RESEARCH ARTICLE

Molecular Investigation of Isocitrate Dehydrogenase Gene (IDH) Mutations in Gliomas: First Report of IDH2 Mutations in Indian Patients

Bibhu Ranjan Das[1]*, Rajiv Tangri[2], Firoz Ahmad[1], Arnab Roy[1], Kamlakar Patole[3]

Abstract

Recent genome wide sequencing has identified mutations in IDH1/IDH2 predominantly in grade II-III gliomas and secondary glioblastomas which are associated with favorable clinical outcome. These mutations have become molecular markers of significant diagnostic and prognostic relevance in the assessment of human gliomas. In the current study we evaluated IDH1 (R132) and IDH2 (R172) in 32 gliomas of various grades and tumor subtypes. Sequencing analysis revealed R132H mutations in 18.7% tumors, while none of the cases showed IDH2 (R172) mutations. The frequency of IDH1 mutations was higher in females (21.4%) than males (11.1%), and it was significantly higher in younger patients. Histological analyses demonstrated presence of necrosis and micro vascular proliferation in 69% and 75% respectively. Interestingly, IDH1 mutations were predominantly present in non-necrotic tumors as well as in cases showing microvascular proliferation. Of the six IDH1 positive cases, three were glioblastomas (IV), and one each were anaplastic oligoastrocytoma (III), anaplastic oligodendroglioma III (n=1) and diffuse astrocytoma. In conclusion, IDH1 mutations are quite frequent in Indian glioma patients while IDH2 mutations are not observed. Since IDH mutations are associated with good prognosis, their use in routine clinical practice will enable better risk stratification and management of glioma patients.

Keywords: Glioma - IDH1 mutation - IDH2 mutation - sequencing - India

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Introduction

Gliomas are the group of neoplasms originating from central nervous system and represent one of the most common and formidable brain tumors. Even with intensive treatment protocol, most of the glioma patients succumb within 2-10 years indicating that gliomas are associated with dismal outcome. However, recent advances in genetics research have greatly expanded our ability to accurately diagnose gliomas and provide more useful prognostic information. In this context, identification of mutations in the isocitrate dehydrogenase (IDH1/IDH2) either in the R132 or R172 residues were recently highlighted, and has already become a molecular marker of significant diagnostic and prognostic relevance in the assessment of human gliomas (Parsons et al., 2008; Yan et al., 2009). Mutations of the IDH1 R132 are reported in 55-80% of grade II/III oligodendroglioma/astrocytomas, over 90% of secondary glioblastomas (GBMs), rarely in primary glioblastoma (Van den Bent et al., 2010), and are associated with favorable prognosis (Uno et al., 2011). In contrast IDH2 mutations are rare, but more common in oligodendroglial tumors, as compared to astrocy¬tomas (Raynaud et al., 2010). Interestingly, a recent study also suggested that presence of IDH mutations may be a protective mechanism in glioma patients which could be the reason for better outcome in patients with mutant IDH than those with the wild-type IDH genes (Zhu et al., 2011). Most of the available studies on IDH1 and 2 mutations are reported from western countries (Bleeker et al., 2009; Metellus et al., 2010; Van den Bent et al., 2010; Cykowski et al., 2012), few reports from Asia (Mukasa et al., 2012; Song Tao et al., 2012), while there is only one report of IDH1 mutations in Indian glioma patients (Jha et al., 2011). In the current study we evaluated IDH1 and IDH2 mutations in glioma patients of different histological types and grades with the aim of assessing their frequency, distribution pattern and their correlation with clinicopathological findings. To the best of our knowledge, this is first study to evaluate IDH2 mutations in Indian glioma patients.

Materials and Methods

The present study was conducted at the Research and Development Division of SRL Ltd., Mumbai, India. IDH1 and IDH2 mutations were evaluated on 32 gliomas of various grades and tumor subtypes. The study population consisted of 18 males (56%) and 14 females (44%) with median age 51.5, ranging from 4-80 years. The study is

*[1]Research and Development, [3]Histopathology Division, SRL Ltd., Mumbai, [2]SRL Ltd., Gurgaon, India *For correspondence: brdas@srl.in*

in accordance with the declaration of Helsinki and written informed consent was taken from each patient. Treatment and outcome were not analyzed. The details of the clinical characteristics of all of the included patients are depicted in Table 1.

Genomic DNA extraction

Genomic DNA was extracted from FFPE tissue using Qiagen extraction kit as per manufacturer's instruction. Prior to DNA extraction, separate hematoxylin and Eosin (HE) slides were reviewed by a pathologist to assure greater than 50% tumor content as suitable for DNA extraction. At least, five FFPE sections of 5μm thickness were processed for genomic DNA extraction.

Screening of IDH1/2 gene mutations

Assessments of IDH1 (R132) and IDH2 (R172) gene mutations were done using the following sets of primers: IDH1_F:5'-CTCCTGATGAGAAGAGGGTTG-3', IDH1_R:5'-TGGAAATTTCTGGGCCATG-3'; IDH2_F:5'-TGGAACTATCCGGAACATCC-3', IDH2_R:5'-AGTCTGTGGCCTTGTACTGC-3'as described earlier (Van den Bent et al., 2010). Briefly, for both IDH1 and IDH2, the PCR was performed in a 50μl volume containing 50ng of genomic DNA, 1.5mmol/L $MgCl_2$, 0.2mM dNTPs, 20 pmol of each primers, and 1.5U of Taq polymerase (Invitrogen Life Technology, Sao Paulo, Brazil) in separate PCR tubes. The PCR conditions consisted of an initial denaturation step at 95ºC for 5 min followed by 40 cycles at 94ºC for 30s, 57ºC for 30s, 72ºC for 1 min, and a final step at 72ºC for 10 min.

Sequencing analysis

Amplified products were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and directly sequenced to identify the different types of mutation by Automated ABI prism 3100 Avant Genetic Analyzer (Applied Biosystems Inc., Foster city, CA) using ABI prism BigDye terminator kit (version 3.1).

Statistical analysis

The data were analyzed using χ^2 test to calculate the significance of association between IDH mutations and other discrete variables among subgroup of patients. The p values <0.05 were considered to be significant.

Results

The current study evaluated IDH1 (R132) and IDH2 (R172) mutations in 32 glioma patients. Heterozygous mutations of IDH1 (R132) were found in 6/32 (18.7%) tumors, while none of the cases showed any evidence of IDH2 (R172) mutations. All six mutations observed in IDH1 gene were single base substitutions c.395G.A occurring at amino acid residue R132, resulting in an arginine to histidine (p.R132H) substitution (Figure 1 and Table 2). The frequency of IDH1 mutations were higher in females (21.4%, 3/14) in comparison to their male counterparts (11.1%, 3/18) (p=0.732). Furthermore, IDH1 mutations were significantly higher in patients who were younger than 50 years when compared with patients who were above 50 years (33.3%, 5/15 *vs* 5.9%, 1/17; p=0.047). Microscopic examination of the tumor samples reveled presence of necrosis and micro vascular proliferation in 69% (22/32) and 75% (24/32) respectively. Interestingly, IDH1 mutations were significantly more frequent in non necrotic tumors in comparison to tumors with necrosis (40%, 4/10 *vs* 9%, 2/22; p=0.038). In contrast, IDH1 mutations were preponderant in tumors showing microvascular proliferation (20.8%, 5/24) in comparison to those without microvascular proliferation (12.5%, 1/8), though the difference was statistically insignificant (p=0.601). As shown in Table 1 and Table 2, the IDH1 mutations were frequent in glioblastomas-WHO grade IV tumors (n=3), followed by equal distribution in anaplastic oligoastrocytoma –WHO grade III (n=1), anaplastic oligodendroglioma –WHO grade III (n=1), and diffuse astrocytoma (n=1), while other tumor type apart from the above did not revealed any mutation. Nevertheless, no significant association of IDH1 mutation with any of

Table 1. Demographic Features of Glioma Patients with IDH1 Mutation Status

Patient Characteristics		No (%)	IDH1 mt, n (%)	WT n (%)	p value
Total No		32 (100)	6 (18.7)	26 (81.3)	
Sex	Male	18 (56)	3 (11.1)	15 (88.9)	
	Female	14 (44)	3 (21.4)	11 (78.6)	0.732
Age (years)	Less than 50	15 (47)	5 (33.3)	10 (66.7)	
	greater than 50	17 (53)	1 (5.9)	16 (94.1)	0.047
	Median (range)	51.5 (4-80)			
Necrosis	Yes	22 (69)	2 (9)	20 (91)	
	No	10 (31)	4 (40)	6 (60)	0.038
Microvasculation proliferation	Yes	24 (75)	5 (20.8)	19 (79.2)	
	No	8 (25)	1 (12.5)	7 (87.5)	0.601
Histological tumor type (grade)	Diffuse astrocytomas (II)	2 (6.3)	1 (50)	1 (50)	
	Anaplastic astrocytomas (III)	1 (3.1)	0 (0)	1 (100)	
	Glioblastomas (IV)	23 (72.0)	3 (13)	20 (87)	
	Gliosarcoma (IV)	1 (3.1)	0 (0)	1 (100)	
	Anaplastic oligodendrogliomas (III)	1 (3.1)	1 (100)	0 (0)	
	Anaplastic oligoastrocytomas (III)	1 (3.1)	1 (100)	0 (0)	
	Pilocytic astrocytoma (I)	1 (3.1)	0 (0)	1 (100)	
	Pilomyxoid astrocytoma (II)	1 (3.1)	0 (0)	1 (100)	
	Pleomorphic xanthoastrocytoma (II)	1 (3.1)	0 (0)	1 (100)	0.17

*mt= Mutant; WT= Wild type

Table 2. Distribution of IDH Mutation and Clinical Characteristics of Glioma Patients

Sl no.	Unique patient ID	Age	Sex	Site of biopsy	Necrosis	Microvasculation proliferation	IHC	Labelling Index	Histopathological Diagnosis	Grade	IDH1 mutation	IDH2 mutation
1	SR-1	48	F	Right temporal tumor	No	Yes	GFAP positive	KI-67(8%)	Anaplastic oligoastrocytoma	III	R132H	WT
2	SR-2	61	M	Left temporal tumor	No	Yes	GFAP/S100 positive	KI-67 (25%)	Anaplastic oligodendroglioma	III	R132H	WT
3	SR-3	40	M	Right temporal tumor	No	No	GFAP positive	KI-67(3.5%)	Diffuse Astrocytoma	II	R132H	WT
4	SR-4	29	F	Left posterior frontal tumor	No	Yes	GFAP positive	KI-67(23%)	Glioblastoma	IV	R132H	WT
5	SR-5	36	F	Corpus callosum tumor	Yes	Yes	GFAP positive	KI-67(22%)	Glioblastoma	IV	R132H	WT
6	SR-6	40	M	Left frontal tumor	Yes	Yes	GFAP positive	Not available	Glioblastoma	IV	R132H	WT

these histological tumor subtype was observed (p>0.05).

Discussion

Malignant gliomas are the most common form of primary brain tumor, accounting for nearly 70% of all primary central nervous system tumors. The disease shows large variation with respect to tumor location, tumor morphology, marked genetic heterogeneity, and clinical outcome. Over the last decade, understanding of glioma on a molecular level has greatly expanded, and infact genome wide sequencing have recently identified mutations in the isocitrate dehydrogenase 1 and 2 gene (IDH 1/2) that occur in the majority of WHO grade II–III gliomas and secondary glioblastomas (Hartmann et al., 2009; Yan etal., 2009), all of which harbor a better prognosis in comparison to their normal counterparts (Sanson et al., 2009). Most of the available reports on IDH1 and IDH2 mutations are from Western countries (Hartmann et al., 2009; Felsberg et al., 2010; Van den Bent et al., 2010; Cykowski et al., 2012) while there is only one Indian published data on IDH1 mutation (Jha et al., 2011). In the current study, we found IDH1 mutations in 18.7% (6/32) of samples tested, while none of the cases demonstrated any mutation in IDH2. The frequency of IDH1 mutations in our cohort was similar to that from Netherlands (20%, 23/113) (Bleeker et al., 2009) and Germany (19%, 50/262) (Felsberg et al., 2010), higher than Brazil (11.8%, 19/161) (Uno et al., 2011), and lower than USA (45.3%, 34/75) (Horbinski et al., 2009), Japan (29%, 73/250) (Musaka et al., 2012) as well as India (46%, 46/100) (Jha et al., 2011). The difference in the frequency of IDH1 mutation may be attributed to sample size, variable sensitivity of the detection assays, or the racial differences can also be taken into consideration. In contrast to IDH1 mutations, IDH2 mutations in gliomas appear to be less frequent with reported incidence ranging between 0.9-4.2% (Hartmann et al., 2009; Metellus et al., 2010; Qi et al., 2011; Musaka et al., 2012). Infact, in the current study none of the cases revealed any mutations in IDH2, which is in agreement with previous report (Krell et al., 2011), thereby further suggesting that IDH2 mutations are rare in gliomas. To the best of our knowledge, this is the first study to evaluate IDH2 mutations in Indian glioma patients.

Sequence analysis demonstrated that all the mutations resulted in G>A change with amino acid substitution of arginine to histidine (p. R132 H) (Figure 1), suggesting that R132H is the most recurrent IDH1 mutation in gliomas. There appears to be a trend towards higher frequency of IDH1 mutations in female patients in comparison to male glioma patients, however the difference was statistically insignificant (p>0.05). Furthermore, IDH1 mutations were significantly higher in younger patients when compared with patients who were above 50 years (33.3%, 5/15 *vs* 5.9%, 1/17; p=0.047), which tallies with several previous studies (Felsberg et al., 2010; Jha et al., 2011). In the present study, the median age of patients with IDH1 mutation lower that patients without mutation (40 years *vs* 52.5 years) which corroborates with previous report by Parson and colleagues, wherein IDH mutations tended to be seen in younger patients (median age 33 years for IDH1 mutation carriers *vs* 53 years for non-carriers) (Parsons et al., 2008). Correlation of IDH1 mutations with histopathological findings reveled that IDH1 mutations were positively associated with absence of tumor necrosis (40%, 4/10 *vs* 9%, 2/22; p=0.038) which is in line with earlier report (Van den Bent et al., 2010), while no significant correlation with presence or absence of microvascular proliferation with IDH1 mutations were observed. Mutations in IDH1 were observed in GBM (IV) tumors, anaplastic oligoastrocytoma (III), anaplastic oligodendroglioma (III), and diffuse astrocytoma (II), while in contrast, no IDH1/2 mutations were identified in pilocytic astrocytomas (I), indicating that these tumors might arise through a different mechanism. This is in agreement with clinical observations that pilocytic astrocytomas rarely if ever undergo malignant transformation (Burger et al., 2000), and with recent data indicating that a duplication at 7q34 producing a BRAF fusion gene occurs frequently in pilocytic astrocytomas but not higher-grade gliomas (Jones et al., 2008).

The molecular pathogenesis of IDH1/2 mutations in the development of gliomas is still unclear. Several theories have been proposed, Turcan et al. (2012) reported that IDH mutations induces extensive DNA hypermethylation by remodeling the methylome to establish glioma hypermethylator phenotype which results in reorganization of the methylome and transcriptome (Turcan et al., 2012). In addition to this, it is also thought that IDH1/2 mutations prevent oxidative decarboxylation of isocitrate to α-ketoglutarate and confer novel enzymatic activity, facilitating the reduction of α-ketoglutarate to

2-hydroxyglutarate, a putative oncometabolite (Koivunen et al., 2012; Losman et al., 2013) and therefore IDH1 and IDH2 mutations are likely the integrally involved in the pathogenesis of malignant transformation (i.e., driver mutations) rather than epiphenomena. Nevertheless, elucidating the pathogenesis of IDH mutations will aid better understanding of the molecular mechanisms of gliomagenesis.

In conclusion, the current study demonstrated that R132H is the most recurrent IDH1 mutations while IDH2 mutations less frequent in Indian glioma patients. The fact that the value of IDH 1/2 mutations relates to prognosis, as demonstrated in terms of longer survival in patients with IDH mutation during German NOA 04 and EORTC 26951 trials (Wick et al., 2009; Van den Bent et al., 2010), we believe that routine clinical testing of IDH mutations will allow assignment of patients to better-defined risk categories.

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