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ORIGINAL ARTICLE

Analysis of Isocitrate Dehydrogenase 1 Mutation and 10q23/PTEN Alterations in Turkish Glioblastoma Patients

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Abstract

Introduction: The object of this study is to identify the frequency isocitrate dehydrogenase 1 gene (IDH1) mutations and 10q23/PTEN locus alterations in Turkish patients with glioblastoma multiforme (GBM).

Methods: For this purpose, DNAs obtained from paraffin-embedded archival materials belong to 54 cases diagnosed as GBM were applied direct sequencing. In addition, overall 25 cases and 10 non-neoplastic brain tissues which were used as controls were analyzed by fluorescent in situ hybridization (FISH).

Results: We detected 5 heterozygous IDH1 c.395G>A mutations (9.3%) with wild-type arginine 132 replaced by histidine (R132H). Although there is no statistical significance between the age of the cases and IDH1 p.R132H mutation, the patients harboring the p.R132H mutation were younger (median age; 38) than patients without this mutation (median age; 52). The median survival time of the patients with this mutation was calculated at 7 months, while it was 5 months for the patients with no mutation; however, this finding was not statistically significant. According to our FISH results, we found a total of 16 10q23/PTEN alterations, of which 8 were hemizygous deletion (32%) and 8 were monosomy (32%).

Discussion and Conclusion: Understanding the prevalence of IDH1 mutations and 10q23/PTEN alterations may have importance in terms of the diagnosis, prognosis, and treatment of GBM patients.

Keywords: 10q23/PTEN; Direct sequencing; FISH; Glioblastoma; IDH1.

Glioblastoma multiforme (GBM) derived from glial cells is one of the most frequent and malignant brain tumors classified as grade IV by World Health Organization and accounts for 80% of all primary malignant central nervous system tumors[1,2]. GBM may develop rapidly (de novo or primary GBM) or slowly from low-grade astrocytomas (secondary GBM)[3]. While primary GBM has been seen in older patients, secondary GBM occurs in younger patients (<45 years)[4]. The overall survival time of patients is 12–15 months[2].

Numerous gene mutations and loss of heterozygosity have been described in the genetic etiology studies of GBM[4-6]. More than 20.000 genes in 22 GBMs were sequenced by a collaborative study and for the first time, an isocitrate dehydrogenase 1 gene (*IDH1*) point mutation has been identified in 12% of the samples analyzed[7]. Thereafter, a myriad of studies has documented the prevalence and molecular basis of *IDH1* mutations in GBM[8-10]. *IDH1* mutations are heterozygous, somatic, missense mutations and affect the isocitrate binding region, which is evolutionarily

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conserved residue R132 (arginine)[8]. *IDH1* R132 mutations are frequently seen in secondary glioblastomas and play an important role in the development of high-grade glial tumors[6,11,12]. *IDH1* R132 mutations inactivate the wild-type enzyme activity, which catalyzes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG)[10]. Little is known about how *IDH1* mutations function in tumorigenesis.

The loss of phosphate and tensin homologous gene (*PTEN*) gene found in 10q23 locus of chromosome 10 was another alteration frequently observed in GBMs[13,14]. *PTEN* gene, a tumor suppressor gene, functions in many cellular processes such as apoptosis, cell proliferation, and response to DNA damage[15].

The frequency and type of mutation in a gene provide significant information about the role of the disease[11]. Therefore, identification of *IDH1* mutations and 10q23/*PTEN* gene alterations suggests that these molecular markers may be useful in terms of diagnosis, prognosis, therapeutic strategies, and role in glioma tumorigenesis. The objective of this study was to identify the prevalence of *IDH1* mutations and 10q23/*PTEN* alterations in glioblastomas.

Materials and Methods

Tumor Samples

We examined a total of 54 glioblastoma patients, diagnosed at the Department of Medical Pathology, Faculty of Medicine Karadeniz Technical University. In this study, the classification of the GBM patients was not done as primary or secondary GBM. Only samples with larger than 60% of tumor cells were included in the study. This study was approved by the Ethics Committee of Karadeniz Technical University Hospital.

PCR Amplification

Genomic DNA was extracted from paraffin-embedded archival materials using QIAmp DNA FFPE Tissue Kit (Qiagen, Germany). Exon 4 of *IDH1* gene corresponding to the catalytic domain including codon 132 was amplified by PCR using two different primer pairs: 5′-GAGCTCTATATGCCATCACTGC-3′ (sense 1), 5′-TATGGTGCCATTTGGTGATTTC-3′ (antisense 1), 5′-TGCTGCAGAAGCTATAAAGAAGC-3′ (sense 2), and 5′-TCATACCTTGCTTAATGGGTGTAG-3′ (antisense 2). Briefly, PCR was performed in a total volume of 25 µl consisting of 62.5 ng/µl of DNA, 0,5 U GoTaq Polymerase (Promega), standard buffer conditions with an initial denaturing step 94°C for 4 min followed by 38 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 6 min (ABI PCR system 9700, USA).

Direct Sequencing for *IDH1* Mutations

The PCR products were purified using Nucleospin® Extract II kit (Macherey-Nagel, Germany). Exon 4 of *IDH1* gene was sequenced by ABI 3130 genetic analyzer (Applied Biosystems, USA) with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

Fluorescent *in situ* Hybridization (FISH) Analyses for *PTEN*/10q Deletions

Single-cell suspensions were obtained from paraffin-embedded archival materials belong to 25 glioblastoma cases using ZytoLight FISH-Tissue Implementation Kit (Zytovision, Germany) and analyzed by FISH. *PTEN* deletions were investigated by ZytoLight SPEC *PTEN*/CEN 10 Dual Color probe (Zytovision, Z-2078-200) corresponding to *PTEN* locus. Signals were scored as 200 non-overlapping intact nuclei. Specimens were observed with an Epi Fluorescent microscope (Nikon E-800, Japan) equipped with the appropriate filters. 10 non-neoplastic brain tissue specimens were used as control (cutoff value) for the probe. Cutoff value was evaluated as 9.6 for 10q23/*PTEN* hemizygous deletion, 6.8 for 10q23/*PTEN* monosomy.

Statistical Analysis

All statistical analyses were performed with GraphPad Prism 8 software. $p<0.05$ were considered statistically significant. Mann–Whitney U test was carried out to analyze the association between *IDH1* mutations and the age of the patients. Kaplan–Meier (log-rank test) method was used for survival analysis.

Results

IDH1 Mutations in Glioblastomas

We studied a total of 54 glioblastomas and heterozygous alterations of *IDH1* were found in 11 tumors. 6 of them corresponded to known SNPs c.315C>T (rs11554137). 5 of them (5/54, 9.3%) were single base substitutions c.395G>A occurring at residue R132, resulting in arginine to histidine substitution (p.R132H) (Fig. 1). Table 1 summarizes clinical characteristic of glioblastomas with and without *IDH1* mutations. The median survival time of GBM patients with *IDH1* mutations (7 months) was longer than those without *IDH1* mutations (5 months) (Fig. 2). Patients carrying *IDH1* mutations were younger (median age 38 years) than patients without *IDH1* mutations (median age: 52 years) (Table 1).

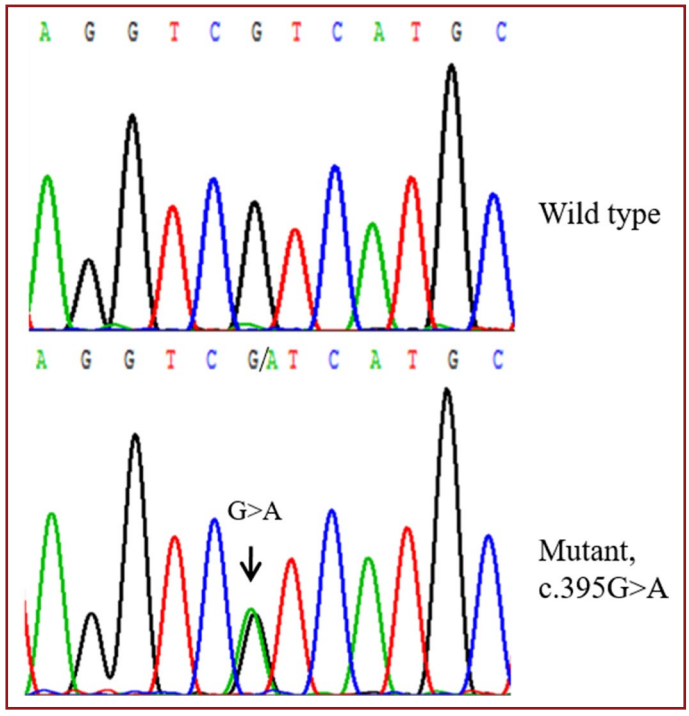

Figure 1. IDH1 mutation in GBM. All mutations were heterozygous c.395G>A and located at codon 132. GBM: Glioblastoma multiforme, IDH1: Isocitrate dehydrogenase 1 gene.

Table 1. Clinical and genetic features of glioblastomas with and without *IDH1* mutations

	GBM with *IDH1* mutation	GBM without *IDH1* mutation
Number of cases	5 cases (9.3%)	49 cases (90.7 %)
Median age	38 years	52 years
Median overall survival	7 months	5 months

GBM: Glioblastoma multiforme; *IDH1*: Isocitrate dehydrogenase 1 gene.

However, there was no significant difference in terms of either age (p=0.078; Mann–Whitney Test) or overall survival (p=0.169, log-rank test) due to a small number of patients with *IDH1* mutations.

10q23/PTEN Alterations in Glioblastomas

All samples showed >90% nuclei with signals. Of 25 glioblastomas analyzed, 16 patients (64%) contained 10q23/*PTEN* alterations. While 8 patients (32%) were hemizygous deletion, 8 of them (32%) were monosomy (Fig. 3). No homozygous deletions were detected in this study.

Discussion

In brain tumors, significant studies have been recently carried out to discover the molecular markers which are associated with diagnosis, treatment, and patient survival. As a result of the comprehensive study conducted by Parsons et al.,[7] *IDH1* mutations were first implicated in young patients, especially secondary GBMs. Subsequent studies have also indicated that *IDH1* mutations are more common in secondary (>80%) compared to primary glioblastomas (<5%). As a result of extensive research, *IDH1* has become an important diagnostic marker for secondary GBMs[3]. However, the role of *IDH1* mutations in cancer remains to be fully elucidated. The *IDH1* enzyme encoded by the *IDH1* gene, which is localized in chromosome 2q33.3, converts the isocitrate to α-KG and provides NADPH production in this reaction[10]. The studies have shown that the vast majority of *IDH1* mutations affect the residue 132 (R132), which is the evolutionarily conserved binding site of the *IDH1* enzyme that plays a key role in cellular metabolic functions, and gain an oncogenic function[8-10,16]. *IDH1* mutations in GBMs (6–30%) are heterozygous and frequently c.C395G>A

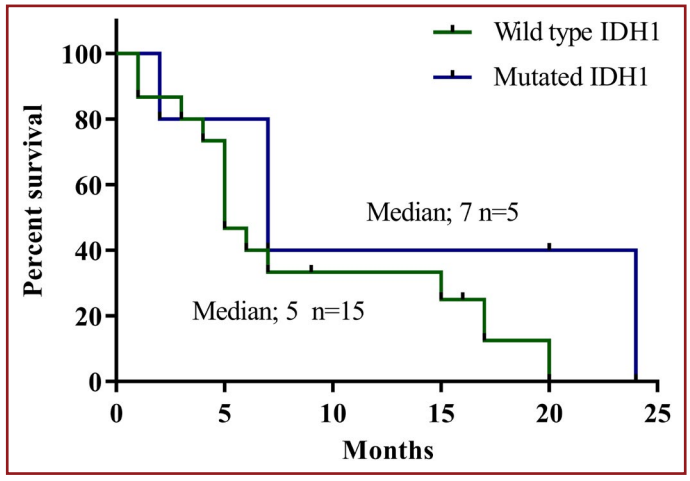

Figure 2. Survival of GBM patients. GBM patients carrying an IDH1 mutation (blue line) had longer overall survival (p=0.169, Log Rank test). GBM: Glioblastoma multiforme, IDH1: Isocitrate dehydrogenase 1 gene.

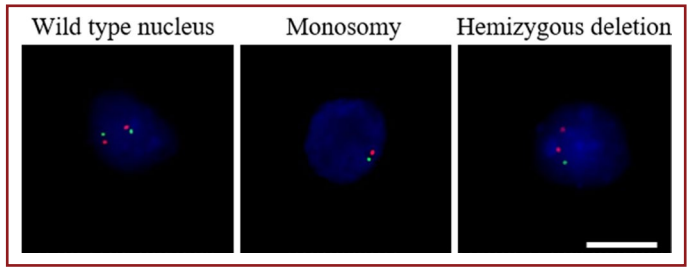

Figure 3. 10q23 PTEN alterations detected by FISH. Wild-type nucleus, monosomy of the chromosome 10, and hemizygous deletion of the 10q23/PTEN locus, respectively. (10q23/PTEN locus; Green, CEN10; Red). Scale bar; 10 µm. PTEN: Phosphate and tensin homologous gene, FISH: Fluorescent in situ hybridization.

(p.R132H) alteration resulting in the replacement of arginine with histidine amino acid[5,7,8,11,17-21]. Other *IDH1* mutations at Arg132 such as R132S, R132C, R132G, and R132L have been seen at lower frequencies[9]. In our study, exon 4 of the *IDH1* gene was sequenced in 54 GBM patients, which were not classified as primary and secondary glioblastoma. We only detected c.395G>A (p.R132H) mutation in five of 54 GBM patients and mutation frequency as 9.3%, which is correlated with the literature.

Genomic studies have identified that GBM patients with *IDH1* mutations exhibit distinct clinical characteristics compared to patients with *IDH1* wild type. Numerous studies showed that the presence of an *IDH1* mutation is associated with longer survival indicating better prognosis and younger age of diagnosis relative to GBMs without this mutation[7,8,12,17,19,21]. In accordance with the literature, in our study, although there is no statistical difference, cases carrying *IDH1* R132H mutation had longer survival (7 months) compared to cases without the mutation (5 months) (p=0.169). In addition, the median age of the patients harboring *IDH1* mutation was 38, while the median age of the patients without *IDH1* mutation was 52 (p=0.078). The lack of statistical significance of the median age and overall survival may be due to a low number of cases included in survival and age analysis in our study.

In the presence of a mutation affecting R132, the wild-type activity of NADP+-dependent enzyme *IDH1*, which catalyzes the oxidative decarboxylation of isocitrate to α-KG to produce NADPH, is decreased[9,10]. One possible reason is the dominant-negative activity of the mutant protein which could form heterodimers with wild-type *IDH1* enzyme, resulting in only 4% of the activity of *IDH1*[10]. This dominant-negative inhibition leads to a decrease in the affinity for isocitrate which, in turn, causes the loss of NADPH production[9]. On the other hand, *IDH1* R132 mutations result in a gain of function and give the enzyme a new ability, leading to the NADPH-dependent reduction of α-KG to oncogenic metabolite R-2-hydroxyglutarate (2HG) [9,22]. NADPH, which is a protective compound against oxidative stress such as radio- and chemotherapy and plays a role in the removal of oxygen radicals, is used to produce 2HG by *IDH1* mutant enzyme[12]. Therefore, the reduced NADPH level results in a decrease in resistance to oxidative stress. This phenomenon may explain why GBM patients harboring *IDH1* mutation, which normally resists radiotherapy and chemotherapy, show longer survival[22,23]. Supportively, despite the oncogenic role of *IDH1* R132H mutations,[16] studies demonstrate that patients harboring this mutation exhibit a better response to radio- and chemotherapy than patients with wild-type *IDH1*[24-26].

Another significant genetic aberration that is related to GBM is 10q23/*PTEN* locus deletions[6]. The *PTEN* tumor suppressor gene, which is found in the 10q23 region of chromosome 10, encodes a lipid phosphatase. *PTEN* plays a role in many cellular functions such as cell aging, apoptosis, cell cycle progression, cell proliferation, cell migration, and cell response to DNA damage. *PTEN* acts as a tumor suppressor by preventing the progression of the cell cycle with lipid phosphatase activity[15]. Losses of 10q23/*PTEN* locus have been seen in 80% of GBMs[14,27]. 10q23/*PTEN* deletions in GBMs occur as homozygous and hemizygous deletions[4,28-30] or monosomy 10[29,31-33] in the literature. In the present study, we detected 16 deletions of 10q23/*PTEN* locus in 25 patients, which were hemizygous deletions (32%) and monosomy 10 (32%). No homozygous 10q23/*PTEN* deletion was observed in this study. 10q23/*PTEN* locus deletion is associated with poor prognosis and older age of diagnosis[28] rather than *IDH1* mutations[7]. A secondary genetic alteration accompanied by *IDH1* mutations may provide valuable molecular biomarkers for diagnosis. For instance, *IDH1* mutations are strongly associated with the presence of TP53 mutations or absence of EGFR amplification, which is related to poor prognosis[4]. Likewise, a strong correlation between *PTEN* deletions/loss of chromosome 10 and the absence of *IDH1* mutation has been shown[7,34,35]. Such interactions may account for why *IDH1* mutations are associated with a better outcome[4]. However, in our study, only four cases with monosomy 10 had no *IDH1* mutation. Therefore, it was not shown such a relationship was due to the small number of cases.

In summary, this study indicated the prevalence of *IDH1* mutations and 10q23/*PTEN* deletions in the Turkish GBM population and confirmed the presence of *IDH1* R132H mutations and 10q23/*PTEN* alterations as a prognostic marker for GBM.

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Conflict of Interest: We have no conflict of interest to disclose.

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