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IDH mutations occur frequently in Chinese glioma patients and predict longer survival but not response to concomitant chemoradiotherapy in anaplastic gliomas

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Abstract. Mutations in the isocitrate dehydrogenase 1 and 2 genes (*IDH1* and *IDH2*) appear to occur frequently and selectively in gliomas. Our aim was to assess whether *IDH* mutations are common in Chinese glioma patients and whether the mutations predict good response to concomitant chemoradiotherapy. In this study *IDH1* and *IDH2* mutations were detected in a series of 203 gliomas. *IDH1* mutations were present in 75 of the 203 cases (36.9%) while *IDH2* mutations in 5 of the 203 cases (2.5%). No tumor was mutated in both *IDH1* and *IDH2*. *IDH1/2* mutations were associated with prolonged overall survival in the whole series of patients exclusive of pilocytic astrocytoma (P<0.001), WHO grade II patients who received no adjuvant therapy after surgery (P=0.014) and WHO grade III patients who received concomitant chemoradiotherapy (standard schedule) after surgery (P=0.033). Furthermore, there was no correlation between *IDH1/2* mutations and reponse to concomitant chemoradiotherapy in anaplastic gliomas. Our results suggest that *IDH1* mutations also occur freuqently in Chinese glioma patients but the frequency of *IDH1* mutations is below the findings reported by North American and European groups. Furthermore, we confirm the prognostic significance of *IDH1/2* mutations in gliomas, but the mutations cannot predict a favorable response to concomitant chemoradiotherapy in anaplastic gliomas.

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Introduction

Gliomas are the most frequent and lethal brain tumors and display a wide diversity with location, morphology, genetic status and response to therapy. These tumors have been classified as grade I to grade IV based on histopathological and clinical criteria established by the World Health Organization (WHO) (1). Despite intensive therapies, including surgery, radiotherapy (RT) and chemotherapy (CT), the outcome of glioma patients remain depressing (2,3). Especially, glioblastoma multiforme (GBM), the most prevalent form of brain tumors, has one of the worst prognosis among all types of gliomas with a median progression-free survival (PFS) of 6.9 months and a median overall survival (OS) of 14.6 months through surgery plus standard concomitant chemoradiotherapy (CCRT) (2,4).

A combined understanding of the genetic basis and pathology of gliomas provides insight into biologically based tumor classification and identifies molecular prognostic biomarkers. In turn, this information is the route by which the most effective therapy can be focused (5). The latest breakthrough came in 2008, when the gene encoding isocitrate dehydrogenase 1 (IDH1) was initially found to be mutated in approximately 12% of GBM (6) followed by the observation that it was mutated in the majority of WHO grade II and III gliomas (7-11). IDH1 (encoded by *IDH1* gene on chromosomal 2q33.3 and located in the cytoplasm and peroxisomes) or its mitochondrial counterpart IDH2, is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate thereby leading to NADPH (Nicotinamide Adenine Dinucleotide Phosphate) production (12). In the vast majority of the cases, *IDH1* mutations affect the amino acid arginine at position 132 of the amino acid sequence while *IDH2* mutations at position 172 of the amino acid sequence. Tumors without *IDH1* mutations often have *IDH2* mutations (11), but *IDH2* mutations are much less common (8). *IDH1* mutation results not only in a dramatic decrease of IDH1 activity (6,11,13), but also in a gain of novel function enabling the conversion of α-ketoglutarate to 2-hydroxyglutarate in a NADPH-consuming manner (14,15).

Table I. Subgroups of patients.

WHO grade	No adjuvant therapy n (%)	RT n (%)	CT n (%)	CCRT n (%)	Total
I	5 (100)	0	0	0	5
II	53 (63.9)	12 (14.5)	9 (10.8)	9 (10.8)	83
III[a]	16 (23.9)	3 (4.5)	3 (4.5)	45 (67.1)	67
IV[a]	19 (42.2)	4 (8.9)	3 (6.7)	19 (42.2)	45
Total[b]	93 (46.5)	19 (9.5)	15 (7.5)	73 (36.5)	200

RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy. [a]Part of patients did not undergo adjuvant therapy after surgery because of their refusal or lack of money; [b]Three patients were excluded from the subgroups because it was unknown whether they received adjuvant therapy.

The impact of *IDH1/2* mutations on clinical outcome has been demonstrated in prospective clinical studies (10,16,17) as well as in various retrospective studies. There are also reports on the correlation between *IDH1/2* mutations and response to temozolomide (TMZ) in gliomas (18,19). However, no report is currently available regarding the predictive value of *IDH* mutations in patients treated with CCRT following surgery. In the present study, we retrospectively analyzed a cohort of 203 Chinese glioma samples for *IDH1/2* mutations. This study aimed: i) to discern whether *IDH* mutations are common in Chinese glioma patients and ii) whether the mutations predict response to CCRT in anaplastic gliomas.

Materials and methods

Tumor samples. The protocol was approved by the Ethics Committee of Nanfang Hospital and all patients provided written informed consent for molecular studies of their tumor. Clinical data were retrieved from the hospital patient records. Formalin-fixed and paraffin-embedded (FFPE) archival tumor specimens were centralized at the Department of Pathology of Nanfang Hospital. After agreed pathological review by two independent neuropathologists, sufficient tissue sections with the highest proportion of malignant cells were cut for analysis.

Molecular analysis of IDH1 and IDH2 mutations. Genomic DNA was extracted from FFPE tissues using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. *IDH1* and *IDH2* alterations of the mutational hotspot codons R132 and R172 were assessed by both High resolution melting (HRM) analysis (20) and bidirectional cycle sequencing of PCR-amplified fragments, which were generated during the HRM procedure with the PCR primers. Primers used were *IDH1*-forward 5'-CGGTCTTCAGAGAAGCCATT-3' and *IDH1*-reverse 5'-GCAAAATCACATTATTGCCAAC-3' and *IDH2*-forward 5'-CCAAGCCCATCACCATTG-3' and *IDH2*-reverse 5'-ACTGGAGCTCCTCGCCTAGC-3'.

Evaluation of response to CCRT. A subgroup of 45 patients with anaplastic glioma received CCRT as first-line post-operative treatment, allowing analysis of molecular predictors of treatment response. Patients received CCRT strictly under the European Organization for Research and Treatment of Cancer (EORTC) TMZ protocol (2). Follow-up was based on clinical examination and brain MRI with gadolinium infusion repeated every 2 months. Response of brain tumors were assessed using MacDonald criteria (21) and disease progression was defined as >25% increase in T2 hypersignal or contrast enhancement, or tumor-related neurologic deterioration exclusive of pseudoprogression and pseudoresponse that easily confused the assessment of outcome (22).

Statistical methods. All statistical analyses were done with SPSS13.0 for Windows. The χ^2 test was used to assess the genotype distribution. The independent-samples T-test was used to compare data acquired in each group for the patient age. PFS and OS were both used to study the prognostic impact of the analyzed variables. PFS was calculated from the start of the surgery until the first unequivocal clinical or radiologic sign of progressive disease or last follow-up (for censored cases). OS was defined as the time between the first surgery and death or last follow-up (for censored cases). Survival distributions were estimated by Kaplan-Meier method and compared among patient subsets using log-rank tests. All statistical tests were two-sided, and the threshold for statistical significance was P=0.05. Patients who died within 2 weeks for high-grade glioma (HGG) and 2 months for low-grade glioma (LGG) after surgery were excluded from analysis to avoid the inclusion of cases in which death may have been attributable to surgical complications.

Results

Frequency and type of IDH1/2 mutations. The main clinical characteristics of patients are summarized in Tables I and II. Gender ratio was 1.26 (113 men and 90 women), and median age was 36.4 years (range 2-78 years). Of the whole series of 203 gliomas, HRM demonstrated 83 samples with *IDH1/2* mutations (Fig. 1, part of the whole samples) while direct sequencing resulted in consensus of 76 cases. Genetic retesting of cases with conflicting findings resulted in a match of 80/80 cases. *IDH1* mutations were found in 75 cases (36.9%). All mutations were located at amino acid residue 132 with 74 of the R132H (G395A Arg132His) and 1 of the R132S (C394A

Table II. Characteristics of the patients.

Variables	All patients	*IDH1* or *2* mutation	No *IDH* mutation	P-value
Age (years)				0.003
Median	36.4	38.4	44.3	
Gender, n (%)				0.671
Male	113 (55.7)	46(57.5)	67 (54.5)	
Female	90 (44.3)	34(42.5)	56 (45.5)	
WHO-PS, n (%)				0.311
0	33 (16.3)	16(20.0)	17 (13.8)	
1-2	98 (48.3)	40(50.0)	58 (47.2)	
3-4	72 (35.5)	24(30.0)	48 (39.0)	
Extent of surgery, n (%)				0.006
Biopsy/PR	33 (16.3)	6 (7.5)	27 (22.0)	
STR/GTR	170 (83.7)	74(92.5)	96 (78.0)	

WHO-PS, World Health Organization-Performance Score; GTR, gross total resection; PR, partial resection; STR, subtotal resection.

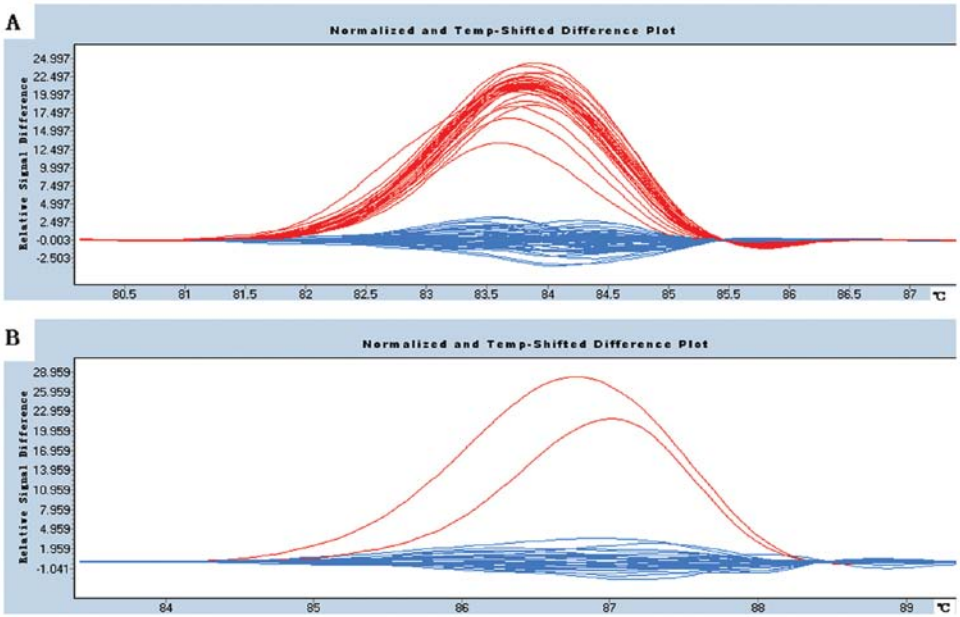

Figure 1. Different plots for the normalized and temperature shifted melting curves for *IDH1* (A) and *IDH2* (B). The melting curves associated with identified mutations of the *IDH1* and *IDH2* gene (shown in red) were clearly distinguishable from the wild-type samples (blue lines).

Arg132Ser) (Fig. 2A, B). When we next detected *IDH2* codon 172 mutation in the whole series, we found a total of 5 *IDH2* mutations (2.5%, 5/203) with 4 of the R172K (G515A Arg172Lys) and 1 of the R172M (G515T Arg172Met) (Fig. 2C, D). No tumor was mutated in both *IDH1* and *IDH2*. There was a significant difference in the median age (exclusive of 33 child patients, 38.4 vs. 44.3 years, P=0.003; Independent-samples T-test) and extent of surgery between patients with and without *IDH1/2* mutations (P=0.006), but no differences in gender and World Health Organization-Performance Score (WHO-PS) between patients with and without *IDH1* mutations. Table III recapitulates the frequency of *IDH1/2* mutations in different pathological types.

Association between IDH1/2 mutations and survival (Table IV). Median follow-up time was 22.6 months (range 2.9-79.9 months). When the correlation of *IDH1/2* mutations with PFS and OS was analyzed within all patients exclusive of pilocytic astrocytoma (because *IDH* mutations rarely occur in pilocytic astrocytoma (23) and no *IDH1/2* mutation was identified in our series), there was apparent difference between patients with and without *IDH1/2* mutations with respect to OS (P<0.001; Fig. 3A) and PFS (P<0.001; Fig. 3B) independent of WHO grade and adjuvant therapy. We next investigated the prognostic impact of *IDH1/2* mutations in WHO grade II patients without adjuvant therapy and WHO grade III patients with CCRT respectively. *IDH1/2* mutations were also signifi-

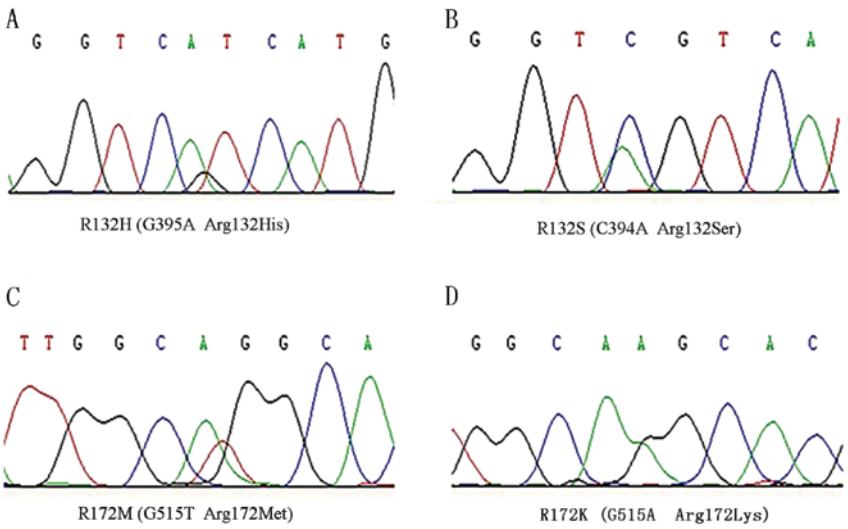

Figure 2. Different types of *IDH1* and *IDH2* mutation by gene sequencing.

Table III. *IDH1* and *IDH2* mutations in different pathological types of gliomas.

Pathological diagnosis	n (203)	WHO grade	*IDH1* mutation no. tumors	*IDH2* mutation no. tumors	Combined mutation (%)
Pilocytic astrocytoma	5	I	0	0	0
Ganglioglioma	4	II	2	0	50
Diffuse astrocytoma	60	II	28	1	48
Oligodendroglioma	8	II	4	1	63
Oligoastrocytoma	14	II	7	1	57
Anaplastic astrocytoma	49	III	17	1	38
Anaplastic oligoastrocytoma	11	III	7	1	73
Anaplastic oligodendroglioma	7	III	4	0	57
Primary glioblastoma	38	IV	4	0	11
Secondary glioblastoma	7	IV	3	0	43

cantly associated with better survival in the former subgroup (OS, P=0.014). In the latter subgroup, patients with *IDH1/2* mutations had a median OS of 38.37 months (P=0.033; Fig. 3C) and a median PFS of 20.53 months (P=0.003; Fig. 3D), whereas patients without *IDH1/2* mutations had a median OS of 17.40 months and a median PFS of 8.23 months.

Correlation between IDH1/2 mutations and response to CCRT in anaplastic gliomas. Among the 45 anaplastic glioma patients with CCRT included in the correlative analysis, 29 (64.4%) had *IDH1/2* mutations and 16 (35.6%) had no *IDH1/2* mutations. The proportion of cases with disease progression within 10 months displayed no significant difference between *IDH*-mutated group and *IDH*-wild group (50.0 and 20.7% respectively, two-sided Fisher's exact test, P=0.053). There was no difference in the proportion of cases with disease progression within 16 months between *IDH*-mutated group and *IDH*-wild group (66.8 and 44.8% respectively, two-sided Fisher's exact test, P=0.212). Therefore, *IDH1/2* mutations cannot predict good response to CCRT in anaplastic gliomas.

Discussion

The frequency of *IDH1/2* mutations in gliomas has recently been firmly established but there is no related reports in Chinese glioma patients to date. The frequency of *IDH1* mutations in this series (grade II-III gliomas, 40-50%) is below the findings reported by North American and European groups (50-70%) (7-11,13,24,25). Besides, the types of *IDH1/2* mutations in our data (Fig. 2; *IDH1*: G395A Arg132His, C394A Arg132Ser; *IDH2*: G515A Arg172Lys, G515T Arg172Met) are also less than the previously reported ones (*IDH1*: G395A Arg132His, C394T Arg132Cys, C394A Arg132Ser, G395T Arg132Leu, C394G Arg132Gly; *IDH2*: Arg172Gly A514G, G515A Arg172Lys, G515T Arg172Met, A514T Arg172Try). Samples without *IDH1/2* mutations were re-tested by HRM and bidirectional cycle sequencing, but no additional *IDH1/2* mutations was found. Interestingly, others also suggested that *IDH* mutations were common in AML but were rather rare in Chinese patients with other types of hematological disorders in contrast with the findings reported by North American and

Table IV. PFS and OS in different subgroups.

Subgroup	No.	Median OS (months)	Log-rank χ^2	Log-rank P-value	Median PFS (months)	Log-rank χ^2	Log-rank P-value
Patients (except grade I)	195		23.56	0.001		26.05	0.001
IDH-mut	80	57.34			56.87		
IDH-wild	115	21.30			13.70		
WHO grade II[a]	52		5.99	0.014		NA	NA
IDH-mut	25	NA			NA		
IDH-wild	28	NA			NA		
WHO grade III[b]	45		4.54	0.033		8.79	0.003
IDH-mut	29	38.37			20.53		
IDH-wild	16	17.40			8.23		

[a]Patients who received no adjuvant therapy after surgery; [b]Patients who received CCRT (standard schedule) after surgery; NA, not available. Part of data can not be available because the follow-up time is not long enough, especially for WHO grade II patients. However, the trend of difference still can be concluded from P-value.

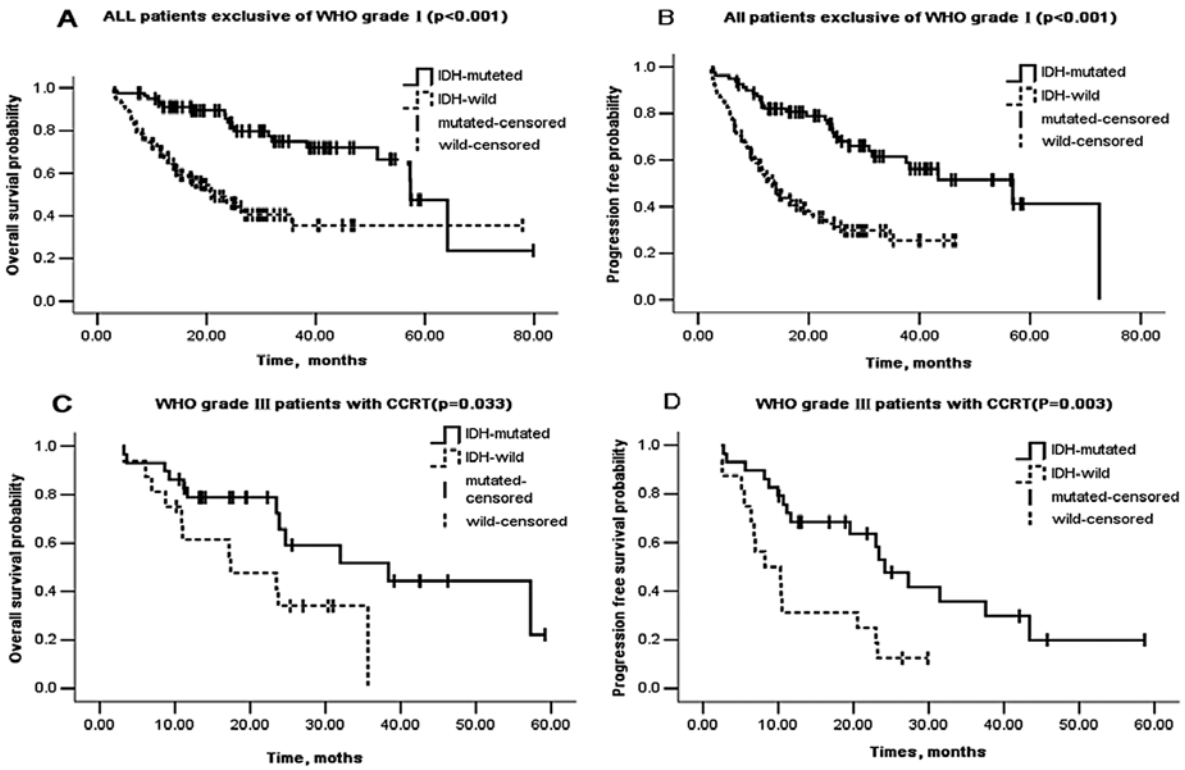

Figure 3. Kaplan-Meier estimate of overall survival and progression-free survival based on *IDH* status for different subgroups of patients. [(A, B), n=195; (C, D), n=45].

European groups (26). These data suggest that *IDH* mutations may have racial and geographical difference. Additional large sample studies in Chinese glioma patients are required to confirm the potential difference. Our data also confirm that *IDH1* mutations occur frequently whereas *IDH2* mutations rarely. As previously reported (27,28), these two mutations are mutually exclusive (100% of cases in our series), suggesting that they are involved in similar tumorigenesis pathways. *IDH1* and *IDH2* mutations were therefore grouped together in our prognostic analysis. Consistent with former reports that *IDH1/2* mutations predominantly occurred in younger patients (6,7,9,11) except for children (29,30), patients harboring *IDH1/2* mutations were younger than those without these alterations in our series after excluding 33 child patients.

High throughput analysis has recently resulted in the identification of *IDH* mutation as a novel prognostic marker

in gliomas (6,7,11,17), and there is consensus on patients with *IDH* mutations performing better than those without (6,9,11,13,18,31-33). In multivariate analyses, *IDH1/2* mutation was an independent favorable prognostic marker in HGGs (13,17,33,34) and LGGs (13,35). Our results are in good agreement with these studies since we found a strong correlation between *IDH1/2* mutations and overall survival in the whole series exclusive of pilocytic astrocytoma, in WHO grade II patients without adjuvant therapy and WHO grade III patients with CCRT, respectively (Table IV, Fig. 3). Furthermore, different from previous reports, in our analysis we have taken into account the potential impact of adjuvant treatment on prognosis in gliomas.

However, few data are available regarding the potential predictive value of *IDH* mutations on adjuvant therapy after surgery either in LGGs or HGGs. Dubbink *et al* (18) retrospectively investigated the correlation of *IDH1/2* mutations with response to TMZ in a cohort of patients with LGG treated with TMZ at the time of progression after RT and indicated that *IDH1* mutations were unrelated to the TMZ response. Others also suggested that the presence of *IDH1* mutations had no predictive significance for outcome to procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea and vincristine (PCV) chemotherapy (16). In contrast, Houillier *et al* (19) studied a group of LGGs who received up-front TMZ before any evidence of anaplastic transformation and concluded that *IDH11/2* mutations predicted response to TMZ in LGGs. Our data show for the first time that *IDH1/2* mutations do not correlate with respose to CCRT in anaplastic gliomas although they are associated with prolonged PFS and OS in this subgroup. Intriguingly, recent studies (16,17) have suggested that *MGMT* promoter methylation, which is a powerful predictor of response to alkylating chemotherapy (36-38), is also prognostic but not predictive for response to CT or CCRT in anaplastic gliomas. All these results may suggest that at present, the prolonged survival in *IDH*-mutated or *MGMT*-methylated gliomas result primarily from a less aggressive biological behavior, but not because of an improved outcome to CT or CCRT treatment.

IDH mutations seem to play a central role in the pathogenesis of gliomas and define a subtype of gliomas with specific biological behavior. But at present, the underlying mechanism of *IDH* mutations in tumorigenesis and their prognostic significance is still not clear (39). Further research should be undertaken to open entire new horizon of potential individualized treatment tailored to gene expression profiling and clinical trials for glioma (40).

In conclusion, we screened *IDH* mutations in a cohort of Chinese glioma patients and found a relatively low frequency of *IDH1* mutations compared with the reports by North American and European groups. Besides we confirm the prognostic significance of *IDH* mutations in gliomas, but the mutations cannot predict a favorable response to CCRT in anaplastic gliomas.

Acknowledgements

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