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Determination of *PDK1, SLC2A1, EGFR, PTEN, CD276* Gene Expression Levels and *IDH1* Gene R132H Polymorphism in Brain Tumor Tissues

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ABSTRACT

AIM: To determine *IDH1* R132H codon and the mRNA levels of *PDK1, SLC2A1, EGFR, PTEN*, and *CD276* genes in brain tumors.

MATERIAL and METHODS: This study included 15 brain tumor tissues [pituitary adenoma (1), pilocytic astrocytoma (1), mixed meningioma (2), mesothelial meningioma (2), atypical meningioma (1), immature teratoma (1), glioblastoma (4), meningioma (2), and bladder cancer metastasis (1)]. The expression levels of genes in brain tumor tissues were analyzed using real-time PCR. Sanger sequencing was performed to identify the *IDH1* gene R132H codon.

RESULTS: All cases were wild-type in terms of *IDH1* R132H: nucleotide 395 G>A; codon CGT>CAT. The mRNA level of *PDK1* was lower in grade I tumor tissues (0.675-fold) and increased in grades II-III-IV (7.135, 16.912, and 7.081-fold, respectively) ($p<0.001$). The mRNA level of *SLC2A1* decreased in all grades I-II-III-IV [(0.424-, 0.093-, 0.234 ($p<0.001$), and 0.141-fold ($p<0.005$), respectively)]. The mRNA level of *EGFR* increased in all grades I-II-III-IV [1.388, 5.452 ($p<0.017$), 4.624-, and 4.137-fold, respectively]. The mRNA level of *PTEN* increased in grades I-II-III [1.802-, 1.702-, and 1.5-fold, respectively] and decreased in grade IV (0.176-fold). The mRNA level of *CD276* increased in all grades I-II-III-IV [1.8-, 5.756-($p<0.001$), 10.303 ($p<0.001$), and 2.5-fold, respectively].

CONCLUSION: We obtained similar findings for previously reported *PDK1, EGFR, PTEN,* and *CD276* gene expression levels. In contrast, *SLC2A1* expression was markedly downregulated, as reported in other tumor studies. These findings may be due to the unique nature of brain tumor tissues. Additionally, a decrease in *PTEN* gene expression has been observed in grade IV brain tumors, including glioblastoma and meningioma. Although the size of the analyzed study group was limited, the gene expression results showed similarities in the behavior of genes during cancer staging.

KEYWORDS: *IDH1, PDK1, SLC2A1, EGFR*, *PTEN, CD276,* brain tumors

ABBREVIATIONS: CAT: Cytosine Adenine Timin, **CGGA:** Chinese Glioma Genome Atlas, **CGT:** Cytosine Guanine Timin, **CD276:** CD276 molecule (B7-H3: B7 homolog 3), **CNS5:** Classification of Tumors of the Central Nervous System, **DNA:** Deoxyribonucleic acid, **EGFR:** Epidermal growth factor receptor, **ERBB2:** Erb-B2 receptor tyrosine kinase, **IDH1:** Isocitrate dehydrogenase (NADP(+)) 1, **IDH1-WT:** IDH-Wild Type, **IDH1-Mut:** IDH-Mutant, **GLUT1:** Glucose transporter-1, **GBM:** Glioblastoma Multiforme, **G395A:** A change from guanine to adenine at position 395, **GAPDH:** glyceraldehyde-3-phosphate dehydrogenase, **mRNA:** Messenger Ribonucleic Acid, **NADPH:** Nicotinamide adenine dinucleotide phosphate, **PI3K:** Phosphatidylinositol 3 Kinase, **PDK1:** Pyruvate dehydrogenase kinase 1, **PTEN:** Phosphatase and tensin homolog, **RNA:** Ribonucleic acid, **SLC2A1:** Solute Carrier Family 2 Member 1, **TCGA:** The Cancer Genome Atlas Research Network, **TERT:** Telomerase Reverse Transcriptase, **WHO:** World Health Organization

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INTRODUCTION

Gliomas originate from glial cells and are the most prevalent malignant tumors of the central nervous system in humans (28). Among brain tumors, malignant gliomas exhibit an exceptional level of aggression and fatality (20). Glioblastoma is the most frequent and deadly primary brain tumor and is a fast-growing grade IV malignant glioma (31,34,35). Grade I tumors have low proliferative potential, and grade II tumors exhibit a low proliferative index. Additionally, grade III tumors show nuclear atypia and mitotic activity, while grade IV tumors are cytologically malignant. These cells have a high mitotic index and undergo necrosis (31).

IDH1 (isocitrate dehydrogenase (NADP(+)) 1) encodes isocitrate dehydrogenase 1 (17), with the most common *IDH1* mutation being R132H (18). Both the 2016 WHO classification and WHO CNS5 have declared that IDH mutational status should be considered in low-grade glioma, emphasizing IDH-wildtype (IDH-WT) as a critical biomarker of high-risk low-grade glioma because its molecular characteristics and clinical manifestations are similar to those of glioblastoma multiforme (52). The molecular classification of gliomas divides these malignancies primarily into IDH-mutant (IDH-Mut) and IDH-WT tumors (8).

Asif et al. suggested that each tumor has a pathophysiological profile that enables targeted therapies based on a unique proteogenomics-based approach. To some extent, this has been achieved using directed treatment strategies against *EGFR* and *ERBB2* in breast cancer. However, this approach has not been successful in the treatment of glioblastoma (2). Amplification and overexpression of the *EGFR* gene are prominent features of glioblastomas and are present in 40% of such tumors. Brennan et al. identified different proteins in glioblastoma samples and different tumor subsets detected by the *EGFR*-related signaling pathway (5).

Targeting *PDK1* may be a therapeutic strategy that can be combined with conventional targets (46). *PDK1* is highly expressed in human glioblastoma multiforme surgical specimens compared to normal brain tissue (49).

The Warburg effect involves an increase in glucose uptake by cancer cells, and glucose transporter proteins are overexpressed in many tumors (10). Modulation of *GLUT1* trafficking is essential for controlling glucose uptake and has been reported in cancer cells. GLUT1/SLC2A1 may also be associated with glioblastoma prognosis. *GLUT1* is required for glycometabolism in the central nervous system (23).

Seaman et al. showed that *CD276* is widely overexpressed in cancer and tumor vascular cells and that anti-CD276 drug conjugates are promising anticancer reagents. The choice of conjugated drugs is crucial as tumor vascular cells may exhibit resistance to susceptible drugs (41).

In addition, Takashima et al. used TCGA expression profiling of 21 immunosuppressive genes, random forest analyses, and Kaplan-Meier analyses of data from 158 patients and suggested that *CD276* could serve as the sole candidate gene marker (44).

PTEN directly antagonizes PI3K signaling and is one of the most frequently altered genes in cancer (25). TCGA data show that approximately 50% of glioblastomas harbor somatic alterations in the phosphatidylinositol 3-OH kinase pathway (8,36). One of the fundamental regulators of this pathway, which is significantly altered in glioblastomas (30-40%), is the *PTEN* tumor suppressor gene (7,47).

The loss of *PTEN* function is associated with metastasis (56). Different epigenetic, transcriptional, and post-translational mechanisms control the levels and function of *PTEN* (50).

Glioblastoma was systematically studied in detail using TCGA. These studies have shown that glioblastomas have a complex signaling network that is critical for rapid growth and differentiation. This network can adapt to the responses to specific targeted molecular therapies. Therefore, an extensive catalogue of molecular changes is essential. Further research is required to fully understand the molecular changes for glioblastoma (2).

The objective of this study was to analyze the *IDH1* gene R132H codon and the expression levels of *PDK1, SLC2A1, EGFR, PTEN, and CD276* genes in brain tumors.

MATERIAL and METHODS

Samples

Fifteen patients who underwent intracranial mass surgery were recruited from the Department of Neurosurgery, Afyonkarahisar Health Sciences University. The histological diagnosis of the brain tumors was confirmed through routine pathological examination (Table I). This study was approved by the Ethics Commission of Afyonkarahisar Health Sciences University (11.09.2020/421). Informed consent was obtained from all patients.

DNA Extraction from Tumor Samples and Sanger Sequencing Analysis

Genomic DNA was extracted from brain tumor tissues that were not in routine pathological analyses (Invitrogen™ PureLink™, USA). QuantiFluor E6090 (Promega, Madison, WI, USA) was used to detect the purity and amount of DNA.

Mutation Analysis of c.395G>A (R132H) of *IDH1*

Mutation analysis of the *IDH1* gene R132H polymorphism was performed using the Applied Biosystems 3130XL Genetic Analyzer (USA), utilizing genomic DNA isolated from the brain tumors. MyTaq™ HS-DNA-Polymerase kit (Bioline, Meridian Bioscience, Tennessee, USA) was used, and the relevant primers were designed by Sentebiolab (Ankara, Turkey).

RNA Extractions and RT-PCR Analyses

RNA was extracted using PureZole reagent (Bio-Rad, USA). All RNA samples were reverse transcribed into cDNA from 1 µg of total RNA using the iScript Reverse Transcription Supermix (Bio-Rad, USA). *PDK1*, *SLC2A1*, *EGFR, PTEN,* and *CD276* genes were analyzed by Rotor Gene-Q (Qiagen, Hilden, Germany) by using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and oligonucleotide primers

[(Oligomere Biotechnology, Ankara, Turkey) (11,48,58) (Table II)]. *GAPDH* was used as the housekeeping gene for normalization.

Statistical Analysis

Gene expression analysis was performed with REST 2009 V2.0.13 software (37).

Table I: Types and Grades of Brain Tumors

No	Types and Grades of Brain Tumors	Grade
1	Pituitary adenoma (used as control for relative expression analyzes)	I
2	Pilocytic astrocytoma	I
3	Mixed Meningioma	I
4	Meningothelial meningioma	I
5	Mixed Meningioma	I
6	Meningothelial meningioma	I
7	Atypical meningioma	II
8	Immature teratome	III
9	Glioblastoma	IV
10	Glioblastoma	IV
11	Glioblastoma	IV
12	Glioblastoma	IV
13	Meningioma	IV
14	Meningioma	IV
15	Bladder cancer metastasis	IV

Table II: Primer Sequences of the Genes

	Primer Sequences 5'→3'
[redacted]	TGAACTGACCTTGCCACAT
[redacted]	TGAAGCAGCACTGAACACG
[redacted]	GGCCAAGAGTGTGCTAAAGAA
[redacted]	ACAGCGTTGATGCCAGACAG
EGFR-F	AAAGTTAAAATTCCCGTCGCTATCAAG
EGFR-R	TCACGTAGGCTTCATCGAGGATTTC
PTEN-F	TGGATTCGACTTAGACTTGACCT
PTEN-R	GGTGGGTTATGGTCTTCAAAAGG
CD276-F	TCACAGGGCAGCCTATGAC
[redacted]	TCCTCAGCTCCTGCATTCTC
[redacted]	CATTGCCCTCAACGACCACTTT
[redacted]	GGTGGTCCAGGGGTCTTACTCC

RESULTS

Mutation Analysis of c.395G>A (R132H) of *IDH1* (codon CGT>CAT)

Sanger sequencing analysis was performed on 15 patients, all of whom were wild-type in terms of *IDH1* R132H: nucleotide 395 G>A; codon CGT>CAT (Figure 1).

mRNA Analysis of *PDK1, SLC2A1, EGFR, PTEN, and CD276* Genes Expressed in Brain Tumor Tissues

Alterations in the mRNA levels of the *PDK1, SLC2A1, EGFR, PTEN,* and *CD276* genes expressed in brain tumor tissues of patients were determined and compared to those in pituitary adenoma as a control tissue.

PDK1 expression decreased in grade I tumor tissues (0.675-fold) and increased in grade II-II-IV tumors (7.135-, 16,912-, and 7.081-fold, respectively, ($p<0.001$). When comparing grades I and IV, *PDK1* expression increased 6.577-fold ($p<0.002$) in grade IV (fold-changes are at Log10 level). *SLC2A1* gene decreased in all grades I-II-III-IV (0.424, 0.093, 0.234 ($p<0.001$), 0.141-fold ($p<0.005$), respectively). When comparing grades I and IV, *SLC2A1* gene expression decreased by 0.333-fold ($p<0.002$) in grade IV tumors. *EGFR* gene expression was increased in all grades I-II-III-IV [1.388, 5.452 ($p<0.017$), 4.624-, and 4.137-fold, respectively]. When comparing grades I and IV, *EGFR* gene expression increased 2.981-fold in grade IV tumors. *PTEN* gene expression increased in grades I, II, and III [1.802-, 1.702-, and 1.5-fold, respectively] and decreased in grade IV (0.176-fold). When comparing grades I and IV, *PTEN* gene expression decreased by 0.25-fold in grade IV tumors. *CD276* expression increased in all grades I-II-III-IV [1.8-, 5.756-($p<0.001$), 10.303 ($p<0.001$), and 2.5-fold, respectively]. When comparing grades I and IV, *CD276* expression increased 2.423-fold in grade IV (Figure 2).

DISCUSSION

Parsons et al. reported that *IDH1* was somatically mutated in glioblastoma multiforme (GBM) tumors in a discovery screen. Several crucial observations have been made regarding *IDH1* mutations. Patients with *IDH1* mutations had a significantly improved prognosis, with a median overall survival of 3.8 years compared to 1.1 years for patients with *IDH1*-WT (36). SongTao et al. suggested that in patients with glioblastoma, these mutations improved the intracellular response to temozolomide compared to cases with the *IDH1-WT* gene (42). The *IDH1*-mutation has an association with improved overall survival than *IDH1*-WT (51). In our study, all the patients harbored the *IDH1*-WT R132H codon.

PDK1 expression is upregulated in some human cancers (3, 21,24,45,49). Baumunk et al. studied *PDK1* mRNA expression in patients with neck and head carcinomas. *PDK1* gene expression was found to be upregulated in patients compared to normal tissues (3). Additionally, it has been suggested that *PDK1* was a novel target for tumor therapy. Most patients with demonstrated *PDK1* expression exhibit poor clinical outcomes (24). In addition, Liu and Yin reported that ectopic overexpression of *PDK1* promoted cell proliferation and

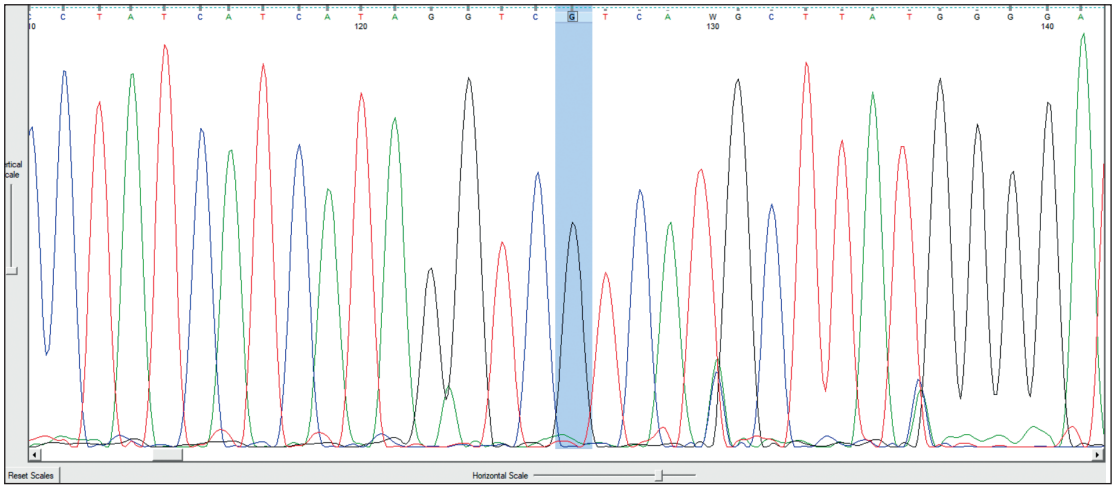

Figure 1: Mutation analysis of c.395G>A (R132H) of *IDH1* (codon CGT>CAT).

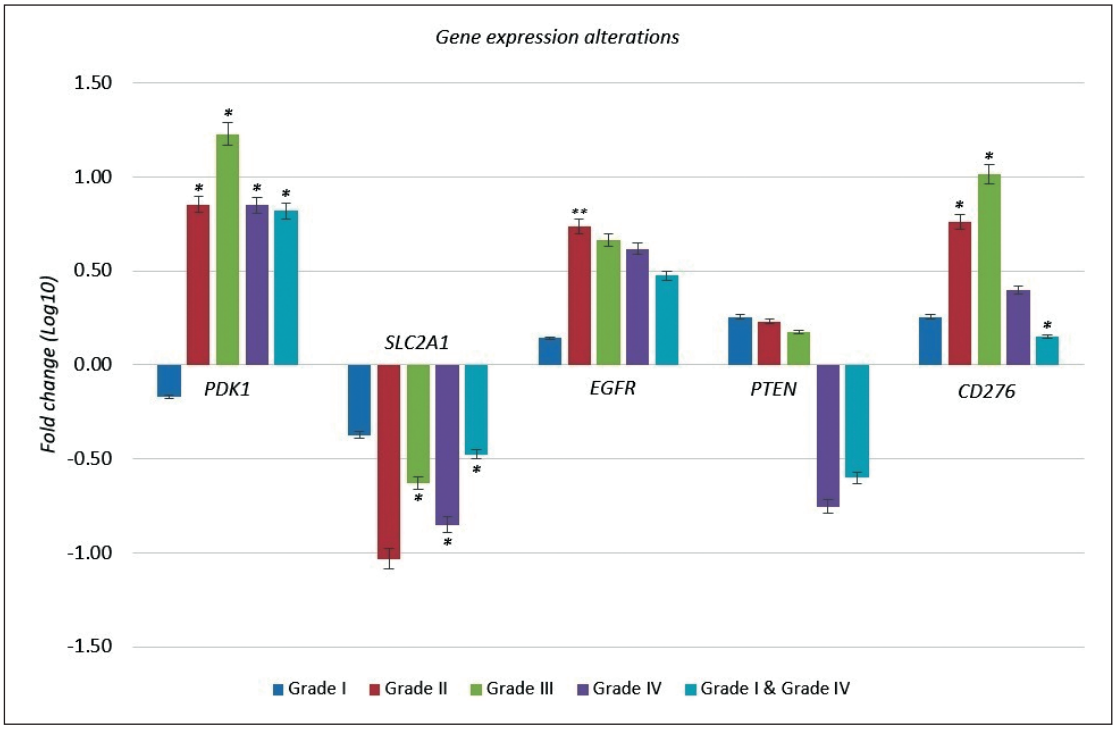

Figure 2: Alterations in *PDK1, SLC2A1, EGFR, PTEN* and *CD276* gene expression in different grades of tumor tissues, as compared to pituitary adenoma tissues used as the control. In the Grade I&Grade IV column, the alterations of related genes in the Grade IV compared to Grade I was shown. *GAPDH* was the reference gene for normalization. *($p<0.005$), **($p<0.02$). (Grade I, II, III, IV, and I and IV, respectively).

inhibited apoptosis (29). Hur et al. reported that *SLC2A1* and *PDK1* expression was significantly associated with tumor progression (21). Velpula et al. suggested that *PDK1* was upregulated in glioblastoma specimens (45). Luo et al. reported that PDK1 protein was significantly upregulated in glioma tissues compared to non-tumorous tissues, and the results indicated that in GBM, *PDK1* functions as an oncogene, promoting proliferation and invasion (30). Similar to the three studies, *PDK1* expression was significantly upregulated in all grades in the present study. In addition, *PDK1* gene expression was significantly upregulated in grade IV brain tumors compared to grade I brain tumors.

The *SLC2A (GLUT)* family includes 14 members from SLC2A1–SLC2A14, known as glucose transporters (9). SLC2A1 has been widely investigated in various cancers (10). The SLC2A family is upregulated in different tumors and exhibits potential oncogenic effect of the SLC2A family (16). *GLUT1* (*SLC2A1*) is overexpressed in various human cancer tissues and is highly expressed in thyroid cancer tissues compared to normal thyroid tissues (32). *SLC2A1* expression is associated with aggressive tumor grade and decreased survival in breast cancer (22,39). SLC2A1 protein is highly expressed in invasive ovarian carcinoma and fallopian tube adenocarcinoma (40), and its association with hepatocellular carcinoma and non–small cell lung cancer has also been reported (1,54). In contrast, we observed a significant downregulation in the brain tissues of our study group. These results conflict with the previous findings. These findings may be owing to the unique nature of brain tumor tissues.

Amplification of the *EGFR* gene represents the first significant molecular genetic alteration identified in human gliomas (27). Elevated *EGFR* levels have been observed in many tumors of epithelial origin (13,53). Studies have shown high *EGFR* expression in GBM and neuroblastoma tumor cells (19,33). A large percentage (40-50%) of GMB characteristically exhibits amplification or overexpression of the *EGFR* gene (12,14). Both wild-type and mutated forms can be amplified, and the levels of mRNA and protein expressed at the cell surface are remarkably higher (4). Similarly, *EGRF* gene expression was upregulated in all grades in our study.

PTEN frequently shows genomic deletions in many tumors, such as brain, prostate, and bladder tumors (26,43). Zhou et al. suggested that high PTEN expression is associated with longer survival in patients with glioma (57). Several studies have identified mutations in the *PTEN* gene in various tumors (15,38). *PTEN* gene expression was upregulated in grade I-II-III brain tumors, but not significantly. In contrast, *PTEN* expression was downregulated in grade IV glioblastoma and meningioma.

Specifically, *IDH*-WT glioblastomas usually show high levels of *EGFR* amplification, TERT promoter mutations, and *PTEN* deletion (6). When considered together, we reported that all patients were *IDH*-WT, *PTEN* expression was downregulated in glioblastomas and meningiomas, and *EGFR* was upregulated in all grades in our study.

CD276 is an immune checkpoint molecule that plays a key role in suppressing T-cells in gliomas (59). Overexpression of *CD276* has been associated with a poor prognosis in glioma patients with CGGA (Chinese Glioma Genome Atlas) and TCGA (55). Our findings indicated that the *CD276* gene expression was upregulated in all brain tumor grades.

The main limitations of this study were the sample size and the heterogeneous pathology of the samples. Despite these limitations, the gene expression results showed similar behavior for the cancer staging genes.

CONCLUSION

We analyzed the expression of *PDK1, SLC2A1, EGFR, PTEN, and CD276* genes by real-time PCR. We observed similar findings for previously reported *PDK1, EGFR,* and *CD276* gene expression levels. In contrast, *SLC2A1* gene expression was markedly downregulated, as reported in other tumor studies. These findings may be owing to the unique nature of brain tumor tissues. Additionally, a decrease in *PTEN* gene expression has been observed in grade IV brain tumors, including glioblastoma and meningioma. This study also reported that all brain tumors were *IDH1*-WT. Nevertheless, replication studies with larger case groups are necessary before claiming that these genes are predictive markers for brain tumors.

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AUTHORSHIP CONTRIBUTION

Study conception and design: SK, ESAS, ZS, MS
Data collection: SK, ESAS, ZS
Analysis and interpretation of results: SK, ESAS, ZS
Draft manuscript preparation: SK, ESAS
Critical revision of the article: SK, ESAS
All authors (SK, ESAS, ZS, MS) reviewed the results and approved the final version of the manuscript.

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