Correlation of Histology and Molecular Genetic Analysis of 1p, 19q, 10q, TP53, EGFR, CDK4, and CDKN2A in 91 Astrocytic and Oligodendroglial Tumors

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ABSTRACT

Purpose: The histological diagnosis of human gliomas is of great importance for estimating patient prognosis and guiding therapy but suffers from being subjective and, therefore, variable. We hypothesized that molecular genetic analysis could provide a more objective means to classify tumors and, thus, reduce diagnostic variability.

Experimental Design: We performed molecular genetic analysis on 91 nonselected gliomas for 1p, 19q, 10q, TP53, epidermal growth factor receptor, and cyclin-dependent kinase 4 abnormalities and compared with the consensus diagnoses established among four independent neuropathologists.

Results: There were six astrocytomas, seven anaplastic astrocytomas, 45 glioblastomas, 21 oligodendrogliomas, eight anaplastic oligodendrogliomas, three oligoastrocytomas, and one anaplastic oligoastrocytoma. Twenty-nine cases had either 1p or 19q loss of heterozygosity (LOH) while retaining both copies of 10q, of which 25 (86%) were histologically oligodendroglioma, anaplastic oligodendroglioma, oligoastrocytoma, or anaplastic oligoastrocytoma. As for the oligodendroglial tumors, unanimous agreement of the initial diagnoses was almost restricted to those cases with combined 1p/19qLOH, whereas all nine tumors without 1p loss initially received variable diagnoses. Interestingly, TP53 mutation was inversely related to 1pLOH in all gliomas (P = 0.0003) but not 19qLOH (P = 0.15).

Conclusions: These data demonstrate that molecular genetic analysis of 1p/19q/10q/TP53 has significant diagnostic value, especially in detecting oligodendroglial tumors. In addition, 1pLOH and TP53 mutations in gliomas may be markers of oligodendroglial and astrocytic pathways, respectively, which may separate gliomas with the same histological diagnosis, especially oligodendroglial tumors and glioblastomas. Testing for those molecular genetic alterations would be essential to obtain more homogeneous sets of gliomas for the future clinical studies.

INTRODUCTION

Treatment of diffuse gliomas still remains one of the toughest challenges in oncology. Surgical cure of these tumors infiltrating into the brain is practically impossible, and their clinical course is primarily determined by the biological behavior of the tumor cells, including growth rate and their responsiveness to radiation therapy and chemotherapy. For the prediction of such biological behavior, the most reliable, proven method has been the histological diagnosis based on the microscopic morphology of the tumor since the era of Bailey and Cushing. However, histological diagnosis suffers from being subjective and, therefore, variable in some cases, which occasionally hampers the clinical studies on gliomas. On the other hand, recent progress in the molecular biology and molecular genetics is showing a potential to provide new means to dissect the biological features of gliomas.

Various genetic alterations have been identified in the tumorigenesis and progression of diffuse gliomas (1–4). Amplification of oncogenes has been observed for EGFR, CDK4, and MDM2 genes (5, 6) and inactivating mutation or deletion of tumor suppressor genes has been discovered for TP53 (17p), RB1 (13q), CDKN2A (9p), PTEN (10q), and DMBT1 (10q) (7–11). Moreover, several chromosomal loci presumed to contain tumor suppressor genes (12, 13) have been reported to be involved in glioma progression or differentiation, including 1p, 9p, 10q, 13q, 17p, 19q, and 22q.

These data demonstrate that molecular genetic analysis of 1p/19q/10q/TP53 has significant diagnostic value, especially in detecting oligodendroglial tumors. In addition, 1pLOH and TP53 mutations in gliomas may be markers of oligodendroglial and astrocytic pathways, respectively, which may separate gliomas with the same histological diagnosis, especially oligodendroglial tumors and glioblastomas. Testing for those molecular genetic alterations would be essential to obtain more homogeneous sets of gliomas for the future clinical studies.

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3 The abbreviations used are: EGFR, epidermal growth factor receptor; CDK, cyclin-dependent kinase; LOH, loss of heterozygosity; AA, anaplastic astrocytoma; AC, astrocytoma; GBM, glioblastoma; OG, oligodendroglioma; AOG, anaplastic oligodendroglioma; OA, oligoastrocytoma; AOA, anaplastic oligoastrocytoma.
suppressor genes, such as 1p, 19q, 11p, and 22q, show frequent allelic losses in gliomas, which are easily detected as LOH (2). Among those, LOH at short arm of chromosome 1 (1p) and long arm of chromosome 19 (19q) is found in 60–70% of OGs (12–15), and recent studies demonstrated that 1pLOH in AOGs, frequently accompanied by 19qLOH, predicted sensitivity to specific chemotherapy and better overall survival (16, 17). Although the underlying molecular biological mechanism for this observation is yet unknown, 1pLOH seems to represent a specific biological feature of tumor cells, at least in OGs. On the other hand, 1pLOH is also found in 50–70% in OAs and 10–20% of astrocytic tumors, including GBMs (4, 18), and its significance within these histological types is not yet clear. Therefore, we studied a series of diffuse gliomas of various grades and histologies for a range of genetic markers known to be important in glioma tumorigenesis. Importantly, we carefully classified these 91 gliomas using independent histological review by four neuropathologists, enabling us to compare genotype with both individual and consensus diagnosis. In this manner, we sought to determine whether a cumulative analysis of those genetic markers could provide insight into the significance of molecular genetic examination in glioma diagnosis.

MATERIALS AND METHODS

Tumor samples were obtained at surgery performed at the Saitama Medical School Hospital, Teikyo University Hospital, and University of Tokyo Hospital and its affiliated hospitals. In total, 91 cases of diffuse gliomas operated during the period of 1996–1999 were studied. Tumor samples were collected on an availability basis and were not selected for specific histological types. Portions of resected tumors were snap frozen in liquid nitrogen and stored at −80°C until use. DNA from the tumor tissue was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) after the manufacturer’s protocol, Constitutional DNA used as a control for the microsatellite analysis was extracted as previously from peripheral blood obtained after written informed consent (19). All experiments using the human samples were approved by the ethical committee at the University of Tokyo Hospital.

Histology slides were reviewed by four independent neuropathologists (T. H., N. F., J. H., and Y. N.), and consensus diagnoses were made following the latest WHO classification (20). In cases with divergent opinions, the senior neuropathologist (Y. N.) made the final diagnosis. For GBM cases, the neuropathologists were also asked to describe if there were oligodendroglial features (OG, AOG, OA, and AOA, respectively). Histological evaluations were blinded to the molecular genetic data.

Molecular Genetic Analysis. LOH and single-strand conformational polymorphism assays were performed using the Genetic Analyzer 310 (PE Biosystems, Norwalk, CT) capillary electrophoresis system, following the manufacturer’s protocols. For the LOH assay, the following microsatellite markers located at the most frequently deleted sites in gliomas were used: D1S244, D1S2734, and D1S402 for 1p (1p36); D19S112, D19S596, D19S412, and D19S219 for 19q (19q13); and D10S1744, D10S1680, and D10S583 for 10q (10q22–23; Refs. 10, 17, and 21). Primer sequences for these markers are available from the Genome Database.4

The single-strand conformational polymorphism assay for exons 5–8 of TP53 was performed using the previously published primer pairs (7). For each primer set, the sense and antisense primers were labeled with two different fluorescent dyes to allow specific detection of each strand. PCR products were separated by capillary electrophoresis and were analyzed with the GeneScan program (PE Biosystems) to detect tumor-specific migration shifts. Exons showing migration shifts were reamplified from the tumor DNA with nonlabeled primers, gel purified, and directly sequenced using the BigDye Terminator Sequencing Kit (PE Biosystems). The sequencing reaction products were separated and analyzed by the Genetic Analyzer 310 following the manufacturer’s protocol.

Previously described, established comparative multiplex PCR assays were used to detect gene amplification for EGFR and CDK4 and homozygous deletion of CDKN2A. (9, 22)

RESULTS

Of the 91 cases, the consensus histological diagnosis was AC (WHO grade 2) in 6, AA (grade 3) in 7, GBM (grade 4) in 45, OG (grade 2) in 21, AOG (grade 3) in 8, mixed OA (grade 2) in 3, and mixed AOA (grade 3) in 1 (Table 1). Complete agreement of the initial diagnoses among all four neuropathologists was seen in 49 of 91 cases (54%): 4 AC (including 2 cases on which one neuropathologist did not make a diagnosis), 0 AA, 36 GBM, 6 OG, 3 AOG, 1 OA, and 1 AOA. Disagreement was much more common in AA (seven of seven, 100%), OA (two of three, 67%), OG (15 of 21, 71%), and AOG (five of eight, 63%), compared with AC (two of eight, 25%) and GBM (9 of 45, 20%; Fig. 1, A–D).

The results of the molecular genetic analysis are also summarized in Table 1. At least one of the three examined 1p markers was informative in 88 cases, of which 30 cases (33%) showed LOH: one of six AC (17%), zero of seven AA (0%), 10 of 42 GBM (24%), 12 of 21 OG (57%), five of eight AOG (63%), two of three OA (67%), and none of one AOA (0%). Similarly, at least one of the four 19q markers was informative in 90 cases, of which 38 cases (42%) showed LOH: one of six AC (17%), two of seven AA (29%), 13 of 44 (31%) GBM, 12 of 21 OG (57%), seven of nine AOG (78%), two of three OA (67%), and one of one AOA.

Among the 30 cases with 1pLOH, 25 cases (86%) also had 19qLOH: one of one AC (100%), 6 of 10 GBM (60%), 11 of 12 OG (92%), five of five AOG (100%), and two of two OA (100%). The positive correlation between 1pLOH and 19qLOH was statistically significant not only in the 34 tumors with oligodendroglial features (OG, AOG, OA, and AOA, P < 0.0001; Fisher’s exact test) but also within the 58 astrocytic tumors (AC, AA, and GBM); of 11 astrocytic tumors with 1pLOH in total, 7 (64%) had 19qLOH (P = 0.0055).

At least one of the three examined 10q markers was informative in 88 cases, and LOH was detected in 29 cases (33%):
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**Table 1: Histology and Molecular Genetic Data on 91 Gliomas**

<table>
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<th>Diagnosis</th>
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*Cons, consensus diagnosis; A-D, diagnosis by neuropathologists A-D; wt, wild type; mut, mutated; amp, gene amplification; ND, not determined; A.Ep, anaplastic ependymoma; DNT, dysembryoplastic neuroepithelial tumor; o-comp, oligo-component; H17040, heterozygous; I, LOH.*
1 AC, 1 AA, and 27 GBM. Among the 43 cases with either 1p or 19qLOH, 14 cases had 10qLOH, and 29 cases retained both copies of 10q. All of the 14 cases (100%) with 10qLOH were GBMs, whereas 25 of 29 cases (86%) retaining both copies of 10q were OG, AOG, OA, or AOA.

Mutation of TP53 was detected in 24 cases: three of six AC (50%), four of seven AA (57%), 9 of 45 GBM (20%), 6 of 21 OG (29%), zero of nine AOG (0%), one of three OA (33%), and one of one AOA (100%). When correlation with 1p status was examined, only one GBM (case 17) had both 1pLOH and TP53 mutation, and none of the remaining 29 cases with 1pLOH had TP53 mutation. This relative exclusiveness between 1pLOH and TP53 mutation was statistically significant when examined in all cases (P = 0.0003) but did not reach statistical significance when the 58 astrocytic tumors (AC, AA, and GBM) were separately analyzed, probably because a considerable number of cases had neither of the two genetic alterations (P = 0.055). On the other hand, TP53 mutation and 19qLOH were not inversely correlated in all gliomas (P = 0.29), with seven cases showing both 19qLOH and TP53 mutation: two AA, three GBM, one OG, and one AOA.

Homzygous deletion of CDKN2A was observed in one AC, one AA, 17 GBM, two OG, three AOG, and in none of OA or AOA. There was no significant association of CDKN2A status with 1pLOH, 19qLOH, or TP53 mutation in any tumor types. However, CDKN2A deletion positively correlated with 10qLOH (P = 0.04).

Gene amplification of EGFR was detected in 11 cases: 10 GBMs and 1 OG. In agreement with previous studies, most GBM cases with EGFR amplification (9 of 10) had 10qLOH, but only one had TP53 mutation (23-25). However, this inverse correlation did not reach statistical significance in this series (P = 0.15). There was no apparent correlation between EGFR...
amplification and 1p/19q status: two had both 1pLOH and 19qLOH, one had 1pLOH alone, two had 19qLOH alone, and four had neither 1p nor 19qLOH. Of the 45 GBMs, 12 cases were described to contain portions with oligodendrogliarial morphological features. Five of the 12 cases (42%) had 19qLOH, and only 1 (8%) had 1pLOH (Table 1; Fig. 1D).

**DISCUSSION**

The data presented here showed that molecular genetic evaluation of chromosomes 1p, 19q, and 10q in diffuse gliomas has significant diagnostic value, especially in identifying OGs and OAs. When 1p or 19qLOH was detected without 10qLOH in diffuse gliomas, the tumor usually demonstrated consensus oligodendrogliarial histological features, compatible with the diagnosis of grade II or III OG or OA. A recent study using real-time quantitative PCR to detect allelic loss also showed this concordance between 1p/19q/10q status and the histological diagnosis of OGs (26). When 1p or 19qLOH was accompanied by additional 10qLOH, on the other hand, such tumors were most likely (86%) GBMs on consensus diagnosis. Interestingly, tumors unanimously agreed as OGs on their initial diagnoses almost always carried combined loss of 1p and 19q; all six OGs and two of three AOGs with unanimous diagnosis had 1p and 19q losses, and the remaining one AOG had 19q loss with noninformative 1p markers (Fig. 1, A and C). Therefore, it appears that tumors showing typical histological features of OGs are likely to carry 1p and 19q losses both in grade II and grade III tumors. On the contrary, all nine consensus OGs without 1p loss had disagreement on the diagnosis initially, indicating that these might be the cases potentially leading to the diagnostic variability (Fig. 1B).

The distinction between OG, OA, and AC can be subjective and sometimes difficult, especially in high-grade, undifferentiated tumors (20, 27). Our study suggested that simple LOH analysis of 1p, 19q, and 10q may provide important supportive information in making diagnoses for such difficult cases, which may affect the clinical management. For instance, a trend in postsurgical treatment for OGs is to start with chemotherapy alone and defer radiation therapy until the tumors show progression (28–30). Whether a similar approach is appropriate in treating patients with OAs or AOAs remains to be investigated, and to do so requires homogeneous defined sets of tumors. Genetic profiles on 1p/19q/10q and TP53 should be one of the important objective factors to be considered in such studies.

There was a small number of outlying cases in our series: one AC, one AA, and one GBM showed 1p and 19qLOH while maintaining allelic balance at 10q, the typical genetic profile for OGs. Therefore, molecular genetic analysis cannot replace histological diagnosis. On the other hand, it is still unknown which of the two measures, histological diagnosis or genetic profiling, would better represent the biological characteristics of a tumor when the two evaluations disagree.

As reported previously, 1p LOH was highly associated with 19qLOH in oligodendrogliomas, but our study further showed that this positive correlation seems to extend to astrocytic tumors, including GBMs (P = 0.0055; Refs. 13 31, and 32). Although they showed such a strong positive correlation, 1pLOH and 19qLOH did have slight differences in their patterns of appearance. 1pLOH demonstrated a tight inverse correlation with TP53 mutation (P = 0.0003), a presumed key genetic event in AC tumorigenesis (2, 33–35). On the other hand, 19qLOH accompanied TP53 mutation more frequently both in oligodendrogial tumors and GBMs. On the basis of such findings, it is tempting to hypothesize that diffuse gliomas with 1pLOH may constitute a genetic subset that is associated with oligodendrogial lineage, whereas TP53 mutation represents another subset associated with astrocytic lineage. Such proposition has been made previously on OA, in which 1p/19qLOH was associated to be more OG predominant, and TP53 mutation was associated with more AC predominant morphology (36). Curiously, however, partial oligodendrogial morphological features in GBMs were more frequently detected in tumors with 19q loss in our study, not in 1p loss, indicating that morphological features do not necessarily follow the genetic profile (Fig. 1D).

Genetic subsets in gliomas were first noticed in GBMs, in which TP53 mutation and EGFR gene amplification occur in a mutually exclusive fashion, thereby defining two genetic subsets (23, 25). Clinical significance of these genetic subsets, one with TP53 mutation and the other with EGFR amplification, was shown by the fact that secondary GBMs mostly belonged to the former, and primary GBMs mostly belonged to the latter (20, 25). Whether the putative subsets by 1p loss and TP53 mutation suggested in our study also would have any clinical relevance remains to be investigated. However, it is noteworthy that AOGs with 1pLOH had already been shown to have better treatment response and prognosis (16, 17), and another recently published study on seven high-grade gliomas with unusual long survival demonstrated 1pLOH in all seven cases (37). Although such association was not proven in OA and astrocytic tumors thus far (17), it is therefore possible that 1pLOH may be a marker indicative of gliomas associated with better treatment response and survival. Unfortunately, we currently do not have sufficient follow-up data on our series to look for correlation between the genetic data and clinical outcomes, such as the difference between GBMs with or without 1pLOH. Additional studies in a larger series with sufficient follow-up should address this clinically important question. Nonetheless, use of molecular genetic markers will allow objective evaluation of diffuse gliomas and, therefore, enable such studies to be done, perhaps without the time-consuming and difficult requirement of independent histological review by multiple neuropathologists.

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