suggested that *MALAT1* induces GB cell aggressiveness by

regulating *SOX2* to promote the stem-like cancer cell phenotype of GB cells, promoting cancer cell aggressiveness. Supporting this finding, we found that empagliflozin not only induced *MALAT1* but also elevated the expression of *SOX2*. Therefore, our results suggest that the aggressive tumor phenotype of GB-DM patients can be explained by low *GLUT3* expression of GB cells, which could maintain the tumor microenvironment to facilitate an aggressive phenotype by the overexpression of *MALAT1*.

Conclusions

Our preliminary findings revealed that GB and GB-DM patients' tumors adopt different metabolic mechanisms to provide ATP for tumor growth. While the tumors of GB-only patients provide the required energy through high glucose uptake *via GLUT3*, GB-DM patients produce ATP from other cellular energy sources through anaerobic metabolism, which increases lactic acid production. Therefore, DM-related metabolic adaptation of GB tumors to glucose shortage caused by low *GLUT3* expression could exaggerate aggressive GB tumor phenotype by promoting stem-like cancer cell phenotype with an induced *MALAT1* and *SOX2* expression. Considering the rarity of GB-DM patients, further studies are warranted to clarify the energy sources mechanism of GB tumors under low-cellular glucose conditions in a large study cohort as well as to investigate the therapeutic targeting potential of *GLUT3*-mediated glucose shortage to treat DM-induced GB tumors.

Funding
This research was funded by the Scientific and Technological Research Council of Turkey (TUBITAK, grant 218S891).

Authors' Contributions
Conception and design of the study: Berrin Tunca, Gulcin Tezcan, Ahmet Bekar, Hasan Kocaeli, Mevlut Ozgur Taskapilioglu. Acquisition of data: Aysen Akkurt Kocaeli, Hasan Kocaeli, Ahmet Bekar, Mevlut Ozgur Taskapilioglu, Sahsine Tolunay. Analysis: Aysen Akkurt Kocaeli; Cagla Tekin, Melis Ercelik, Secil Ak Aksoy. Interpretation of data: Aysen Akkurt Kocaeli. Drafting the article: Aysen Akkurt Kocaeli, Gulcin Tezcan. Critical revisions: Berrin Tunca, Hasan Kocaeli. Supervision: Berrin Tunca, Gulcin Tezcan. Validation: Cagla Tekin, Melis Ercelik, Secil Ak Aksoy. Final approval of the version of the article to be published: Berrin Tunca, Hasan Kocaeli.

ORCID ID
Aysen Akkurt Kocaeli: 0000-0001-7604-0605
Cagla Tekin: 0000-0002-2568-3667
Melis Ercelik: 0000-0003-0366-2424
Gulcin Tezcan: 0000-0002-5956-8755
Secil Ak Aksoy: 0000-0002-3760-9755
Hasan Kocaeli: 0000-0003-4140-5955
Ahmet Bekar: 0000-0002-2716-1985
Mevlüt Özgür Taşkapılıoğlu: 0000-0001-5472-9065
Şahsine Tolunay: 0000-0002-9038-0515
Berrin Tunca: 0000-0002-1619-6680

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
All data generated or analyzed during this study are included in this published article. The data supporting this study's findings are available from the corresponding author upon request.

Ethics Approval
This study was approved by the Local Ethics Committee of Bursa Uludag University (approval number 2017-13/98).

References

1) Rahman I, Athar MT, Islam M. Type 2 Diabetes, Obesity, and Cancer Share Some Common and Critical Pathways. Front Oncol 2021; 20: 10: 600824.
2) Alfarouk KO, Bashir AH. Diabetes mellitus type 2 through oncology lens. Med Hypotheses 2011; 76; 761-762.
3) Murea M, Ma L, Freedman BI. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. Rev Diabet Stud 2012; 9: 6-22.
4) Klil-Drori AJ, Azoulay L, Pollak MN. Cancer, obesity, diabetes, and antidiabetic drugs: is the fog clearing? Nat Rev Clin Oncol 2017; 14: 85-99.
5) Divella R, De Luca R, Abbate I, Naglieri E, Daniele A. Obesity and cancer: the role of adipose tissue and adipo-cytokines-induced chronic inflammation. J Cancer 2016; 26: 2346-2359.
6) Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer 2009; 16: 1103-1123.
7) Adamson DC, Rasheed BA, McLendon RE, Bigner DD. Central nervous system. Cancer Biomark 2010; 9: 193-210.
8) Dunn J, Grider MH. Physiology, Adenosine Triphosphate, Treasure Island (FL): StatPearls Publishing, 2022
9) Yiu KH, Tse HF. Specific role of impaired glucose metabolism and diabetes mellitus in endothelial progenitor cell characteristics and function. Arterioscler Thromb Vasc Biol 2014; 34: 1136-1143.

10) Bielecka-Wajdman AM, Ludyga T, Smyk D, Smyk W, Mularska M, Świderek P, Majewski W, Mullins CS, Linnebacher M, Obuchowicz E. Glucose Influences the Response of Glioblastoma Cells to Temozolomide and Dexamethasone. Cancer Control 2022; 29: 10732748221075468.
11) Simpson IA, Dwyer D, Malide D, Moley KH, Travis A, Vannucci SJ. The facilitative glucose transporter GLUT3: 20 years of distinction. Am J Physiol Endocrinol Metab 2008; 295: E242-E253.
12) Haber RS, Weinstein SP, O'Boyle E, Morgello S. Tissue distribution of the human GLUT3 glucose transporter. Endocrinology 1993; 132: 2538-2543.
13) Shah K, Desilva S, Abbruscato T. The role of glucose transporters in brain disease: diabetes and Alzheimer's Disease. Int J Mol Sci 2012; 13: 12629-12655.
14) Boado RJ, Black KL, Pardridge WM. Gene expression of GLUT3 and GLUT1 glucose transporters in human brain tumors. Brain Res Mol Brain Res 1994; 27: 51-57.
15) Kipmen-Korgun D, Bilmen-Sarikcioglu S, Altunbas H, Demir R, Korgun ET. Type-2 diabetes down-regulates glucose transporter proteins and genes of the human blood leukocytes. Scand J Clin Lab Invest 2009; 69: 350-358.
16) American Diabetes Association. 2. Classification and Diagnosis of Diabetes [published correction appears in Diabetes Care. 2016; 39; 1653. Diabetes Care 2016; 39 Suppl 1: 13-22.
17) Ak Aksoy S, Tunca B, Erçelik M, Tezcan G, Ozturk E, Cecener G, Ugras N, Yilmazlar T, Yerci O. Early-stage colon cancer with high MALAT1 expression is associated with the 5-Fluorouracil resistance and future metastasis. Mol Biol Rep 2022; 49: 11243-11253.
18) Rio DC, Ares M Jr, Hannon GJ, Nilsen TW. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc 2010; 2010: 5439.
19) Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 2008; 13: 472-482.
20) Chaichana KL, McGirt MJ, Woodworth GF, Datoo G, Tamargo RJ, Weingart J, Olivi A, Brem H, Quinones-Hinojosa A. Persistent outpatient hyperglycemia is independently associated with survival, recurrence, and malignant degeneration following surgery for hemispheric low-grade gliomas. Neurol Res 2010; 32: 442-448.
21) Vannucci SJ, Clark RR, Koehler-Stec E, Li K, Smith CB, Davies P, Maher F, Simpson IA. Glucose transporter expression in brain: relationship to cerebral glucose utilization. Dev Neurosci 1998; 20: 369-379.
22) Barron CC, Bilan PJ, Tsakiridis T, Tsiani E. Facilitative glucose transporters: Implications for cancer detection, prognosis, and treatment. Metabolism 2016; 65: 124-139.
23) Bao Z, Chen K, Krepel S, Tang P, Gong W, Zhang M, Liang W, Trivett A, Zhou M, Wang JM. High Glucose Promotes Human Glioblastoma Cell Growth by Increasing the Expression and Function of Chemoattractant and Growth Factor Receptors. Transl Oncol 2019; 12: 1155-1163.
24) Richardsen E, Andersen S, Al-Saad S, Rakaee M, Nordby Y, Pedersen MI, Ness N, Grindstad T, Movik I, Dønnem T, Bremnes R, Busund LT. Evaluation of the proliferation marker Ki-67 in a large prostatectomy cohort. PLoS One 2017; 15: e0186852.
25) Soini Y, Niemelä A, Kamel D, Herva R, Bloigu R, Pääkkö P, Vähäkangas K: p53 immunohistochemical positivity as a prognostic marker in intracranial tumours. APMIS 1994; 102: 786-792.
26) Wang Y, Wu S, Huang C, Li Y, Zhao H, Kasim V. Yin Yang 1 promotes the Warburg effect and tumorigenesis via glucose transporter GLUT3. Cancer Sci 2018; 109: 2423-2434.
27) Hsu CC, Tseng LM, Lee HC. Role of mitochondrial dysfunction in cancer progression. Exp Biol Med (Maywood) 2016; 241: 1281-1295.
28) Koepsell H. Glucose transporters in brain in health and disease. Pflugers Arch - Eur J Physiol 2020; 472: 1299-1343.
29) Iadecola C. Sugar and Alzheimer's disease: a bittersweet truth. Nat Neurosci 2015; 18: 477-478.
30) Pavlova NN, Thompson CB. The Emerging Hallmarks of Cancer Metabolism. Cell Metab 2016; 12: 27-47.
31) Romero-Garcia S, Prado-Garcia H, Valencia-Camargo AD, Alvarez-Pulido A. Lactic Acidosis Promotes Mitochondrial Biogenesis in Lung Adenocarcinoma Cells, Supporting Proliferation Under Normoxia or Survival Under Hypoxia. Front Oncol 2019; 17: 1053.
32) Romero-Garcia S, Lopez-Gonzalez JS, Báez-Viveros JL, Aguilar-Cazares D, Prado-Garcia H. Tumor cell metabolism: an integral view. Cancer Biol Ther 2011; 1: 939-948.
33) Sridhar VS, Fraser DJ. SGLT2 inhibition, glucose transport and peritoneal dialysis: Finding the sweet spot. Perit Dial Int 2023; 43: 115-118.
34) Kumar P, Baquer N. Role of metformin in diabetic aging female rat brain: a future therapy for neurodegenerative diseases [abstract]. Mov Disord 2018: 33 (suppl 2).
35) Nesci V, Russo E, Arcidiacono B, Citraro R, Tallarico M, Constanti A, Brunetti A, De Sarro G, Leo A. Metabolic Alterations Predispose to Seizure Development in High-Fat Diet-Treated Mice: the Role of Metformin. Mol Neurobiol 2020; 57: 4778-4789.
36) Wu CA, Chao Y, Shiah SG, Lin WW. Nutrient deprivation induces the Warburg effect through ROS/AMPK-dependent activation of pyruvate dehydrogenase kinase. Biochim Biophys Acta 2013; 1833: 1147-1156.
37) Choi SY, Collins CC, Gout PW, Wang Y. Cancer-generated lactic acid: a regulatory, immunosuppressive metabolite? J Pathol 2013; 230: 350-355.
38) Bengal E, Aviram S, Hayek T. p38 MAPK in Glucose Metabolism of Skeletal Muscle: Beneficial or Harmful? Int J Mol Sci 2020; 4: 6480.

39) Marquez J, Kratchmarova I, Akimov V, Unda F, Ibarretxe G, Clerigué AS, Osinalde N, Badiola I. NADH dehydrogenase complex I is overexpressed in incipient metastatic murine colon cancer cells. Oncol Rep 2019; 41: 742-752.

40) Shi HZ, Wang DN, Ma LN, Zhu H. MicroRNA-362 inhibits cell growth and metastasis in glioblastoma by targeting MAPK1. MicroRNA-362 inhibits cell growth and metastasis in glioblastoma by targeting MAPK1. Eur Rev Med Pharmacol Sci 2020; 24: 8931-8939.

41) Zhang C, Zhao D. MicroRNA-362-5p promotes the proliferation and inhibits apoptosis of trophoblast cells via targeting glutathione-disulfide reductase. Bioengineered 2021; 12: 2410-2419.

42) Zhang TN, Wang W, Yang N, Huang XM, Liu CF. Regulation of Glucose and Lipid Metabolism by Long Non-coding RNAs: Facts and Research Progress. Front Endocrinol (Lausanne) 2020; 16: 457.

43) Biswas S, Coyle A, Chen S, Gostimir M, Gonder J, Chakrabarti S. Expressions of Serum lncRNAs in Diabetic Retinopathy - A Potential Diagnostic Tool. Front Endocrinol (Lausanne) 2022; 7: 851967.

44) Feichtenschlager V, Zheng YJ, Ho W, Chen L, Callanan C, Chen C, Lee A, Ortiz J, Rappersberger K, Ortiz-Urda S. Deconstructing the role of MALAT1 in MAPK-signaling in melanoma: insights from antisense oligonucleotide treatment. Oncotarget 2023; 26: 543-560.

45) Biswas S, Thomas AA, Chen S, Aref-Eshghi E, Feng B, Gonder J, Sadikovic B, Chakrabarti S. MALAT1: An Epigenetic Regulator of Inflammation in Diabetic Retinopathy. Sci Rep 2018; 8: 6526.

46) Malakar P, Stein I, Saragovi A, Winkler R, Stern-Ginossar N, Berger M, Pikarsky E, Karni R. Long Noncoding RNA MALAT1 Regulates Cancer Glucose Metabolism by Enhancing mTOR-Mediated Translation of TCF7L2. Cancer Res 2019; 15: 2480-2493.

47) Jia Y, Yi L, Li Q, Liu T, Yang S. LncRNA MALAT1 aggravates oxygen-glucose deprivation/reoxygenation-induced neuronal endoplasmic reticulum stress and apoptosis via the miR-195a-5p/HMGA1 axis. Biol Res 2021; 9: 8.

48) Kölling M, Genschel C, Kaucsar T, Hübner A, Rong S, Schmitt R, Sörensen-Zender I, Haddad G, Kistler A, Seeger H, Kielstein JT, Fliser D, Haller H, Wüthrich R, Zörnig M, Thum T, Lorenzen J. Hypoxia-induced long non-coding RNA Malat1 is dispensable for renal ischemia/reperfusion-injury. Sci Rep 2018; 21: 3438.

49) Akkurt Kocaeli A, Ak Aksoy S, Erçelik M, Tezcan G, Tekin C, Kocaeli H, Bekar A, Taskapilioglu MO, Tolunay S, Tunca B. Diabetes mellitus mediated MALAT1 expression induces glioblastoma aggressiveness. Turk Neurosurg 2023. Epub ahead of print. DOI: 10.5137/1019-5149.JTN.41845-22.2.

50) Xiong Z, Wang L, Wang Q, Yuan Y. LncRNA MALAT1/miR-129 axis promotes glioma tumorigenesis by targeting SOX2. J Cell Mol Med 2018; 22: 3929-3940.

51) Baspinar Y, Elmaci I, Ozpinar A, Altinoz MA. Long non-coding RNA MALAT1 as a key target in pathogenesis of glioblastoma. Janus faces or Achilles' heal? Gene 2020; 20: 144518.