

Analysis of the *IDH1* codon 132 mutation in brain tumors

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Abstract A recent study reported on mutations in the active site of the isocitrate dehydrogenase (*IDH1*) gene in 12% of glioblastomas. All mutations detected resulted in an amino acid exchange in position 132. We analyzed the genomic region spanning wild type R132 of *IDH1* by direct sequencing in 685 brain tumors including 41 pilocytic astrocytomas, 12 subependymal giant cell astrocytomas, 7 pleomorphic xanthoastrocytomas, 93 diffuse astrocytomas, 120 adult glioblastomas, 14 pediatric glioblastomas, 105 oligodendrogliomas, 83 oligoastrocytomas, 31 ependymomas, 58 medulloblastomas, 9 supratentorial primitive neuroectodermal tumors, 17 schwannomas, 72 meningiomas and 23 pituitary adenomas. A total of 221 somatic *IDH1* mutations were detected and the highest frequencies occurred in diffuse astrocytomas (68%), oligodendrogliomas (69%), oligoastrocytomas (78%) and secondary glioblastomas (88%). Primary glioblastomas and other entities were characterized by a low frequency or absence of mutations in amino acid position 132 of *IDH1*. The very high frequency of *IDH1* mutations in WHO grade II astrocytic and oligodendroglial gliomas suggests a role in early tumor development.

Keywords *IDH1* · Glioma · Progression · Astrocytoma · Oligodendroglioma · Medulloblastoma

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Introduction

Mutations in the gene encoding cytosolic NADP⁺ dependent isocitrate dehydrogenase (*IDH1*) emerged as an unsuspected finding in sequence analysis of glioblastoma (GBM) [9]. Isocitrate dehydrogenase catalyzes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate thereby reducing NADP⁺ to NADPH. Mutations affected the amino acid arginine in position 132 of the amino acid sequence which belongs to an evolutionary conserved region locating to the binding site of isocitrate. In the vast majority of the cases wild type arginine in position 132 was replaced by histidine (R132H). The mutations reported always were heterozygous and alterations suggestive for protein inactivation such as splice site or nonsense mutations were not detected thus prompting speculations on an activating nature of the mutation [9]. However, site directed mutagenesis leading to a R132E exchange in rat *IDP2* which is homologous to human *IDH1* completely abrogated enzyme activity [3]. Further, a mutation in porcine NADP-isocitrate dehydrogenase at position 133, R133Q, corresponding to human position 132 also resulted in down-regulation of enzyme activity [14]. Thus the effect on enzyme activity of R132H currently is not resolved.

The subcellular localization of *IDH1* encoded isocitrate dehydrogenase is the cytoplasm and the peroxisome [1]. In the cytoplasm, the role of *IDH1* protein might be to provide NADPH under conditions not favorable for generation of NADPH by the hexose monophosphate shunt. In the peroxisome *IDH1* is the only known source of NADPH which is required by several enzymes such as hydroxymethyl-CoA-, 2,4-dienoyl-CoS- and acyl-CoA-reductases. An important role of *IDH1* in protection from oxidative stress may be concluded from the demonstration of increased resistance of *IDPc*, the mouse homolog of *IDH1*, overexpressing and

sensitivity of IDPc deficient NIH3T3 cells to exposure of hydrogen peroxide [5]. Further, IDPc negative HL-60 cells exhibited augmented caspase-3 activation upon oxidative stress suggesting a role in apoptosis [4].

IDH1 mutations were reported in 12% of GBM. However, the incidence of *IDH1* mutations in secondary glioblastoma (secGBM) was much higher with five of six tumors carrying this alteration than that in primary glioblastoma (prGBM). By definition secGBM arise from diffuse astrocytoma WHO grade II (A II) or anaplastic astrocytoma WHO grade III (A III). This prompted us to analyze A II, A III, GBM subtypes and other brain tumors for alterations in the mutational hotspot of *IDH1* with a focus on A II and A III as precursor lesions for secGBM.

Materials and methods

Tumor specimens

DNA from human brain tumors diagnosed at the departments of Neuropathology at the University Bonn, the Charité Berlin, the Burdenko Neurosurgical Institute in Moscow and the University Heidelberg were analyzed. The series included 41 pilocytic astrocytomas WHO grade I (PA I), 12 subependymal giant cell astrocytomas WHO grade I (SEGA), 7 pleomorphic xanthoastrocytomas WHO grade II (PXA), 46 diffuse astrocytomas WHO grade II (A II), 47 anaplastic astrocytomas WHO grade III (A III), 99 primary glioblastomas WHO grade IV (prGBM), 8 secondary glioblastomas WHO grade IV (secGBM), 8 giant cell glioblastomas WHO grade IV (gcGBM), 14 pediatric glioblastomas (pedGBM), 5 gliosarcomas WHO grade IV (GS), 51 oligodendrogliomas WHO grade II (O II), 54 anaplastic oligodendrogliomas WHO grade III (O III), 46 oligoastrocytomas WHO grade II (OA II), 37 anaplastic oligoastrocytomas WHO grade III (OA III), 6 myxopapillary ependymomas WHO grade I (E myx I), 15 ependymomas WHO grade II (E II), 10 anaplastic ependymomas WHO grade III (E III), 58 medulloblastomas WHO grade IV (MB IV), 9 supratentorial primitive neuroectodermal tumors WHO grade IV (PNET), 17 schwannomas WHO grade I (S I), 38 meningiomas WHO grade I (M I) including the meningothelial and transitional variant, 17 atypical meningiomas WHO grade II (M II), 17 anaplastic meningiomas WHO grade III (M III) and 23 pituitary adenomas WHO grade I of the null cell adenoma type (PIAD). prGBM and secGBM were defined according to Scherer [12]. pedGBM were defined as arising in patients up to the age of 17. One patient with morphology of gcGBM was aged 10 years and therefore grouped with pedGBM. Data on *TP53*, combined LOH1p/19q and *EGFR* from tumors included in this study have been published previously [8, 13, 15].

PCR amplification

A fragment of 129 bp length spanning the catalytic domain of *IDH1* including codon 132 was amplified using 60 ng each of the sense primer IDH1f CGGTCTTCAGA GAAGCCATT and the antisense primer IDH1r GCAAAA TCACATTATTGCCAAC. PCR using standard buffer conditions, 20 ng of DNA and GoTaq DNA Polymerase (Promega, Madison, USA) employed 35 cycles with denaturing at 95°C for 30 s, annealing at 56°C for 40 s and extension at 72°C for 50 s in a total volume of 15 µl.

For confirmation, the sense primer IDH1f ACCA AATGGCACCATACGA and antisense primer IDH1rc TTCATACCTTGCTTAATGGGTGT generating a 254 bp fragment at the same PCR conditions were employed.

Direct sequencing

A total of 2 µl of the PCR amplification product was submitted to the sequencing reaction using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, USA). Twenty-five cycles were performed employing 12 ng of the sense primer IDH1f CGGTC TTCAGAGAAGCCATT, with denaturing at 95°C for 30 s, annealing at 56°C for 15 s and extension at 60°C for 240 s. A second round of sequencing analysis was performed using the antisense primer IDH1rc TTCATACCTTGCTT AATGGGTGT and the sequencing reaction conditions as described above. Sequences were determined using the semiautomated sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems, Foster City) and the Sequence Pilot version 3.1 (JSI-Medisys, Kippenheim, Germany) software.

Statistics

The Fisher exact test was used to examine associations between nominal variables referring to absence or presence of genetic alterations. The *t* test was used to examine the relation of nominal variables referring to absence or presence of genetic alterations with age within distinct tumor groups.

Results and discussion

IDH1 mutations in brain tumors

Altogether, 685 tumors were analyzed and 221 mutations in *IDH1* were detected. All mutations were heterozygous with one wild type allele being present. Only codon 132 of *IDH1* was affected by mutations and 205 mutations were of the R132H type, however, we also found 8 mutations leading to

Table 1 Type of 221 *IDH1* mutations in brain tumors

Nucleotide change	Amino acid change	N (%)
G395A	R132H	205 (92.7)
C394T	R132C	8 (3.6)
C394A	R132S	4 (1.8)
C394G	R132G	2 (0.9)
G395T	R132L	1 (0.5)
C394G G395T	R132V	1 (0.5)

N (%) Number of tumors and percentage of mutation among all mutations

R132C, 4 mutations leading to R132S, 2 mutations leading to R132G, and one mutation each resulting in R132L and R132V. There was no clear association of the rare mutation types with a distinct tumor entity, although six of the eight R132C mutations were seen in astrocytomas. Type and frequency of mutations are listed in Table 1 and the sequence alterations leading to the R132V are depicted in Fig. 1. Frequent mutations of *IDH1* were observed in A II (74%), A III (62%), secGBM (88%), O II (71%), O III (67%), OA II (78%) and in OA III (78%). In order to control for false positive results due to potential contamination, all A II and all O III with *IDH1* mutations and all tumors with rare *IDH1* mutation types detected in the first round were confirmed with a second primer pair spanning a larger fragment. Because no differences between initial and confirmatory analyses were detected, the confirmatory series was not further extended.

The frequency of mutations in PNET (33%) and gcGBM (25%) is noteworthy, although, the number of tumors included in the study is too low for a clear statement. No *IDH1* mutations were detected in SEGA, PXA, GS, E myx I, E II, E III, MB IV S I, M I, M II, M III and PIAD. Only single or few mutations occurred in PA I (3%), prGBM (7%) and pedGBM (7%). Constitutional DNA from peripheral leukocytes was examined from all patients with a

tumor carrying an *IDH1* mutation. In all patients with alterations in *IDH1* the mutation was confirmed to be of somatic origin. A compilation of *IDH1* mutations in our series of human brain tumors is given in Table 2.

Unexpected findings were the high frequency of *IDH1* mutations in diffuse astrocytic, oligodendroglial and mixed gliomas of the WHO grades II and III. The WHO grade II tumors already carry *IDH1* mutations thereby demonstrating that this alteration does not occur during tumor progression. In contrast, the high incidence of *IDH1* mutations in A II, O II and OA II may allow the speculation, that this mutation is involved in early steps of tumorigenesis. The frequency of mutations in these tumors exceeded the one observed in GBM. However, *IDH1* mutations showed an unbalanced distribution among the different subtypes of GBM. A very high frequency was observed in secGBM confirming the previous report [9]. In contrast, only few prGBM carried *IDH1* mutations. This distribution may even suggest that in those prGBM with *IDH1* mutation, the diagnosis of a previous lower grade astrocytoma has been missed. No mutations were detected in GS.

The analysis of PIAD was of interest because of the observation, that *IDH1* expression was strongly up-regulated in non-functional PIAD [7]. While *IDH1* mutations have been discussed to be of activating nature [9], they might also have an effect on transcription or stability of mRNA. However, analysis of 23 PIAD lacking expression of pituitary hormones revealed no mutation, suggesting that up-regulated *IDH1* expression in these tumors is independent to the status in the mutational hotspot coding for amino acid position 132.

Distribution of *IDH1*, TP53 and EGFR mutations and of LOH1p/19q in gliomas

IDH1 mutations did not show any association with TP53 mutations or LOH1p/19q in A II, A III, O II, O III and

Fig. 1 Example for a mutation in *IDH1* in amino acid position 132. wt Wild type sequence (green background, mut mutated sequence (shaded in red)

Amino acid - pos.	129	130	131	132	133	134	135
Amino acid - wt	I	I	G	R	H	A	Y
Nucleotide - wt	T C A T C A T A G G T C G T C A T G C T T A T						
Amino acid - mut	I	I	G	V	H	A	Y
Nucleotide - mut	T C A T C A T A G G T G T T C A T G C T T A T						
c-DNA - pos.	385	390		395		400	

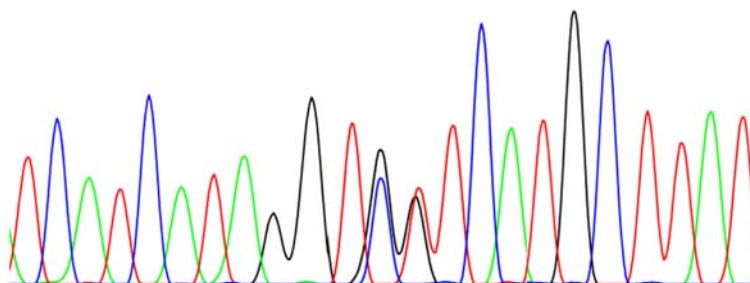


Table 2 *IDH1* mutations in 685 brain tumors

Diagnosis	N	<i>IDH1</i>		
		Wt	Mut	Mut (%)
Pilocytic astrocytoma WHO grade I (PA I)	41	40	1	2
Subependymal giant cell astrocytoma WHO grade I (SEGA I)	12	12	0	0
Pleomorphic xanthoastrocytoma WHO grade II (PXA II)	7	7	0	0
Astrocytoma WHO grade II (A II)	46	13	34	74
Anaplastic astrocytoma WHO grade III (A III)	47	18	29	62
Primary Glioblastoma WHO grade IV (prGBM)	99	92	7	7
Secondary glioblastoma WHO grade IV (secGBM)	8	1	7	88
Giant cell glioblastoma WHO grade IV (gcGBM)	8	6	2	25
Pediatric Glioblastoma WHO grade IV (pedGBM)	14	13	1	7
Gliosarcoma WHO grade IV (GS)	5	5	0	–
Oligodendroglioma WHO grade II (O II)	51	15	36	71
Anaplastic oligodendroglioma WHO grade III (O III)	54	18	36	67
Oligoastrocytoma WHO grade II (OA II)	46	10	36	78
Anaplastic oligoastrocytoma WHO grade III (OA III)	37	8	29	78
Myxopapillary ependymoma WHO grade I (E myx I)	6	6	0	–
Ependymoma WHO grade II (E II)	15	15	0	0
Anaplastic ependymoma WHO grade III (E III)	10	10	0	0
Medulloblastoma WHO grade IV (MB IV)	58	58	0	0
Primitive neuroectodermal tumor WHO grade IV (PNET)	9	6	3	33
Schwannoma WHO grade I (S I)	17	17	0	0
Meningioma WHO grade I (M I)	38	38	0	0
Atypical meningioma WHO grade II (M II)	17	17	0	0
Anaplastic meningioma WHO grade III (M III)	17	17	0	0
Pituitary adenoma WHO grade I (PIAD)	23	23	0	0

N Number of tumors, *IDH1* isocitrate dehydrogenase 1, Wt wild type, Mut mutated, Mut (%) percentage of tumors with mutation. Percentages are given for entities with seven or more tumors included

mixed OA II and OA III. On the other hand LOH1p/19q typically is detected in oligodendrogliomas but rare in astrocytomas and, vice versa, *TP53* mutations are frequent in astrocytomas but rare in oligodendrogliomas [8]. Both alterations are quite frequent in oligoastrocytomas but usually do not co-occur. Thus, the hallmark molecular findings in these tumors so far segregated between oligodendroglial and astrocytic tumors and this distribution suggested that these tumors originated from different precursor populations. In contrast, *IDH1* mutations seem to group together A II, A III, O II, O III, OA II and OA III and suggest a common pathway in their genesis. It might be that *IDH1* mutations occur in the O2A precursor cell giving rise to astrocytic and oligodendroglial lineage [11] and that *TP53* mutations and LOH1p/19q arise at a later point in cells already committed to either lineage. However, such speculation implies that oligodendroglial tumors arise from precursor cells of oligodendroglia and this has not yet been demonstrated.

With regard to GBM it is of interest that *IDH1* mutations are much more frequent in A II and A III than in prGBM which parallels the distribution of *TP53* mutations in these entities. In contrast, the frequency of mutations in both, *IDH1* and *TP53* are high in secGBM as can be expected

from tumors which by definition progress from A II or A III.

IDH1 mutations and *EGFR* amplification were inversely associated upon pooling all GBM. However, this is to be expected given the high frequency of *IDH1* in secGBM and the well established absence of *EGFR* amplification in this subgroup [16, 17]. Within the prGBM, there was no association between *IDH1* and *EGFR* mutational status.

Mutations of single genes or disruption of signal transduction pathways have been used to establish models for the molecular subclassification of gliomas [2, 6, 10, 16, 17]. The addition of the mutational status of *IDH1* adds to these models. For example, the frequency of *IDH1* mutations clearly differs between secGBM and pedGBM both of which are characterized by frequent *TP53* mutations and rare or absent *EGFR* amplifications. In contrast, oligodendroglioma, oligoastrocytoma and astrocytoma which so far were on a molecular basis distinguished on their frequency of *TP53* mutations and LOH1p/19q are unified by a high frequency of *IDH1* mutations in all three entities. Data on *TP53*, combined LOH1p/19q and *EGFR* from tumors included in this study have been published previously [8, 13, 15] and are compiled in Table 3.

Table 3 Incidence of mutations in *IDH1*, *TP53* and *EGFR* and occurrence of LOH 1p/19q in gliomas [8, 13, 15]

	<i>IDH1</i>	<i>TP53</i>	LOH 1p/19q	<i>EGFR</i> amp
Glioblastoma				
pedGBM	1/14 (7%)	2/3	0/3	0/2
prGBM	7/99 (7%)	15/88 (17%)	6/73 (8%)	26/71 (37%)
secGBM	8/8 (88%)	7/8 (88%)	1/8 (13%)	0/8 0(%)
gcGBM	2/8 (25%)	6/7 (86%)	0/6	0/7 (0%)
Astrocytoma				
A II	33/46 (79%)	13/25 (52%)	6/34 (18%)	0/17 (0%)
A III	29/47 (62%)	13/30 (43%)	5/43 (12%)	1/22 (5%)
Oligodendroglioma				
O II	36/51 (71%)	3/31 (10%)	30/50 (60%)	0/11 (0%)
O III	36/54 (67%)	4/31 (13%)	35/53 (66%)	0/4
Oligoastrocytoma				
OA II	36/46 (78%)	6/26 (23%)	24/45 (53%)	0/10 (0%)
OA III	29/37 (78%)	6/22 (27%)	26/36 (72%)	0/8 (0%)

Given are the numbers of tumors with mutations among the tumors examined for this parameter, and in brackets percentages of mutations in this series

IDH1 Isocitrate dehydrogenase 1, *TP53* tumor protein 53, *LOH 1p/19q* combined loss of heterozygosity on chromosomal arms 1p and 19q, *EGFR*amp amplification of the *EGFR* gene, *pedGBM* pediatric glioblastoma WHO grade IV, *prGBM* primary glioblastoma WHO grade IV, *secGBM* secondary glioblastoma WHO grade IV, *gcGBM* giant cell glioblastoma WHO grade IV, *A II* astrocytoma WHO grade II, *A III* anaplastic astrocytoma WHO grade III, *O II* oligodendroglioma WHO grade II, *O III* anaplastic oligodendroglioma WHO grade III, *OA II* oligoastrocytoma WHO grade II, *OA III* anaplastic oligoastrocytoma WHO grade III

Association of *IDH1* mutations with age

There was a strong association of age and presence or absence of *IDH1* mutations in patients with prGBM. Patients with prGBM and *IDH1*mut averaged 40.3 years and those with *IDH1*wt averaged 52.6 years ($P < 0.005$). This confirms previous observations [9]. Patients with A III and *IDH1*mut averaged 35.0 years and those with *IDH1*wt averaged 44.4 years ($P < 0.05$). Patients with O III and *IDH1*mut averaged 47.7 years and those with *IDH1*wt averaged 54.8 years (not significant). Patients with OA III and *IDH1*mut averaged 44.3 years and those with *IDH1*wt averaged 63.5 years ($P < 0.0005$). The average ages in patients with A II, O II and OA II did not differ significantly in the groups with and without *IDH1* mutations. An explanation for the association of *IDH1* mutations with age only in the highly malignant entities may be that these tumors sometimes are under-diagnosed due to lack of presence of necrosis resulting from tumor sampling. Therefore, these entities might contain some prGBM which do have a low frequency of *IDH1* mutations but predominantly occur in patients of advanced age. Interestingly, no significant difference is

observed in O III which among anaplastic astrocytic and oligodendroglial tumors poses least problems in differentiating from prGBM.

IDH1 and tumor progression

IDH1 mutations were frequent in A II (74%) suggesting that this alteration already is of importance for earlier steps in tumor formation. Mutations in A III (62%) were somewhat less frequent than in A II, however, this difference was not significant. *IDH1* mutations in secGBM (88%) were very frequent. In contrast, only few *IDH1* mutations were detected in prGBM (7%). Thus, all tumors belonging to the progression series from A II and A III to secGBM exhibited *IDH1* mutations in a high and comparable frequency. Similar to A II, O II (71%) and OA II (78%) exhibit a high frequency of *IDH1* mutations. Comparable frequencies are observed in O III (67%) and OA III (78%). These findings suggest that *IDH1* mutations are more likely to be important for tumor formation in diffuse astrocytomas and oligodendroglial tumors than for progression towards malignancy. The evaluation of a prognostic and predictive value of *IDH1* mutations will require analyses of large and well-documented trial studies with focus on diffuse gliomas including A II, A III, O II, O III, OA II and OA III.

Conclusions

IDH1 mutations typically occur in diffuse gliomas of astrocytic, oligodendroglial and mixed oligoastroglial differentiation and in glioblastomas having progressed from these tumors. This is contrasted by the low frequency of *IDH1* mutations in primary glioblastoma. The very high frequency of *IDH1* mutations in WHO grade II astrocytic and oligodendroglial gliomas suggests a role in early tumor development.

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