

## RAPID COMMUNICATION

**IDH1 mutations in a Brazilian series of Glioblastoma**

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## INTRODUCTION

Diffusely infiltrating astrocytomas are the most common primary brain tumors in adults, being classified into four grades according to the World Health Organization (WHO).<sup>1</sup> Glioblastoma (GBM), grade IV astrocytoma, is the most frequent,<sup>2</sup> and presents median survival rarely exceeding 12 months in spite of currently available treatment approaches.<sup>3</sup> GBM may manifest rapidly *de novo* (primary GBM), or may develop slowly from grade II or grade III astrocytomas (secondary GBM), suggesting that they are distinct disease entities that evolve through different genetic pathways.

In recent genome-wide analyses, high rates of spontaneous mutations in the gene encoding cytosolic NADP-dependent isocitrate dehydrogenase 1 (*IDH1*) have been reported in diffuse gliomas including WHO grades II and III astroglial and oligodendroglial lineages.<sup>4-6</sup> Mutations of *IDH1* are rare in primary GBM (<10%) and frequent in secondary GBM (>80%).<sup>4,7,9-11</sup> Thus, *IDH1* mutations are strong predictors of more favorable prognosis and a highly selective molecular marker of secondary GBM that complements clinical criteria for distinguishing them from primary GBM. Intriguingly, mutations of *IDH1* predominantly occurred in younger patients and were preferentially found in tumors harboring *TP53* mutations.<sup>12</sup> These results corroborate the fact that primary and secondary GBMs originate from different progenitor cells.

*IDH1*, located on 2q33.3, encodes the cytosolic NADP<sup>+</sup> specific isocitrate dehydrogenase, which catalyzes the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate.<sup>13</sup> *IDH1* is configured as a homodimer with two enzymatically active sites, and most of its activity is detected in the cytosol and in peroxisomes. The other four members of the IDH family are exclusively localized in mitochondria.<sup>14</sup> Glioma-specific mutations in *IDH1* always affect the amino acid arginine 132 located in an evolutionarily highly conserved region at the binding site for isocitrate.<sup>11</sup> Mutations in *IDH1*

are of somatic origin and heterozygous, and inactivate enzyme activity.<sup>7</sup>

We studied the frequency of *IDH1* mutations in a series of 161 GBM patients from the Brazilian population according to patient age, gender, GBM type and survival time.

Tumor samples were collected during surgical procedures by the Neurosurgery Groups of different institutions from the state of São Paulo: 93 from Hospital das Clínicas, School of Medicine of University of São Paulo; 38 from Barretos Cancer Hospital; 13 from Paulista School of Medicine, Federal University of São Paulo; 10 from Albert Einstein Jewish Hospital; and 7 from Nove de Julho Hospital. Informed consent was obtained from each patient, and the study was approved by the local ethics committee. The samples included frozen tissues, collected upon surgical removal and immediately snap-frozen in liquid nitrogen<sup>15</sup> and paraffin-embedded blocks. The mean age of 161 GBM patients was 56 years, with 59 females and 102 males. A total of 155 cases were primary GBMs (mean age 55 years), and 6 cases were diagnosed as secondary GBM (mean age 31 years) with histological evidence of a previous less malignant astrocytoma.

DNA was extracted from the frozen tissues by a standard phenol/chloroform method or by Trizol (Invitrogen Inc, Carlsbad, CA, USA), following the manufacturer's instructions, and by QiaAmp DNA Micro kit (Qiagen, Hilden, Germany) from paraffin-embedded sections.

Polymerase chain reaction (PCR) followed by DNA sequencing was applied to detect *IDH1* mutation. Primers sequences synthesized by IDT (Integrated DNA Technologies, Inc, Coralville, IA, USA) for PCR amplification of exon 4 were (5'-3'): CCATCACTGCAGTTGTAGGTT and CATAACAAGTTGGAAATTTCTGG. PCR products were generated in a 25  $\mu$ L reaction mixture including 100 ng of DNA, 50 mM KCl, 50  $\mu$ M of each dNTP, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl<sub>2</sub>, 10 pmol of each primer and 1 unit of Taq DNA polymerase (GE Healthcare, Piscataway, NJ, USA). The PCR was performed with an initial denaturing step at 94°C for 5 min, followed by 35 cycles consisting of 94°C for 30 s, 54°C for 30 s and at 72°C for 30 s. After the final cycle, an extension period of 10 min at 72°C was performed. The PCR products (436 bp) of amplification were checked, purified with a GFX column (GE Healthcare) and sequenced on an ABI Prism 3130 DNA automated sequencer using the

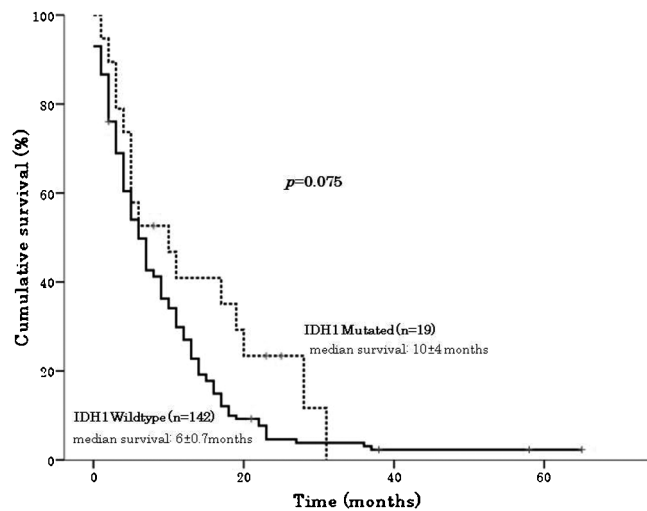
Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, Foster City, CA, USA). Primers used for the sequencing were the same as those used for PCR. Results were analyzed and compared with the public sequence of *IDH1* cDNA (GenBank), accession no. NM\_005896.

The statistical analyses and their associations with patient characteristics were performed by chi-square test ( $\chi^2$ ). 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 *IDH1* mutation status, according to the Kaplan–Meier method. Calculations were performed using STATA, version 7 (STATA Corp., College Station, TX, USA) and SPSS 15.0 software (SPSS, Chicago, IL, USA), with statistical significance of  $p < 0.05$ .

We found *IDH1* mutations in 11.8% (19 out of 161) of samples tested, with a higher mutation rate in GBMs diagnosed as secondary, 66.7% (4 out of 6), than in cases of primary GBMs, 9.7% (15 out of 155),  $p < 0.001$ . All mutations were heterozygous, located at codon 132, resulting in amino acid change from arginine to histidine. We found a higher frequency of *IDH1* mutation in females (18.6%) than in males (7.8%) ( $p = 0.041$ ). GBM patients carrying *IDH1* mutations were significantly younger (diagnosed before age 50 years), mean age of 44 years, than patients with wild-type *IDH1* (diagnosed at age 50 years or older), mean age of 56 years,  $p = 0.011$  (Table 1). The mean survival time of all GBM patients with and without *IDH1* mutations was 12 months (19 cases) and 9 months (142 cases), respectively ( $p = 0.075$ , log-rank test), as shown in Figure 1.

**DISCUSSION**

A higher rate of *IDH1* mutation in secondary compared with primary GBM cases (66.7% vs. 9.7%) was observed in our study. Additionally, patients carrying *IDH1* mutations were younger (44 years) than those patients without the mutation (56 years), and *IDH1* status has been shown to have an association trend with an increase in the overall survival of GBM patients, as described by others.<sup>4-7,9-12</sup> The lack of statistical impact of the overall survival time might be attributed to a low number of cases with *IDH1* mutation reflecting a low incidence of secondary GBM in our series.



**Figure 1** - Survival of glioblastoma patients according to their *IDH1* mutation status (positive vs. negative). Glioblastoma patients carrying an *IDH1* mutation had longer overall survival (log-rank test; Mantel–Cox test;  $p = 0.075$ ).

We have also previously reported a low prevalence of *TP53* mutations, usually detected among secondary GBM cases, because of a higher frequency of primary GBM in our series.<sup>16</sup> Both results concerning *TP53* and *IDH1* mutation status point out the molecular differences between primary and secondary GBM. These results reinforce the concept that, despite the histological similarities, primary and secondary GBMs are genetically and clinically distinct entities.<sup>6,12</sup>

In summary, this study established the frequency of *IDH1* mutation in a Brazilian series of GBM, confirmed *IDH1* mutation as a genetic marker for secondary GBM, and therefore as complementary information to help predict the outcome of patients with GBM.

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**Table 1** - *IDH1* mutation status of glioblastomas according to age, gender and tumor subtype in Brazilian patients.

Characteristics	GBM patients (%)	<i>IDH1</i> mutation		%	<i>p</i> value
		Positive*	Negative		
	161	19	142	11.8	
Gender					
Female	59 (36.7)	11	48	18.6	0.041
Male	102 (63.3)	8	94	7.8	
Age at diagnosis (years)					
<50	52 (32.3)	11	41	21.1	0.011
≥50	109 (67.7)	8	101	7.3	
(Age, mean ± SE)	54.6 ± 13.4	43.9 ± 19.1	56.1 ± 11.9		
Tumor subtype					
Primary	155 (96.3)	15	140	9.7	<0.001
Secondary	6 (3.7)	4	2	66.7	

\**IDH1* mutation at R132H in heterozygous form.

procedures of all patients included in this study. We also thank the Psychiatry Institute for the logistical help in the surgical therapy at USP-SP, and Diagnostika Laboratory for preparation of paraffin-embedded material from Albert Einstein Jewish Hospital and Nove de Julho Hospital.

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