Research Article

IDH2 Mutations in Primary Glioblastoma

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Summary

Abstract Purpose: To establish the frequency of IDH2 mutations in primary glioblastoma at a population level.

Experimental Design: We screened primary glioblastoma in a population-based study for IDH2 mutations and correlated them with clinical data.

Results: No IDH2 gene mutation was detected in tumor samples. Since the analyzed tumor samples were the primary GBMs, the data of undetected IDH2 gene mutation was in agreement with the literature.

Conclusion: In summary, our study is the first study to investigate the IDH2 status in primary GBMs in Turkish patients. Anaplastic astrocytoma or glioblastoma patients with IDH1/IDH2 mutations have been reported to be younger than those carrying the wild-type allele. Accordingly IDH1/IDH2 mutations were observed more frequently in patients at an early age. In our study, mutations were not detected in our study group, thus a statistical ratio cannot be derived. The average age for our patient sample was 54.5 ± 2.

Key words: Glioblastoma multiforme, IDH2, sequencing

Primer Glioblastoma Hastalarında IDH2 Mutasyon Sıklığı

Özet


Anahtar Kelimeler: Glioblastoma, IDH2, DNA dizi analizi
INTRODUCTION

Glioma develops in the supporting cells in the brain as a result of neoplastic changes. These cells are called neuroglia or glia (2). Gliomas are the most malignant astrocytic tumors consisting of poorly differentiated astrocytes. Glioblastoma is not only the most malignant but also the most common brain tumors in adults (7). Glioblastoma constitutes approximately 12-15% of all intracranial tumors and 50-60% of all astrocytic tumors (6). The peak incidence is between the ages of 45-70. The average age is 53. Men are affected more often than women (male/female ratio of 1.5/1). The average survival time in patients with glioblastoma is 12-18 months. Congenital cases of glioblastoma are rare. Tumors can be detected at 29 weeks of gestation by ultrasonography (6,7).

Glioblastoma is classified into two groups as primary and secondary glioblastoma. The two sub-groups and their definitions were first distinguished in 1940 by the German neuropathologist Hans-Joachim Scherer (15). Primary glioblastoma are advanced tumors that arise de novo, without any clinical and/or histological malignant precursor lesions and that were not present three months prior to diagnosis. The main period of the first symptom to histological diagnosis was 6.3 months (7,9). Secondary glioblastomas constitute up to 40% of glioblastomas in patients younger than 45 years old, low-grade (WHO grade II) or anaplastic astrocytoma. The average time to progression has been reported to be 4-5 years. Moreover, the average survival time is 12-15 months in patients with secondary glioblastoma (6,12,14). In comparison to primary glioblastoma patients, this survival time is longer. The reason for this difference has been theorized to be the younger age of secondary glioblastoma patients when compared against all other astrocytic neoplasms, there are more genetic aberrations in glioblastomas. In addition, the development of molecular methods, glioblastomas that originate from different genetic pathways have been identified. There has been piling evidence that secondary and primary glioblastomas are two different disease entities (7).

In recent years, studies have further focused on the genetic background of glioma. In 2008, IDH1 gene was identified that encodes for a metabolic enzyme isocitrate dehydrogenase. IDH genes code for five different proteins in the human genome. These five genes are coenzymes of nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotides (NAD). IDH1 is localized in the cytosol and peroxisomes IDH2/IDH3 are localized in the mitochondria. Isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation of forming isocitrate to α-ketoglutarate and NAD(P) + and NAD(P)H (14,18). This process involves the oxidation of isocitrate to oxalosuccinate (a ketone). After that α-ketoglutarate decarboxylated will return to the form oxalosuccinate. IDH2 plays a key role in tri-citric acid cycle regulation in multiple tissues and α-ketoglutarate transformation assumes the function of isocitrate in TCA cycle.

IDH1/IDH2 mutation results in a new enzymatic activity that transforms -ketoglutarate into 2-hydroxyglutarate (2-HG). IDH2 play a prominent yet a distinctive role in cellular metabolism. IDH2 mutations disrupt the normal function of the wild type, lowering the affinity to the substrate. Catalytically inactive heterodimer formation occurs as a result of mutation and transformation is inhibited of isocitrate to α-ketoglutarate. Studies show that transfected cells with IDH2 mutation show decrease in the level of α-KG. IDH1/2 mutation bearing cancer tissue samples when compared to non-cancerous cells have 100 times higher concentration 2-HG. Progressive accumulation of 2-HG can lead to neuronal defects and increased the risk for brain
tumors such as gliomas. Increased levels of 2-HG in the brain due to increased ROS levels that can potentially lead to an increased risk of cancer development\(^7\). IDH2 is involved in the regulation of oxidative respiration. As a result of IDH mutations, cells become exposed more to the effects of oxidative stress. The abnormal metabolites produced as a result of mutations and lead to increased risk for glioma development. As a result of abnormal cellular function both due to the oxidative stress and new enzymatic activities that lead to accumulation of unnatural metabolites (2-Hydroxy Glutarate (2-HG)), cells are more susceptible for external agents (chemotherapy, radiotherapy, ROS etc.)\(^7,8,12,18\).

IDH1/2 mutation is observed at a percentage of 70% in WHO grade II and III astrocytoma, oligodendroglioma and secondary glioma. IDH1 mutations were detected in tumors more often than IDH2, these tumors without mutations encoding amino acid analog with IDH1. Most frequent mutations in glioma are R132H (G395) in IDH 1 and R172K (G515) in IDH2 (11,20). Arg132 of IDH1 is aligned with Arg172 of IDH2 and this arginine is conserved in all known homologs of IDH. Recent studies indicates that IDH1/2 mutations as a new prognostic marker. IDH1 and IDH2 mutations could also contribute to tumorigenesis and cancer progression through increased mutagenesis. Loss of a wild-type IDH1 or IDH2 allele, combined with a new enzyme activity that consumes NADPH and α-ketoglutarate, could lead to depletion of these two compounds that normally help to defend the cell against oxidative stress. Unchecked oxidative stress could lead to mutagenesis as ROS interact with the genome. Alternatively, 2-hydroxyglutarate itself may act as a mutagen through an as-yet unknown mechanism. Grade 2 and 3 astrocytomas and secondary glioblastomas frequently contain IDH mutations and later develop missense mutations in TP53 and other genes supporting the idea that early IDH mutations could promote later advantageous mutations that underlie the formation and progression of these cancers\(^10,12,13,15,16\).

Ichimura et al reported IDH1/2 mutations carrying glioma patients have a longer life expectancy than those lacking the mutation. In this study IDH2 gene mutation analysis were carried out on the samples of the Turkish GBM patients and evaluated IDH2 gene mutations were compared with clinical and histopathological features\(^5\).

**MATERIAL AND METHODS**

We studied the frequency of IDH2 mutations in a series of 40 primary GBM patients from the Turkish population correlating it to patient age, gender, GBM type and survival time. Tumor samples were collected during surgical procedures by the Department of Neurosurgery of Eskisehir Osmangazi University. Informed consent was obtained from each patient, and the study was approved by the local ethics committee. The clinical and radiologic data were carefully reviewed, compared, and correlated with the histological results. Clinical data included sex, date of birth, Karnofsky performance score (KPS) and type of treatment (surgical resection— gross total, subtotal, partial, as per radiologic report; radiotherapy and/or chemotherapy). The samples included frozen tissues, collected upon surgical removal. The mean age of 40 GBM patients was 54,89 ± 3,34 years, with 51,71 ± 3,98 females and 58,07 ± 2,71 males. A total of 40 cases were primary GBMs.

DNA was extracted from the frozen tissues by Magna Pure Compact DNA isolation kits and Roche Magna Pure Compact DNA isolation machine and its quantity was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). Polymerase chain reaction (PCR) followed by DNA sequencing was applied to detect IDH2 mutation, with primers described previously\(^14\). PCR
products were generated in a 50 µl reaction mixture including 100 ng of DNA, 2.5 mM dNTP, 25 mM MgCl₂, Forward and Reverse primers, 10x PCR buffer, Taq DNA polymerase and PCR grade water. The PCR was performed with an initial denaturation step at 95°C for 7 min, followed by 32 cycles consisting of 94°C for 35 s, 60°C for 45 s and at 72°C for 45 s. After the final cycle, an extension period of 10 min at 72°C was performed.

The PCR products of amplification were checked, purified with a ExoSAP-IT (Affymetrix Inc. USB) and sequenced on an ABI Prism 3130 DNA automated sequencer using the Big DyeTM Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, Foster City, CA, USA). Primers used for the sequencing were the same as those used for PCR. Results were analyzed and compared with NCBI human genomic database and BioEdit v5 software program.

Evaluation of the results of any polymorphisms and mutations were detected in workgroup in tissue samples. Survival times of the patients were obtained from patient files. Mutations were not detected for IDH2 gene in our study group. The average survival time were 17 months.

The statistical analyses and their associations with patient characteristics were performed by chi-square test (x²) and t-test. Overall survival (OS) was calculated as the interval between the surgery and day of death, in months. The log-rank test was used for univariate analysis to estimate differences in survival time for IDH2 mutation status, according to the Kaplan–Meier method. Calculations were performed using SPSS 15.0 software (SPSS, Chicago, IL, USA), with statistical significance of p<0.05.

RESULTS

Mutation screening was performed by DNA sequence analysis. IDH2 gene mutations in exon 4 were studied in particular due to the settlement is made to this region in our study. Results were compared with NCBI human genome sequence database and results were evaluated by BioEdit v5 software program. Evaluation of the results of any polymorphisms and mutations were detected in workgroup in tissue samples.

Survival times of the patients were obtained from patient files. Mutations were not detected for IDH2 gene in our study group. The average survival time were 17 months.

Figure 1: Sample 43, wild type sequence exon 4 of IDH2 gene 172 codon.
DISCUSSION

IDH1 and IDH2 mutations in glioblastoma have been reported by many studies\(^{(3,7,17,18,19,21)}\). Our study is the first study to investigate the IDH2 status in primary GBMs in Turkish patients.

IDH2 contains an N-terminal mitochondrial signal peptide and localizes to the mitochondria. IDH2 with mutations result is the new gain of function reported to contribute to gliomagenesis. However IDH1 / 2 mutations are mostly observed in secondary tumors. IDH1 mutations ratio is 5% in primary tumor, IDH2 mutation has been reported also negligibly low level (0.1%)\(^{(4)}\). In our study, sequencing results IDH2 gene mutation was not detected in tumor samples. These data are consistent with the literature because all of our clinical cases were patients with a diagnosis of primary GBM in this study. Also this condition was confirmed by IDH mutations. However, we think that IDH 1 and IDH 2 in routine diagnostic analyzes will help clinicians in determining the subtype of GBM.

Anaplastic astrocytoma or glioblastoma patients with IDH1/IDH2 mutations have been reported to be younger age than carrying wild-type allele. Ichimura et al. reported for patients with mutations the average age of 41, patients with wild-type alleles, average age of 56\(^{(5)}\). Parsons and colleagues mutations seen 33 years of age, in patients with wild-type allele reported as the average age was 53\(^{(14)}\). According to these result IDH1/IDH2 mutations observed more frequently in patients at an early age. This situation also conforms to the definition of secondary GBM. In our study, mutations were not detected in our study group so no statistical ratio can be extrapolated. But all of our samples were wild-type allele therefore the average age was 54.5 ± 2, respectively. These findings are consistent with GBM patients which presented in the literature with the wild-type allele.

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