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ORIGINAL PAPER

Type and frequency of *IDH1* and *IDH2* mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas

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Abstract Somatic mutations in the *IDH1* gene encoding cytosolic NADP+-dependent isocitrate dehydrogenase have been shown in the majority of astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III. *IDH2* encoding mitochondrial NADP+-dependent isocitrate dehydrogenase is also mutated in these tumors, albeit at much lower frequencies. Preliminary data suggest an importance of *IDH1* mutation for prognosis showing that patients with anaplastic astrocytomas, oligodendrogliomas and oligoastrocytomas harboring *IDH1* mutations seem to fare much better than patients without this mutation in their tumors. To determine mutation types and their frequencies, we examined 1,010 diffuse gliomas. We detected 716 *IDH1* mutations and 31 *IDH2* mutations. We found 165 *IDH1* (72.7%) and 2 *IDH2* mutations (0.9%) in 227 diffuse astrocytomas WHO grade II, 146 *IDH1* (64.0%) and 2 *IDH2* mutations (0.9%) in 228 anaplastic astrocytomas WHO grade III, 105 *IDH1* (82.0%) and 6 *IDH2* mutations (4.7%) in 128 oligodendrogliomas WHO grade II, 121 *IDH1* (69.5%) and 9 *IDH2* mutations (5.2%) in 174 anaplastic oligodendrogliomas WHO grade III, 62 *IDH1* (81.6%) and 1 *IDH2* mutations (1.3%) in 76 oligoastrocytomas WHO grade II and 117 *IDH1* (66.1%) and 11 *IDH2* mutations (6.2%) in 177 anaplastic oligoastrocytomas WHO grade III. We report on an inverse association of *IDH1* and *IDH2* mutations in these gliomas and a non-random distribution of the mutation types within the tumor entities. *IDH1* mutations of the R132C type are strongly associated with astrocytoma, while *IDH2* mutations predominantly occur in oligodendroglial tumors. In addition, patients with anaplastic glioma harboring *IDH1* mutations were on average 6 years younger than those without these alterations.

Keywords *IDH1* · *IDH2* · Mutation · Glioma · Astrocytoma · Oligodendroglioma · Oligoastrocytoma

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Introduction

Extraordinary high rates of spontaneous mutations in the gene encoding cytosolic NADP+-dependent isocitrate dehydrogenase (*IDH1*) have been reported in diffuse gliomas of World Health Organization (WHO) grades II and III of astrocytic and oligodendroglial lineages [1, 8, 17, 21] and in lower frequency mutations in the gene encoding mitochondrial NADP+-dependent isocitrate dehydrogenase (*IDH2*) [21]. In contrast, mutations of *IDH1* are rare in primary glioblastoma [1, 2, 8, 9, 14, 17, 21]. These mutations appear to be of significant importance for survival of patients. Patients with anaplastic astrocytoma and glioblastoma show significantly longer overall survival in the presence of *IDH1* or *IDH2* mutations [21]. The analysis of a prospective study demonstrated that the absence of *IDH1* mutations in anaplastic astrocytoma, oligoastrocytoma and oligodendroglioma of WHO grade III is a strong indicator for poor prognosis [20].

Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate.

Five genes encoding human isocitrate dehydrogenases have been identified. *IDH1* on 2q33.3 encodes cytosolic NADP+-specific isocitrate dehydrogenase [13]. IDH1 is configured as a homodimer with two enzymatically active sites and most of its activity is detected in the cytosol and in peroxisomes [4]. A major function of IDH1 is believed to be the synthesis of NADPH required for reducing reactions and for lipid synthesis [16]. *IDH2* on 15q26.1 encodes the mitochondrial NADP+-specific isocitrate dehydrogenase [5]. Similar to IDH1, this enzyme functions as a homodimer. Recent findings suggest that the IDH2 may be the main catalyst for the oxidation of isocitrate to α-ketoglutarate in the citric acid cycle [6]. IDH3 is composed of three subunits encoded by *IDH3A* (subunit alpha) on 15q25.1–q25.2 [7], by *IDH3B* (subunit beta) on 20p13 [10] and by *IDH3G* (subunit gamma) on Xq28. IDH3 is a multi-tetrameric enzyme ($2\alpha1\beta1\gamma$) with α-subunits being catalytic and the β- and γ-subunits being believed to be regulatory [15, 19]. IDH3 utilizes NAD+ as a coenzyme. The function of IDH3 in the citric acid cycle is well established.

Glioma-specific mutations in *IDH1* always affected the amino acid arginine in position 132 of the amino acid sequence which belongs to an evolutionary highly conserved region located at the binding site for isocitrate [14]. Mutations in *IDH2* were exclusively detected in arginine at position 172 which is the analogous site to arginine 132 in *IDH1* [21]. Mutations in both *IDH1* and *IDH2* are heterozygous and of somatic origin. The role of *IDH1* mutations in tumor biology currently is intensely studied. Mutations inactivate enzyme activity [21]. This inactivation is due to impaired substrate affinity, and moreover, *IDH1* mutations exert a dominant negative effect rendering heterodimers inactive as well [22]. Enzyme deficiency results in depletion of α-ketoglutarate which is required for prolylhydroxylases promoting degradation of hypoxia inducible factor 1α (HIF-1α). Increased HIF-1α levels in gliomas carrying the *IDH1* R132H mutations have been demonstrated [22]. However, there are many other oxygenases which are dependent on α-ketoglutarate and which are involved in different processes such as histone modification or fatty acid metabolism [11]. Therefore, several other tumor relevant mechanisms besides HIF-1α stabilization may result from *IDH1* mutation-mediated depletion of α-ketoglutarate.

In order to determine the different types of mutations and their frequencies, we examined 1,010 human gliomas for mutations in codons 132 and 172 in the genes for *IDH1* and *IDH2*, respectively. Because high frequencies of *IDH1* mutations have been described only in few glioma subtypes, we focussed on these entities including WHO grades II and III astrocytomas, oligodendrogliomas and oligoastrocytomas in the present study.

Materials and methods

Tumor specimens average patient age and sex ratio

DNA samples from human brain tumors diagnosed at the Departments of Neuropathology of the Universities of Heidelberg, Bonn, Düsseldorf, Nijmegen, Magdeburg and at the Charité Berlin were analyzed. All tumors were diagnosed and classified according to the WHO classification of tumors of the nervous system [12]. The series consisted of 1,010 diffuse gliomas including 227 diffuse astrocytomas WHO grade II (A II), 228 anaplastic astrocytomas WHO grade III (A III), 128 oligodendrogliomas WHO grade II (O II), 174 anaplastic oligodendrogliomas WHO grade III (O III), 76 oligoastrocytomas WHO grade II (OA II) and 177 anaplastic oligoastrocytomas (OA III). The *IDH1* mutation data of 281 patients in this series have been reported in a preceding study [1]. The mean ages and female to male sex ratios were 37 years and 44–56% for A II, 42 years and 40–60% for A III, 44 years and 43–57% for O II, 49 years and 47–53% for O III, 43 years and 62–38% for OA II and 47 years and 45–55% for OA III.

PCR amplification

Primer design was based on accession numbers NM_005896 for *IDH1* and NM_002168 for *IDH2* (http://www.ncbi.nlm.nih.gov). A fragment of 129 bp length

spanning the sequence encoding the catalytic domain of *IDH1* including codon 132 was amplified using 60 ng each of the sense primer IDH1f CGGTCTTCAGAGAAGCC ATT and the antisense primer IDH1r GCAAAATCACAT TATTGCCAAC. PCR using standard buffer conditions, 20 ng of DNA and GoTaq DNA Polymerase (Promega, Madison, WI, 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 IDH1fc ACCAAA TGGCACCATACGA and antisense primer IDH1rc TTC ATACCTTGCTTAATGGGTGT generating a 254 bp fragment at the same PCR conditions were employed.

A fragment of 150 bp length spanning the sequence encoding the catalytic domain of *IDH2* including codon 172 was amplified using 60 ng each of the sense primer IDH2f AGCCCATCATCTGCAAAAAC and the antisense primer IDH2r CTAGGCGAGGAGCTCCAGT. 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 58°C for 40 s and extension at 72°C for 50 s in a total volume of 15 µl.

For confirmation, the sense primer IDH2fc GCTGC AGTGGGACCACTATT and antisense primer IDH2rc TG TGGCCTTGTACTGCAGAG generating a 293 bp fragment at the same PCR conditions were employed.

Direct sequencing

Two microliters of the PCR amplification product were subjected to sequencing using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Twenty-five cycles were performed employing 12 ng of the sense primers IDH1f CGGTCTTCAGAGA AGCCATT or IDH2f CTAGGCGAGGAGCTCCAGT, with denaturing at 95°C for 30 s, annealing at 56°C for 15 s and extension at 60°C for 240 s. In case of ambiguous readings, a second round of sequencing analysis was performed using the antisense primer IDH1rc TTCATACC TTGCTTAATGGGTGT or IDH2rc TTCATACCTTGC TTAATGGGTGT and the sequencing reaction conditions as described above. Sequences were determined using the semiautomated sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems) and the Sequence Pilot version 3.1 software (JSI-Medisys, Kippenheim, Germany).

Statistics

The Fisher exact test was used to examine associations between nominal variables referring to absence or presence of genetic alterations. The relationship of *IDH* mutations with patient age was examined by Student's *t* test.

Results and discussion

We detected 716 *IDH1* mutations and 31 *IDH2* mutations in 1,010 patients with astrocytoma, oligodendroglioma or oligoastrocytoma of WHO grades II and III. Only codon 132 of *IDH1* and codon 172 of *IDH2* were affected by mutations. Previous studies did not detect *IDH1* and *IDH2* mutations in constitutional tissues [1, 8, 14, 17, 21]. Therefore, we did not analyze corresponding blood samples from the glioma patients included in this study. In the majority of the cases, mutations in codon 132 of *IDH1* and codon 172 of *IDH2* were obvious upon sequencing in forward direction. However, we encountered cases yielding only weak peaks in addition to the signal of the wild-type sequence. These cases suspected to carry a mutation were sequenced a second time in reverse direction. Reappearance of an additional signal at the site of suspect was then scored as positive for mutation. Based on direct sequencing, all mutations appeared to be heterozygous meaning that in each tumor with a mutation only one of the two gene copies of *IDH1* or *IDH2*, respectively, was altered.

Type and frequency of *IDH1* and *IDH2* mutations

The predominant amino acid sequence alteration in *IDH1* was R132H accounting for 92.7% of the detected mutations followed by R132C for 4.1%, R132S for 1.5%, R132G for 1.4% and R132L for 0.2% of all *IDH1* mutations. Type and distribution of the mutations are given in Table 1. We did not detect the R132V mutation reported and illustrated in our previous series [1]. The distribution of mutations in the present series matches well with those of other studies demonstrating the vast majority of *IDH1* mutations being of the R132H type followed by R132C. The discrepancies in literature regarding the low frequencies of R132S, R132G and R132L may be due to differences in sample size and different types of tumors analyzed.

Table 1 Type of 716 *IDH1* and 31 *IDH2* mutations and frequency among mutations in 1,010 WHO grades II and III astrocytomas, oligodendrogliomas and oligoastrocytomas

Gene	Nucleotide change	Amino acid change	*N* (%)
IDH1	G395A	R132H	664 (92.7%)
	C394T	R132C	29 (4.2%)
	C394A	R132S	11 (1.5%)
	C394G	R132G	10 (1.4%)
	G395T	R132L	2 (0.2%)
IDH2	G515A	R172K	20 (64.5%)
	G515T	R172M	6 (19.3%)
	A514T	R172W	5 (16.2%)

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

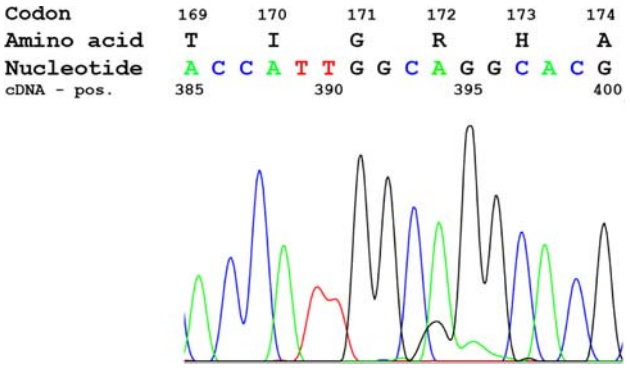

Fig. 1 Example for a sequence with a small G signal at nucleotide position 394 of *IDH2*, which we did not score as evidence for a point mutation but rather considered as a sequencing artifact due to stuttering of polymerase induced by the flanking G signals

In *IDH2*, the distribution of mutation types is less off-center. R172K made up 65%, R172M amounted to 19% and the previously not described R172W to 16% of all *IDH2* mutations. In contrast to the initial report on *IDH2* mutations, we did not find the R172G exchange. While in several instances we detected a shallow G peak in nucleotide position 514, we interpreted this signal as artificial and caused by the flanking G nucleotides in positions 511, 512, 515 and 516 of the wild-type sequence of *IDH2*. An example for such a signal interpreted as sequencing artifact is shown in Fig. 1.

The amino acid sequences of *IDH1* and *IDH2* at the sites of mutations are identical while the nucleotide sequences differ. It is noteworthy that the most frequent mutations in both *IDH1* and *IDH2* derive from a G to A transition in nucleotide position 395 of *IDH1* and 515 of *IDH2*, respectively.

IDH1 and *IDH2* mutations in astrocytoma, oligodendroglioma and oligoastrocytoma

We detected 716 *IDH1* mutations in our series. Type and distribution of the mutations are given in Table 2. A II carried mutations in 72.7% comparing well with frequencies of 74% [1], 83% [21], 88% [17] and 59% [8] reported in previous studies. *IDH1* mutations in A III were observed in 64.0% comparable to 62% [1], 69% [21], 78% [17] and 52% [8] in earlier studies. The present frequency was 82% for O II comparing to 71% [1], 82% [21], 79% [17] and 68% [8] and 69.5% for O III comparing to 67% [1], 86% [21], 75% [17] and 60% [8]. OA II carried *IDH1* mutations in 81.6 % comparing to 78% [1], 100% [21], 94% [17] and 50% [8] and OA III in 66.1% while the previous studies detected mutations in 78% [1], 100% [21], 71% [17] and 78% [8]. Thus, the frequencies in the present study are very similar to our previous series and well within the range of data published. This difference may in part be attributed to small numbers of particular subtypes of gliomas in the other studies; however, the use of different thresholds in scoring weaker signals as sufficient for a mutation may also play a role. Glioma tissues always contain a non-neoplastic cell compartment including vascular cells, reactive astrocytes, lymphocytes and microglial cells. In addition, the diffuse and infiltrative nature of glioma frequently results in a substantial fraction of residual brain in the tissue samples. Therefore, the mutant signal in gliomas heterozygous for mutations usually is less intense than the wild-type signal requiring a more or less arbitrary threshold level to separate between a signal indicating a mutation and a background peak.

Mutations in *IDH2* mutations were much less common than those in *IDH1*. We found 31 *IDH2* mutations in our series. A II and A III carried *IDH2* mutation in 0.9% each while 4.7% of O II, 5.2% of O III, 1.3% of OA II and 6.2% of OA III had *IDH2* mutations. Type and distribution of the mutations are given in Table 3. A II carried *IDH2* mutations in 0.9% which is considerably lower than the frequency of 7% previously observed [21]. Mutations in A III were observed in 0.9% and thus were also less frequent than the 4% reported in the preceding study [21]. The present frequency for O II and O III were 4.7 and 5.2% comparing well to 4 and 8% [21]. OA II and OA III carried mutations in 1.3 and 6.2% and have not previously been reported.

Table 2 716 *IDH1* codon 132 mutations in 1,010 WHO grades II and III astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III

Amino acid exchange	All tumors ($N = 1{,}010$)	A II ($N = 227$)	A III ($N = 228$)	O II ($N = 128$)	O III ($N = 174$)	OA II ($N = 76$)	OA III ($N = 177$)
R132H	664 (61.7%)	143 (63.0%)	132 (57.9%)	103 (80.5%)	111 (63.8%)	60 (78.9%)	115 (65.0%)
R132C	29 (2.9%)	17 (7.5%)	7 (3.1%)	0 (0%)	2 (1.1%)	1 (1.3%)	1 (0.6%)
R132S	11 (1.1%)	3 (1.3%)	3 (1.3%)	2 (1.5%)	4 (2.3%)	0	0
R132G	10 (1.0%)	2 (0.9%)	3 (1.3%)	0	3 (1.7%)	1 (1.3%)	1 (0.6%)
R132L	2 (0.2%)	0	1 (0.4%)	0	1 (0.6%)	0	0
All	716 (70.9%)	165 (72.7%)	146 (64.0%)	105 (82.0%)	121 (69.5%)	62 (81.6%)	117 (66.1%)

N number of tumors analyzed

Table 3 31 *IDH2* codon 172 mutations in 1,010 WHO grades II and III astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III

Amino acid exchange	All tumors ($N = 1,010$)	A II ($N = 227$)	A III ($N = 228$)	O II ($N = 128$)	O III ($N = 174$)	OA II ($N = 76$)	OA III ($N = 177$)
R172K	20 (2.0%)	2 (0.9%)	2 (0.9%)	3 (2.3%)	6 (3.4%)	1 (1.3%)	6 (3.4%)
R172M	6 (0.6%)	0	0	1 (0.8%)	2 (1.1%)	0	3 (1.7%)
R172W	5 (0.5%)	0	0	2 (1.6%)	1 (0.6%)	0	2 (1.1%)
All	31 (3.1%)	2 (0.9%)	2 (0.9%)	6 (4.7%)	9 (5.2%)	1 (1.3%)	11 (6.2%)

N number of tumors analyzed

IDH1 R132C mutations associate with astrocytoma of WHO grade II

IDH1 mutations exhibited a non-random distribution among astrocytic and oligodendroglial tumors. In our previous study, we hinted at a trend for R132C mutations to favor astrocytomas. In the present series, a total of 29 R132C mutations were observed in 17 A II, 7 A III, 2 O III and 1 OA II and OA III each. This distribution favoring astrocytomas was highly significant ($p < 0.0001$). The significance of the association of R132C with astrocytoma is further supported by a recent report. In a series of Li-Fraumeni patients with astrocytoma, only R132C mutations in *IDH1* but not the more frequent R132H mutation were observed [18]. Because sporadic astrocytomas carry somatic mutations in the *TP53* gene more frequently than oligodendrogliomas and oligoastrocytomas, and because Li-Fraumeni patients by definition have a germ line mutation in *TP53*, there seems to be an association of the *IDH1* R132C mutation with mutations in *TP53*. Whether and how *TP53* mutations favor the occurrence of *IDH* R132C mutations, as supported in the Li-Fraumeni setting, or whether *IDH1* mutations favor subsequent *TP53* mutations, as suggested by the significantly higher incidence of *IDH1* mutations than the one of *TP53* mutations in sporadic astrocytomas, remains yet unresolved. R132C mutations were more frequent in WHO grade II tumors than in WHO grade III tumors ($p < 0.05$).

IDH2 mutations associate with oligodendroglial tumors

In our series, *IDH2* mutations predominantly occurred in tumors with an oligodendroglial component. Six O II, 9 O III, 1 OA II and 11 OA III but only 2 A II and A III each carried *IDH2* mutations ($p < 0.001$). This partially contrasts the initial report of *IDH2* mutations in gliomas [21] reporting 2/51 O II, 3/36 O III, also 2/30 A II and 2/52 A III with *IDH2* mutations. OA II and OA III were not analyzed for *IDH2* in that study. Our findings are very similar with regard to O II and O III; however, they are different from those reported for A II and A III. Divergent results may to some extent be explained by the considerable interobserver variability in the distinction of astrocytoma from oligoastrocytoma [3]. The WHO criteria do allow for a significant diagnostic overlap [12] and the problem is further aggravated by tissue sampling with usually only parts of the tumor tissue being available for histological analysis. Another reason for this discrepancy may be our threshold in scoring mutations resulting in elimination of all R172G mutations reported previously.

In our series, 22 of 31 *IDH2* mutations occurred either in A III, O III or OA III. This represents a trend for an association with anaplastic tumors. While there is no doubt that *IDH1* mutations occur early in tumor formation, because the majority of A II, O II and OA II already harbor this alteration, the time point of the occurrence of *IDH2* mutations cannot definitely be set at a similar early point.

IDH1 and *IDH2* are inversely associated

In the initial report on *IDH2* mutations, only glioma samples not carrying *IDH1* mutations were analyzed for *IDH2* [21]. In order to analyze the potentially complementary effects of these mutations, we examined our entire series for both *IDH1* and *IDH2* mutations. We detected 712 tumors with an *IDH1* mutation but wild type for *IDH2*, 27 tumors with *IDH2* mutation but wild type for *IDH1* and only 4 tumors, 1 A III, 1 O III and 2 OA III, characterized by both *IDH1* and *IDH2* mutations. Interestingly, the four tumors with mutations in both *IDH1* and *IDH2* were all anaplastic gliomas. This clearly is a non-random distribution ($p < 0.00001$). Such partition indicates that either *IDH1* or *IDH2* mutations independently provide a growth advantage for mutant cells and that one of them is sufficient to mediate this advantage.

IDH1 and *IDH2* mutations and age

In patients under the age of 18 years, *IDH1* mutations were rare occurring in only 4 of 32 (12.5%) tumors and *IDH2* mutations were absent. This finding suggests that pediatric astrocytomas, oligodendrogliomas and oligoastrocytomas genetically differ from their adult counterparts. The mean age of adult patients aged 18 or older with anaplastic

gliomas of WHO grades III carrying *IDH1* mutations was 43.9 years while anaplastic glioma patients without mutations averaged 50.6 years ($p < 0.0001$). In all three groups, A III, O III and OA III patients with mutations on average were younger than patients without mutations (A III, $p < 0.01$; O III, not significant; OA III, $p < 0.01$). The average age of patients with gliomas of WHO grade II carrying *IDH1* mutations was 41.3 years while glioma patients without mutations averaged 42.8 years (not significant). Patients carrying the most common R132H mutation averaged 42.9 years while patients with the R132C mutation were significantly younger averaging 34.9 years ($p < 0.01$), those with the R132G mutation were 37.9 years old (not significant) and those carrying R132S averaged 36.2 years ($p < 0.01$).

Conclusions

The present study provides a reliable basis for the frequencies and types of *IDH1* codon 132 and *IDH2* codon 172 mutations in diffusely infiltrating astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III. The data confirm a high frequency of *IDH1* mutation in these tumors, with 70.9% of all investigated tumors carrying an *IDH1* codon 132 mutation, most commonly of the R132H type. In contrast, *IDH2* mutations were restricted to 3.1% of the tumors. Our data confirm a mutually exclusive presence of either *IDH1* or *IDH2* mutation in gliomas. Furthermore, we show that the R132C *IDH1* mutation is significantly associated with astrocytic histology, while *IDH2* mutations are more common in oligodendroglial tumors as compared to astrocytomas. Further studies need to address the clinical impact of the individual *IDH1* and *IDH2* mutations with respect to their potential role as prognostic markers in patients with diffuse gliomas.

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References

1. Balss J, Meyer J, Mueller W et al (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602
2. Bleeker FE, Lamba S, Leenstra S et al (2009) IDH1 mutations at residue p.R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat 30:7–11
3. Coons SW, Johnson PC, Scheithauer BW et al (1997) Improving diagnostic accuracy and interobserver concordance in the classification and grading of primary gliomas. Cancer 79:1381–1393
4. Geisbrecht BV, Gould SJ (1999) The human PICD gene encodes a cytoplasmic and peroxisomal NADP(+)-dependent isocitrate dehydrogenase. J Biol Chem 274:30527–30533
5. Grzeschik KH (1976) Assignment of a gene for human mitochondrial isocitrate dehydrogenase (ICD-M, EC 1.1.1.41) to chromosome 15. Hum Genet 34:23–28
6. Hartong DT, Dange M, McGee TL et al (2008) Insights from retinitis pigmentosa into the roles of isocitrate dehydrogenases in the Krebs cycle. Nat Genet 40:1230–1234
7. Huh TL, Kim YO, Oh IU et al (1996) Assignment of the human mitochondrial NAD+-specific isocitrate dehydrogenase alpha subunit (IDH3A) gene to 15q25.1→q25.2 by in situ hybridization. Genomics 32:295–296
8. Ichimura K, Pearson DM, Kocialkowski S et al (2009) IDH1 mutations are present in the majority of common adult gliomas but are rare in primary glioblastomas. Neurol Oncol. 12 May 2009. [Epub ahead of print]
9. Kang MR, Kim MS, Oh JE et al (2009) Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. Int J Cancer 125(2):353–355
10. Kim YO, Park SH, Kang YJ et al (1999) Assignment of mitochondrial NAD(+)-specific isocitrate dehydrogenase beta subunit gene (IDH3B) to human chromosome band 20p13 by in situ hybridization and radiation hybrid mapping. Cytogenet Cell Genet 86:240–241
11. Loenarz C, Schofield CJ (2008) Expanding chemical biology of 2-oxoglutarate oxygenases. Nat Chem Biol 4:152–156
12. Louis DN, Ohgaki H, Wiestler OD et al (2007) WHO classification of tumours of the central nervous system. In: World Health Organization classification of tumours, 4th edn. International Agency for Research on Cancer, Lyon
13. Narahara K, Kimura S, Kikkawa K et al (1985) Probable assignment of soluble isocitrate dehydrogenase (IDH1) to 2q33.3. Hum Genet 71:37–40
14. Parsons DW, Jones S, Zhang X et al (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812
15. Ramachandran N, Colman RF (1980) Chemical characterization of distinct subunits of pig heart DPN-specific isocitrate dehydrogenase. J Biol Chem 255:8859–8864
16. Shechter I, Dai P, Huo L et al (2003) IDH1 gene transcription is sterol regulated and activated by SREBP-1a and SREBP-2 in human hepatoma HepG2 cells: evidence that IDH1 may regulate lipogenesis in hepatic cells. J Lipid Res 44:2169–2180
17. Watanabe T, Nobusawa S, Kleihues P et al (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:1149–1153
18. Watanabe T, Vital A, Nobusawa S et al (2009) Selective acquisition of IDH1 R132C mutations in astrocytomas associated with Li-Fraumeni syndrome. Acta Neuropathol 117:653–656
19. Weiss C, Zeng Y, Huang J et al (2000) Bovine NAD+-dependent isocitrate dehydrogenase: alternative splicing and tissue-dependent expression of subunit 1. Biochemistry 39:1807–1816
20. Wick W, Stoffels M, Engel C et al (2009) NOA-04 randomized phase III study of sequential radiochemotherapy of anaplastic glioma with PCV or temozolomide. J Clin Oncol (in press)
21. Yan H, Parsons DW, Jin G et al (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773
22. Zhao S, Lin Y, Xu W et al (2009) Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 324:261–265