Mutation Analysis of IDH1 in Paired Gliomas Revealed IDH1 Mutation Was Not Associated with Malignant Progression but Predicted Longer Survival

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Abstract

Recurrence and progression to higher grade lesions are characteristic behaviors of gliomas. Though IDH1 mutation frequently occurs and is considered as an early event in gliomagenesis, little is known about its role in the recurrence and progression of gliomas. We therefore analysed IDH1 and IDH2 status at codon 132 of IDH1 and codon 172 of IDH2 by direct sequencing and anti-IDH1-R132H immunohistochemistry in 53 paired samples and their recurrences, including 29 low-grade gliomas, 16 anaplastic gliomas and 8 Glioblastomas. IDH1/IDH2 mutation was detected in 32 primary tumors, with 25 low-grade gliomas and 6 anaplastic gliomas harboring IDH1 mutation and 1 low-grade glioma harboring IDH2 mutation. All of the paired tumors showed consistent IDH1 and IDH2 status. Patients were analyzed according to IDH1 status and tumor-related factors. Malignant progression at recurrence was noted in 22 gliomas and was not associated with IDH1 mutation. Survival analysis revealed patients with IDH1 mutated gliomas had a significantly longer progression-free survival (PFS) and overall survival (OS). In conclusion, this study demonstrated a strong tendency of IDH1/IDH2 status being consistent during progression of glioma. IDH1 mutation was not a predictive marker for malignant progression and it was a potential prognostic marker for gliomas of Chinese patients.

Introduction

Gliomas are the most common primary brain tumors, accounting for 80% of malignant central nervous system neoplasms [1]. Recent genome-wide mutational analysis has demonstrated that the incidence of IDH1 mutations in gliomas ranges from 5% in primary glioblastoma (GBM) to 70% in anaplastic astrocytomas (AA) and 80% in secondary GBM [2-6]. Patients with high-grade astrocytomas with IDH1 mutations were reported to have a better survival [6]. The IDH1 gene is located on 2q33.3 and its mutation has been described in a very restricted number of human cancers including gliomas [3,7,9]. The most common IDH1 mutation is a heterozygous missense mutation with a change of guanine to adenine at position 395 (G395A), leading to the replacement of arginine by glycine at position 132 (G395A) [11]. Accumulation of this oncometabolite induce extensive DNA hypermethylation, leading to genome-wide epigenetic changes and predisposing cells toward neoplastic transformation [12].

In spite of all the studies, the role of IDH1 mutation in the recurrence of gliomas is unknown. There have been few studies in which paired gliomas at primary presentation and recurrence were studied by molecular means. In the present study, we investigated the mutational status of IDH1 and IDH2 in 53 pairs of primary and recurrent gliomas. All pairs showed consistent IDH1/IDH2 status. Correlation analysis with clinicopathological parameters revealed that IDH1 mutation was not associated with malignant progression but was a potential prognostic marker for progression-free survival (PFS) and overall survival (OS) in astrocytomas.
Patients and Methods

Ethics Statement
This study was approved by the Ethics Committee of Shanghai Huashan Hospital and the New Territories East Cluster-Chinese University of Hong Kong Ethics Committee.

Patients and Tissue Samples
Records of patients with glioma diagnosed in the Department of Neurosurgery, Huashan hospital (Shanghai, China) and Department of Anatomical and Cellular Pathology, Prince of Wales Hospital (Hong Kong) between 1990 and 2011 were reviewed. 53 paired cases were retrieved where formalin-fixed paraffin embedded (FFPE) tissues were available from primary presentations and recurrences (Table S1). Haematoxylin & eosin (H&E) stained sections of each tumor were reviewed and graded according to the 2007 WHO classification of tumors of central nervous system.

Mutation Analysis of IDH1/IDH2
Mutational hotspots of IDH1 at codon 132 and IDH2 at codon 172 were evaluated by direct sequencing. Representative tumor area scrapped off from dewaxed sections into microfuge tubes were resuspended in 10 mMTris-HCl buffer, pH 8.5. Proteinase K was added to a final concentration of 2g/l and the mixture was incubated at 55°C for 2 hours and then at 98°C for 10 min. The PCR mixture of 10 μl volume contained 1–2 μl of crude cell lysate, 10 mMTris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.2 mM of each deoxyribonucleoside triphosphate, 0.4 mM of each primer (IDH1-F: 5’-CGGTCTTCAGAGAAGCCATT-3’ and IDH1-R: 5’-CACATTATTGCCAACATGAC-3’; IDH2-F: 5’-AGGCGCATCCTGCAAAAAC-3’ and IDH2-R: 5’-CTAGGCGAGGCTCCAGT-3’) [5,9] and 0.2 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Hong Kong). PCR was initiated at 95°C for 10 min, followed by 45 cycles of 95°C for 20 sec, 60°C for 20 sec and 72°C for 30 sec, and a final extension step of 72°C for 3 min. Products were then treated with exonuclease I and alkaline phosphatase (TakaRa, Japan). Sequencing was performed using BigDye Terminator Cycle Sequencing kit v1.1. The products were resolved in the Genetic Analyzer 3130xl and analyzed by Sequencing Analysis software. All base changes were confirmed by sequencing of a newly amplified fragment.

Immunohistochemistry of IDH1-R132H
FFPE tissue sections of 4 micron thickness were deparaffinized in xylene and rehydrated in graded alcohols. Antigen retrieval was carried out by treating the sections in 1 m Methylenediamine solution (pH 8.0) in a microwave oven. After antigen retrieval, the slides were processed by BenchMark XT automated tissue staining systems (Ventana Medical Systems, Inc., Tucson, U.S.A.) using validated protocols. Tissue sections were incubated at 37°C for 32 min with mouse monoclonal anti-IDH1-R132H antibody (1:50 dilution; Dianova, Hamburg, Germany) followed by incubation with UltraView HRP-conjugated multimer antibody reagent (Ventana). Antigen detection was performed using Ultra View diaminobenzidine chromogen step (Ventana). Tissues were counterstained with hematoxylin. The presence of cytoplasmic staining indicated positivity for IDH1-R132H.

Statistical Analysis
Statistical analysis was performed by PASW Statistics 18 (version 18.0.0; SPSS, Inc.). The Chi square test (or Fisher exact test when one subgroup was ≤5) was used to examine association
Table 2. IDH1/IDH2 status of primary and recurrent gliomas.

| Tumor grade | Initial tumor | Recurrent tumor |
|-------------|---------------|-----------------|
|             | IDH1/IDH2 mutant | IDH1/IDH2 wild type | Total no. | IDH1/IDH2 mutant | IDH1/IDH2 wild type | Total no. |
| LGG         | 26 (90%)       | 3 (10%)         | 29       | 10 (77%)       | 3 (23%)         | 13       |
| AG          | 6 (38%)        | 10 (63%)        | 16       | 11 (61%)       | 7 (39%)         | 18       |
| GBM         | 0 (0%)         | 8 (100%)        | 8        | 11 (50%)       | 11 (50%)        | 22       |

LGG: low grade glioma; AG: anaplastic glioma; GBM: Glioblastoma.

Only one case of oligoastrocytoma (WHO grade II) harbored IDH2 mutation and progressed to anaplastic oligoastrocytoma upon recurrence.

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Results

Primary Tumors

The primary tumor cohort consisted of 29 low grade gliomas (WHO grade II) (17 diffuse astrocytomas, 7 oligoastrocytomas and 5 oligodendrogliomas), 16 anaplastic gliomas (WHO grade III) (8 anaplastic astrocytomas, 3 anaplastic oligodendrogliomas, 1 anaplastic oligoastrocytoma, 1 anaplastic ganglioglioma and 3 anaplastic ependymomas) and 8 glioblastomas (GBM) (WHO grade IV). WHO grade II was defined as low grade glioma (LGG), while WHO grade III and IV were defined as high grade. The mean and median age of the patients was 39.5 and 38 years, respectively (range 5 to 67). The male/female ratio of the cohort was 1:0.83. 96% (51/53) of cases were supratentorial tumors and 4% (2/53) of cases were infratentorial tumors.

Recurrent Tumors

Recurrent tumors with malignant progression. Malignant progression occurred in 42% (22/53) of the primary tumors. 55% (16/29) of LGGs underwent malignant transformation upon recurrence, with eight cases recurred as anaplastic gliomas while eight cases progressed to GBM. Similarly, 38% (6/16) of anaplastic gliomas progressed to GBM upon recurrence. (Table 1).

Recurrent tumors with histological grade same as primary tumor. 58% (31/53) of the primary tumors had recurrence with same histological grade as the corresponding primary tumors, including 45% (13/29) of LGGs, 63% (10/16) of anaplastic gliomas and 100% (8/8) of GBM. (Table1).

IDH1/IDH2 Mutation

IDH1/IDH2 mutation analysis by direct sequencing and anti-IDH1-R132H immunohistochemistry revealed 60% (32/53) of the primary tumors harboring IDH1 or IDH2 mutations, which included 90% (26/29) of LGG, 38% (6/16) of anaplastic gliomas and none of the primary GBM. All of the recurrent tumors showed consistent IDH1/IDH2 status as the corresponding primary tumors. Result of anti-IDH1-R132H immunohistochemistry was 100% concordant with direct sequencing. Among the 32 mutations detected, 91% (29/32) was IDH1-R132H, 3% (1/32) was IDH1-R132S, 3% (1/32) IDH1-R132G and 3% (1/32) was IDH2-R172K. IDH1/IDH2 mutation was observed in 90% (26/29) of primary LGGs, 38% (6/16) of primary anaplastic gliomas and none (0/8) of the primary GBM. Similarly among the recurrent tumors, IDH1/IDH2 mutation was detected in 77% (10/13) of LGGs, 61% (11/18) of anaplastic gliomas and 50% (11/22) of GBM, with 79% (11/14) of secondary GBM harbored the mutation. (Table 2).

Relationship between IDH1 Mutation and Malignant Transformation

In LGGs, 94% (15/16) of tumors with malignant transformation upon recurrence harbored IDH1 mutation, whereas 77% (10/13) of tumors recurring without malignant transformation harbored IDH1 mutation (p = 0.299). One case of oligoastrocytoma (WHO grade II) harbored IDH2 mutation and recurred as anaplastic oligoastrocytoma (WHO grade III). In patients with anaplastic gliomas, 50% (3/6) of tumors progressing to GBM upon recurrence were IDH1 mutated and 30% (3/10) of tumors without malignant transformation upon recurrence had IDH1 mutation (p = 0.607). Therefore, we did not observe any association between IDH1 mutation and malignant transformation (Table 3).

Survival analysis

Survival data was available in all of the patients in this study. The median follow-up time, PFS and OS were 161.6 months, 25.4 months and 63.1 months, respectively. Univariate analysis showed advanced WHO grade, age over 50 years, astrocytic phenotype and wild type IDH1 were poor prognostic factors for OS (Figure S1a to 1c, Figure 1A, Table 4). Advanced WHO grade, age over 50 years and wild type IDH1 were associated with shorter PFS (Figure S1d to 1f, Figure 1B, Table 4). Further analysis in astrocytomas (AII and AIII) revealed the association between...
IDH1 mutation and prognostic outcome. Patients with IDH1 wild-type astrocytomas had shorter OS (median 65 months) and PFS (median 33.9 months) than those with IDH1 mutated astrocytomas (median OS 23 months, \( p = 0.001 \); median PFS 14 months, \( p = 0.001 \)) (Figure 2A and 2B).

Multivariate analysis by Cox-proportional hazards model identified age (\( p = 0.01 \)), WHO grade (\( p = 0.001 \)), tumor phenotype (\( p < 0.001 \)) and IDH1 status (\( p = 0.002 \)) as independent prognostic factors in OS in our cohort of gliomas. Age (\( p = 0.03 \)) and IDH1 status (\( p = 0.006 \)) were shown to be independent prognostic factors in PFS. (Table 5).
Discussion

Watanabe et al. dissected multiple biopsies from the same patients and found that IDH1 mutations always preceded acquisition of TP53 mutation or loss of 1p/19q [5]. This genetic evidence suggests that IDH1 mutations are early genetic events in the development of glioma from a cell-of-origin that can give rise to both astrocytes and oligodendrocytes. To date, little is known about the role of IDH1 and its clinical implications in the processes of glioma progression, particularly in Chinese patients. Previous reports were mainly focused on analysis of IDH1 status in primary gliomas or secondary gliomas. Thus, the significance of IDH1 in paired gliomas, especially its role in predicting malignant progression, remains to be further defined. In our study, we investigated the IDH1 and IDH2 status of 53 pairs of primary and recurrent gliomas by direct sequencing and anti-IDH1-R132H immunohistochemistry. All of the primary gliomas showed consistent IDH1/IDH2 status as the corresponding recurrent gliomas, including the three cases of rare mutant (IDH1-R132S, IDH1-R132G and IDH2-R172K). No association was observed between IDH1 mutation and malignant transformation. Together with the fact that IDH1 mutation is an early event in gliomagenesis, its constant status throughout the tumor evolution and absence of association with malignant transformation suggest that IDH1 mutation is likely involved in tumor initiation instead of malignant progression [22]. Interestingly, a very recent paper by Lass et al. [22] showed that a small number of gliomas changed its IDH1 status in recurrence.

Evidence has accumulated in the literature regarding the prognostic impact of IDH1 mutation in gliomas, particularly high grade gliomas [4,6,21,23–26]. The prognostic significance of IDH1 mutation in LGG is more debatable. Dubbink et al. investigated the IDH1/IDH2 status in 49 low grade astrocytomas and demonstrated the association between IDH1 mutation and improved OS [27]. In another study, Houllier et al. analysed the clinical and molecular data of 271 LGGs and identified IDH1/IDH2 mutation as an independent prognostic marker in OS of LGG after adjusting for age, gender, Karnofsky performance status (KPS), histology, type of surgery, chromosome 1p/19q methylation [18]. By studying 404 gliomas (including 100 LGGs), Sanson et al. also showed the independent prognostic significance of IDH1 mutation in OS of gliomas by multivariate analysis adjusting for age, histological grade, type of surgery, postoperative treatment and molecular alterations (including 1p/19q codeletion, MGMT methylation and EGFR amplification) [23]. In a study investigating various molecular markers (including TP53 mutation, MGMT promoter methylation, 1p/19q codeletion and IDH1 mutation) of 139 LGGs, Hartmann et al. found that IDH1 mutation was the strongest prognostic marker for OS regardless of histology [31]. On the other hand, IDH1/IDH2 mutation was of no prognostic value in a study by Kim et al. investigating IDH1/IDH2 mutation, 1p/19q codeletion and TP53 mutation in 360 LGGs [28]. Ahmadi et al. also evaluated 100 diffuse astrocytomas and found the lack of association between IDH1 mutation and clinical outcome in terms of OS, DFS and time to malignant progression [29]. Differences in
methodology perhaps partially explained such discrepancy in their conclusions regarding prognostic impact of IDH1 mutation. OS was calculated from the date of first symptom in the study by Ahmadi et al. but most other studies, including ours, calculated OS from the date of histological diagnosis or date of first surgery. Secondly, patients in Ahmadi’s study were treated with nitrosourea-based chemotherapy as adjuvant treatment but in Hartmann’s study which demonstrated survival benefit of IDH1 mutated LGG, adjuvant treatment was alkylating agents. Additionally, in contrast to most studies about IDH1/IDH2 mutation in gliomas in the literature which investigated primary samples, we studied paired primary and recurrent gliomas. Such differences in methodology potentially influenced the evaluation of prognostic impact of IDH1 mutation in LGG. In our study, IDH1 mutation was associated with longer OS and PFS in 53 patients suffering from various grades of glioma, particularly in astrocytic tumors. Due to the relatively small size of our cohort and only 3 LGGs (2 adult AII, 1 paediatric OAI) were IDH1 wild type, statistical analysis of IDH1 mutation in LGG in our cohort was not performed. Further study with larger cohort would be needed to address the prognostic value of IDH1 mutation in LGG of Chinese patients. Nevertheless, our study has provided further evidence for the prognostic impact of IDH1 mutation in gliomas in general in Chinese patients.

In contrast to chromosome 1p/19q codeletion requiring fluorescence in-situ hybridization (FISH) analysis and MGMT promoter methylation requiring methylation-specific PCR (MSP), which are important diagnostic and predictive markers of glioma, IDH1 status could be readily evaluated by anti-IDH1-R132H immunohistochemistry for the most common mutant or by PCR followed by direct sequencing for all the mutant of the two mutation hotspots of IDH1 and IDH2. Our study examined the IDH1/IDH2 status of 53 pairs of primary and recurrent gliomas. The concordance rate of the two assays was 100%, confirming the reliability of mutation analysis in our study.

Malignant progression at recurrence is a crucial phenomenon in patients suffering from gliomas. We have previously evaluated various molecular alterations in a series of microdissected primary GBM and paired astrocytic tumors and revealed that low grade areas and high grade areas of primary GBM had more similar genetic abnormalities comparing with paired low and high-grade tumors underwent malignant progression, suggesting that additional molecular aberrations accumulate during malignant transformation [32]. Authors of several recent studies have examined the histological grade and molecular alterations in order to identify biomarkers for predicting malignant progression. In a study of 33 WHO grade II astrocytomas by Yue et al. [15], expression of Ki-67 was significantly associated with malignant progression, suggesting that tumors expressing higher Ki-67 may have an inherently faster growth rate and thus recur faster in the setting of gross-total or subtotal resection. Ishii et al. reported that the presence of TP53 mutation in WHO Grade II astrocytoma was associated with malignant progression and shorter PFS, whereas tumors without TP53 mutation recurred and progressed to malignancy without the change in TP53 status [16]. In this study, we evaluated the relationship between progression of glioma and IDH1 status but no association between IDH1 status and malignant progression was observed.

Though many studies demonstrated that IDH1 mutation was an important biomarker in glioma, mechanism of IDH1 mutation in glioma was not yet fully determined. Zhao et al. demonstrated the accumulation of hypoxia-inducible factor subunit (HIF-1α) due to reduced formation of α-KG in IDH1-mutated glioma cells, suggesting that activation of the HIF-1 pathway may be one of the oncogenic mechanisms of IDH1 mutation [10]. Dang et al. [11] further discovered the neomorphic gain of function of the IDH1-R132H mutant protein in converting α-KG to α-KG, an oncometabolite inhibiting multiple α-KG-dependent dioxygenases and leading to genome-wide histone and DNA methylation alterations [12]. Turcan et al. [13] unmasked the in vivo effect of IDH1 mutation in primary human astrocytes by showing the IDH1-R132H mutation induced histone alterations and extensive DNA hypermethylation, which actually remodel the methylome and establish the glioma CpG island methylator phenotype (G-CIMP), a subset of glioma with distinct genomic and clinical characteristics [30].

In summary, our study is the first study in investigating the IDH1/IDH2 status in paired primary and recurrent gliomas in Chinese patients. We have shown consistent IDH1/IDH2 status in the progression of gliomas and lack of association between IDH1 mutation and malignant progression. Patients with IDH1 mutated gliomas had longer OS and PFS, suggesting IDH1 mutation as a potential prognostic marker in gliomas in Chinese patients.

Supporting Information

Figure S1 Kaplan-Meier survival curves comparing OS and PFS in gliomas with advanced WHO grade, age and astrocytic phenotype. (a–c) Comparison of Kaplan–Meier OS curves according to advanced WHO grade, age over 50 years and astrocytic phenotype. (d–f) Comparison of Kaplan–Meier PFS curves according to advanced WHO grade, age over 50 years and astrocytic phenotype. (TIF)

Table S1 Clinical data and IDH status of 53 patients with paired primary and recurrent gliomas. (DOCX)

Author Contributions

Conceived and designed the experiments: LFZ H-KN. Performed the experiments: AK-YC XZ HML. Analyzed the data: YY XZ HML YW. Contributed reagents/materials/analysis tools: JC-SP AK-YC. Wrote the paper: YY AK-YC LCC. Provided clinical samples: ZYQ YM H-KN. Revised for important intellectual content: H-KN JC-SP.

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