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ORIGINAL ARTICLE

Genetic and Epigenetic Features of Rapidly Progressing *IDH*-Mutant Astrocytomas

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Abstract

IDH-mutant astrocytomas are significantly less aggressive than their *IDH*-wildtype counterparts. We analyzed The Cancer Genome Atlas dataset (TCGA) and identified a small group of *IDH*-mutant, WHO grade II–III astrocytomas (n = 14) with an unexpectedly poor prognosis characterized by a rapid progression to glioblastoma and death within 3 years of the initial diagnosis. Compared with *IDH*-mutant tumors with the typical, extended progression-free survival in a control group of age-similar patients, the tumors in the rapidly progressing group were characterized by a markedly increased level of overall copy number alterations ([CNA]; p = 0.006). In contrast, the mutation load was similar, as was the methylation pattern, being consistent with *IDH*-mutant astrocytoma. Two of the gliomas (14%) in the rapidly progressing, *IDH*-mutant group but none of the other grade II–III gliomas in the TCGA (n = 283) had pathogenic mutations in genes (*FANCB* and *APC*) associated with maintaining chromosomal stability. These results suggest that chromosomal instability can negate the beneficial effect of *IDH* mutations in WHO II–III astrocytomas. The mechanism of the increased CNA is unknown but in some cases appears to be due to mutations in genes with a role in chromosomal stability. Increased CNA could serve as a biomarker for tumors at risk for rapid progression.

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Key Words: Glioblastoma (GBM), Isocitrate dehydrogenase, Prognosis, WHO 2016.

INTRODUCTION

Infiltrating gliomas are among the most common primary intracranial tumors. The prognosis depends on the histological grade, age of the patient, and genetic features in particular 1p/19q codeletion and isocitrate dehydrogenase 1/2 (*IDH1/2*) mutation status (1–3). With the publication of the WHO Classification of Tumors of the Central Nervous System revised fourth edition in 2016, infiltrating gliomas are molecularly subgrouped into *IDH*-mutant and *IDH*-wildtype groups (1). Diffuse astrocytomas corresponding to WHO grades II and III with wildtype *IDH1/2* have a significantly worse prognosis than their *IDH*-mutant counterparts and are associated with a more rapid progression to glioblastoma ([GBM]; WHO grade IV astrocytoma) (1). The average overall survival ranges between 81 and 151 months for lower-grade (WHO grade II–III) astrocytomas with *IDH* mutations and 19–60 months for WHO grade II–III astrocytomas without *IDH* mutations (4–6).

Much work has been done in recent years to determine additional prognostic biomarkers and potential targetable mutations for these tumors (7–11). Factors such as GBM-like genetic alterations (12, 13) and high nestin expression (14) have been implicated in worse prognosis in some WHO grade II–III astrocytomas. In a previous study, *IDH*-mutant GBMs showed increased copy number alterations (CNA) compared with *IDH*-mutant WHO grade II–III astrocytomas (12), suggesting that increased CNA is associated with progression to grade IV in *IDH*-mutant astrocytomas. However, it was previously unknown if increased CNA levels precede progression to GBM and are therefore associated with a worse prognosis in *IDH*-mutant grade II–III astrocytomas. We recently described a small cohort of 4 cases *IDH*-mutant WHO grade II–III astrocytomas from our own institution with rapid progression to GBM and unexpectedly short survival (<3 years) (15). All 4 of these tumors had very high levels of CNA at their initial WHO grade II–III presentation compared with more classically indolent-behaving *IDH*-mutant lower-grade

gliomas, suggesting that increased CNA levels are an adverse prognostic feature.

Herein, we identified and analyzed 14 *IDH*-mutant WHO grade II–III astrocytomas from The Cancer Genome Atlas (TCGA) online repository (16) and cBio cancer genomics portal (17, 18) with very short intervals to recurrence, progression, and ultimately patient death compared with a group of 16 age-similar *IDH*-mutant WHO grade II–III astrocytomas from the same database which followed the expected, more indolent clinical course.

MATERIALS AND METHODS

TCGA Case Selection

We performed a search of the 516 lower-grade glioma cases in the TCGA database and identified a total of 14 patients meeting the criteria of an *IDH* mutation, initial WHO grade of II or III, genetic alterations consistent with astrocytoma, as well as progression to GBM and overall survival <3 years. A control group of 16 patients was selected from the remaining TCGA cases that met the criteria of an *IDH* mutation and a more conventional clinical course, that is, a disease-free interval >5 years. The original histologic diagnoses reported in TCGA included astrocytomas, oligoastrocytomas, and oligodendrogliomas. All cases were molecularly reclassified according to the WHO 2016 criteria as astrocytomas, WHO grade II–III, based on an intact 1p/19q status and the presence of *ATRX* and/or *TP53* mutations. Mean age at presentation was 40.7 ± 2.7 years and 37.8 ± 2.9 years in the rapidly progressing and control groups, respectively ($p = 0.48$). No significant difference in tumor grade at initial presentation was identified between these 2 groups ($p = 0.52$). The study was granted an exemption by the UT Southwestern Institutional Review Board (IRB STU 042014-067).

Genetic and Epigenetic Analysis

The gene expression (Illumina HiSeq, RNASeq) and DNA methylation data (Illumina Human Methylation 450) was downloaded for the selected TCGA low-grade glioma cases and analyzed with TCGAbiolinks (19). The Affymetrix SNP 6.0 micorarray data normalized to germline for copy number analysis for the same TCGA cases was downloaded from Broad GDAC Firehose (http://gdac.broadinstitute.org/runs/stddata__2016_01_28/). The fraction of copy number altered genome was calculated from the above data as the fraction of the genome with log2 of copy number >0.3 following the procedure used in cBioportal (18). The mutation load is the number of nonsynonymous mutations seen in a sample. The differential analysis and visualization of mutations was done using Maftools (Mayakonda A, Koeffle HP, bioRxiv doi:10.1101/052662). The Ideogram for visualization of genome-wide copy number variation results was generated using Genome Decoration Page (https://www.ncbi.nlm.nih.gov/genome/tools/gdp). The pathway and network analyses were conducted using Qiagen's IPA tool (www.qiagen.com/ingenuity) and R 3.4.1 (http://www.R-project.org/).

GISTIC Analysis

The GISTIC (Genomic Identification of Significant Targets in Cancer) 2.0 algorithm was used to identify regions of the genome that are significantly amplified or deleted across the samples in the rapidly progressing and conventional course groups (20). Each CNA is assigned a G-score that considers the amplitude of the alteration as well as the frequency of its occurrence across samples. The false discovery rate (FDR) was then used to determine the relative significance of each abnormality. Each region predicted to be significantly different between the conventional course and rapidly progressing astrocytomas was screened for tumor suppressor genes, oncogenes, and other genes associated with glioma and malignancy (20, 21). GISTIC 2.0 analysis was run in GenePattern (22).

Mutation Analysis of Genes Involved in Maintenance of Genomic Stability

A group of 43 genes previously known to be involved in maintaining chromosomal stability was identified by a literature review and included the following genes: *APC*, *ATM*, *ATR*, *BLM*, *BRCA1* (*FANCS*), *BRCA2* (*FANCD1*), *BUB1B*, *CHK1*, *Claspin*, *DNA-PK*, *EME1*, *FANCA*, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ* (*BRIP1*), *FANCL*, *FANCM*, *FANCN* (*PALB2*), *FANCO* (*RAD51C*), *FANCP* (*SLX4*), *FANCQ*, *FANCR*, *FANCT* (*UBE2T*), *HUS1*, *Ligase IV*, *MUS81*, *NBN*, *Pol η*, *Pol κ*, *RAD51*, *RAD52*, *REV3*, *SMC1*, *SNM1B*, *TOP1*, *TP53*, *WRN*, and *XLF* (23, 24). Variant annotation was performed using COSMIC (25), dbSNP (26), ClinVar (27), CanProVar 2.0 (28), The 1000 Genomes Project (29), and FATHMM-MKL (30).

Statistical Analysis

Differences in mutation and CNA were evaluated using the Mann-Whitney test in GraphPad Prism version 7.03 (GraphPad, La Jolla, CA). Kaplan-Meier analysis was used for survival analysis. Significance levels of the survival analysis were tested using the Log-rank test.

RESULTS

Clinical Outcome

As expected based on our experimental design, the rapidly progressing glioma patients had both shorter disease-free intervals ($p = 2.6e\text{-}9$; Fig. 1A) and overall survival times ($p = 1.7e\text{-}9$; Fig. 1B).

CNA Analysis

Overall CNA levels were increased in the rapidly progressing *IDH*-mutant astrocytomas compared with the control *IDH*-mutant astrocytomas. The total percentage of the genome affected by CNA was significantly higher in the rapidly progressing *IDH*-mutant astrocytoma group compared with the *IDH*-mutant control group ($16.6\% \pm 2.8\%$ and $8.1\% \pm 2.5\%$, respectively; mean ± STD; $p = 0.006$; Fig. 2A). Most of the CNA were spread widely throughout the genome, and were considered significant with an amplification or deletion level ≥0.3. (Fig. 3A, B).

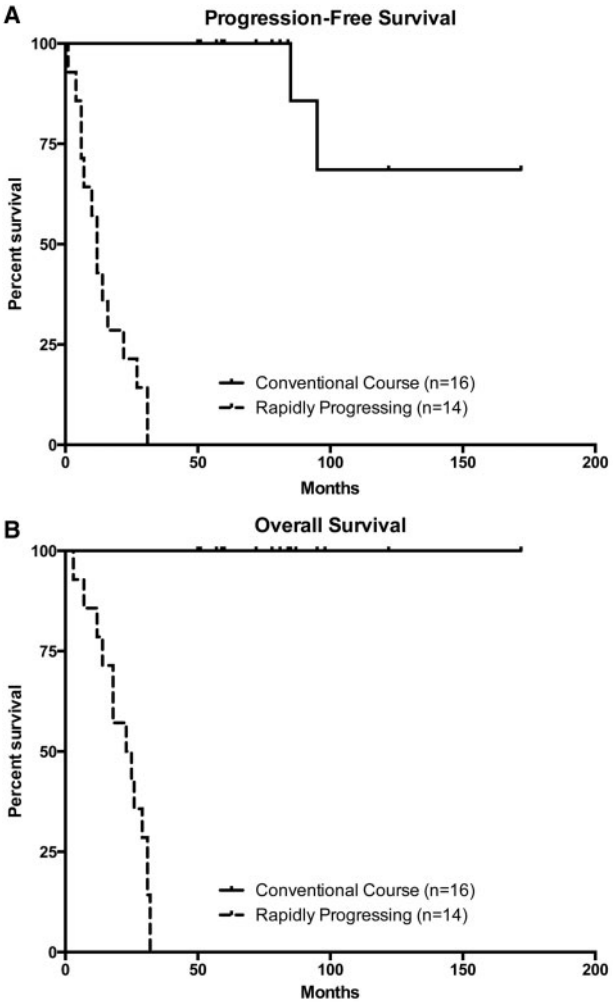

FIGURE 1. Kaplan-Meier survival plots demonstrating a significant difference in both **(A)** disease-free interval ($p = 2.6e\text{-}9$) and **(B)** total survival ($p = 1.7e\text{-}9$) between the rapidly progressing group ($n = 14$) and the control group ($n = 16$).

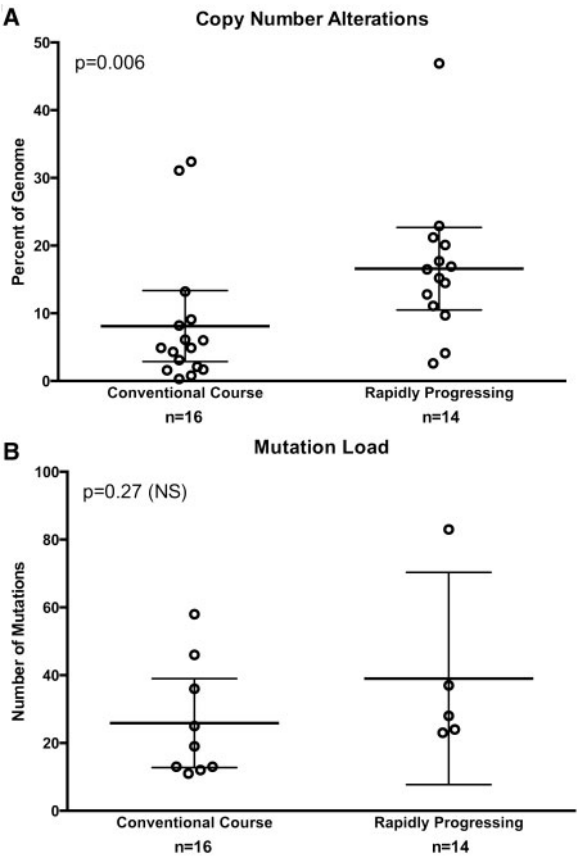

FIGURE 2. (A) Box plot illustrating an increased amount of overall copy number alterations (CNA) in the rapidly progressing group ($n = 14$) as compared with the control group ($n = 16$; $p = 0.006$), and **(B)** box plot showing no significant difference in the number of overall mutations between the rapidly progressing ($n = 5$) and control glioma groups ($n = 9$; $p = 0.27$).

In addition, multiple specific chromosomal alterations such as areas of deletion frequently present in secondary GBM (13, 31, 32), including on chromosome 9p (reported in 33%–38% of secondary GBMs), chromosome 10q (80%–86%), and chromosome 13q (31%–33%), were identified in some of these lower-grade gliomas before histologic progression to GBM (Fig. 3C). Using GISTIC algorithms (20, 21), we confirmed that the astrocytomas that progressed rapidly to GBM and had short survival intervals had increased frequencies of 9p, 10q, and 13q deletions, as well as less frequent amplifications in cytobands, including 4q12 and 12q14.1, which were not seen in the conventional course group (Figs. 3C and 4). We identified several consistently altered genes within these regions relevant to progression to GBM not seen in the conventional course lower-grade gliomas, including *PDGFRA* amplification on chromosome 4p12 (which is associated with aggressive and high-grade astrocytomas, including those with H3K27M mutation) (33–36), *CDKN2A/B* homozygous deletion on chromosome 9p21.3 (7, 8), *CDK4* amplification on chromosome 12q14.1 (Fig. 4) (37, 38), as well as less common amplification of *BRAF* and *CDK6* on chromosome 7q (39, 40). These genes are all associated with higher grade and, importantly, have multiple specific inhibitors already under investigation or in use clinically (41–44).

MGMT Methylation Analysis

None of *MGMT* methylation probes demonstrated a significantly different (FDR ≤ 0.05 and difference in mean beta value ≥ 0.25) methylation status between the conventional and rapidly progressing cohorts, indicating that *MGMT* gene methylation was not significantly different between the 2 groups.

Mutation Load

No significant difference was identified between the overall number of mutations (mutational burden) between a subset of tumors for which full mutation data are available ($p = 0.27$) in the group of control astrocytomas (25.9 ± 5.7 mutations/case; $n = 9$) and the group of rapidly progressing astrocytomas

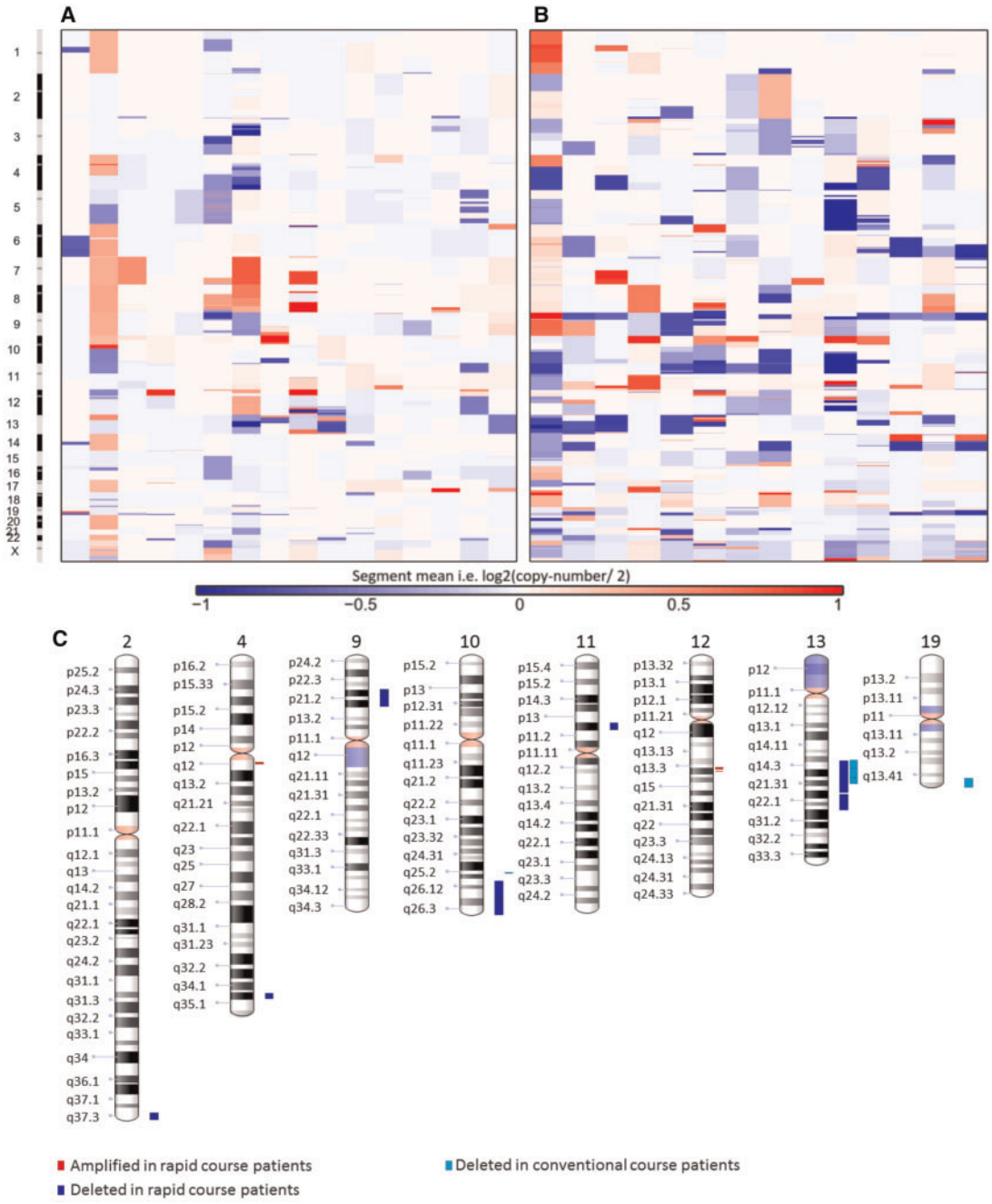

FIGURE 3. Overall amplification and deletion levels and chromosomal locations in all of the patients included in the conventional course glioma **(A)** and rapidly progressing glioma **(B)** groups. **(C)** Chromosomal regions with the most significant areas of alteration in both groups including those commonly associated with glioblastoma, 9p, 10q, and 13q.

(39.0 ± 11.2 mutations/case; n = 5; Fig. 2B). A full list of genes with mutations and the number of cases with mutations in these genes is presented in Supplementary Data Table S1.

Mutation Analysis of Genes Involved in Maintenance of Genomic Stability

Two of the gliomas (14%) among the rapidly progressing *IDH*-mutant gliomas harbored a pathogenic mutation in genes from a 43 gene panel (*FANCB* and *APC*) known to be associated with chromosomal instability (Fig. 5) (45–49). In contrast, none of the gliomas in the *IDH*-mutant control group and no tumor among the 283 other *IDH*-mutant and *IDH*-wildtype WHO grade II–III gliomas with full mutation sequencing data available in the TCGA dataset harbored pathogenic mutations in *FANCB*, *APC*, or any of the remaining genes associated with chromosomal instability. The *APC* (N1808T) and *FANCB* (P586S) mutations were both missense

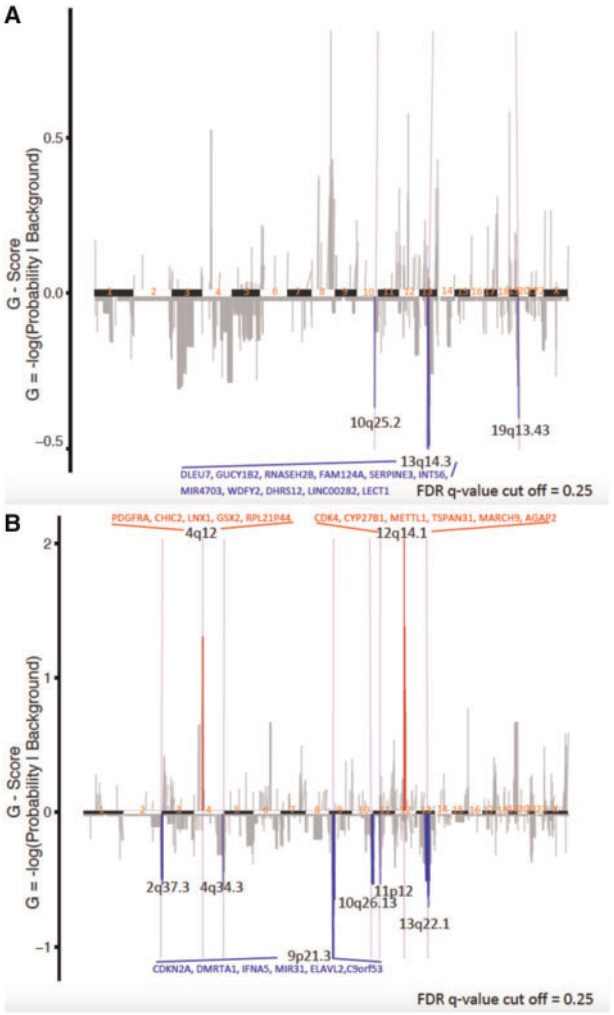

FIGURE 4. GISTIC analysis showing the most consistent and relevant cytoband/gene alterations in conventional course astrocytomas **(A)** and rapidly progressing astrocytomas **(B)**. All cytobands shown met the criterion of false discovery rate (FDR) ≤ 0.25. The 4 cytobands with gene names annotated met the criterion of FDR ≤ 0.05.

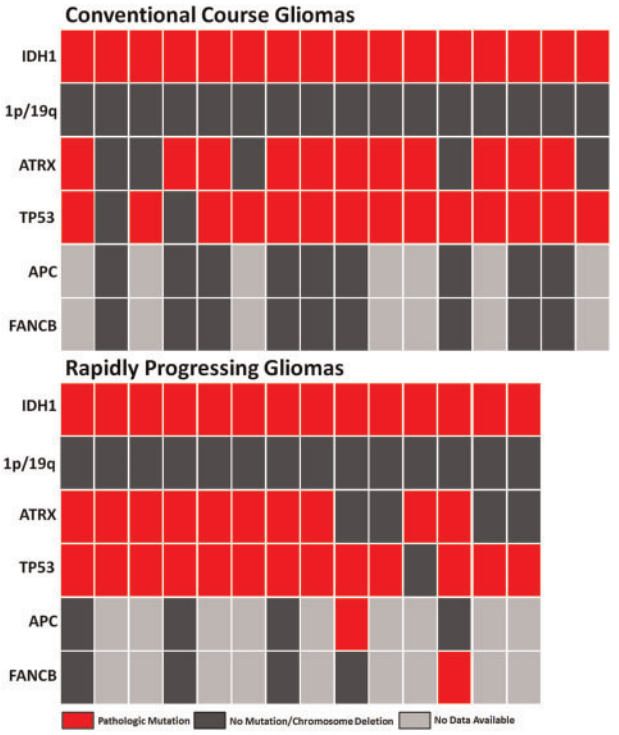

FIGURE 5. Relevant mutations (*IDH1*, *ATRX*, *TP53*, *APC*, and *FANCB*) identified in each case of conventional course (n = 9) and rapidly progressing (n = 5) lower-grade glioma in which partial or complete mutational data were available.

mutations predicted to be pathogenic with a high specificity (0.98 and 0.96, respectively) based on their locations in highly conserved regions and other factors (FATHMM-MKL algorithm) (30).

Pathway Analysis

Pathway analysis accounting for both mRNA expression levels and gene amplification/deletion levels was performed (Supplementary Data Fig. S1). The relative deletions and amplifications in rapidly progressing gliomas, as compared with our conventional course glioma control group, shows an overall increase in pathways driving gliomagenesis and tumor progression leading to increased cell survival, growth, and proliferation after accounting for directionality of these alterations.

DISCUSSION

Representing ~29% of all primary intracranial tumors and 80% of all malignant brain tumors (2), glial tumors are subject to a wide array of ongoing research studies. Recently, *IDH1/2* mutational status has emerged as an important prognostic factor in gliomas, particularly lower-grade (WHO grade II–III) astrocytomas. Astrocytomas with mutations in either *IDH1* or *IDH2* have a significantly better prognosis than *IDH*-wildtype astrocytomas of comparable grade (1, 5, 6). However, in rare cases, lower-grade *IDH*-mutant astrocytomas may follow an aggressive clinical course (15).

More recently, new genome-wide analysis methods based on next-generation sequencing and methylation pattern analysis have provided a new approach to the diagnosis and classification of these tumors, resulting in redefinition of many of the diagnostic categories (31, 50, 51). This technology has been used to address the overall state of the genome across *IDH*-mutant and *IDH*-wildtype grade II–IV gliomas. These data showed that there were increased levels of overall CNA as well as localized rearrangements (chromothripsis) in *IDH*-mutant GBMs compared with lower-grade *IDH*-mutant astrocytomas (12, 16). In contrast, no increase in CNA levels was found with increasing histologic grade in *IDH*-wildtype astrocytomas (12), an observation that corresponds well with the relatively weak association between increasing histologic grade and worsening clinical outcome in *IDH*-wildtype astrocytomas (1). If the increased levels of overall chromosomal

fracturing and instability occurred prior to or after progression to GBM was not clear from previous studies.

In a small cohort from our own institution, we previously demonstrated that a subgroup of astrocytomas with the *IDH1* R132H mutation actually harbored significantly increased levels of CNA prior to progression to secondary GBM. This early chromosomal instability was associated only with tumors that progressed in an unexpectedly rapid manner from lower-grade astrocytoma to GBM and were rapidly fatal, despite the favorable *IDH*-mutant status (15).

In the present study, we identified 14 cases of *IDH*-mutant grade II–III astrocytoma with rapid progression to GBM and short patient survival (defined here as 36 months or less) in the TCGA dataset and compared this group to a cohort of age-similar 16 *IDH*-mutant grade II–III astrocytomas with a clinical course typical for their favorable *IDH*-mutational status (defined here as patients with a disease-free interval of 60 months or more) from the same dataset (Fig. 1). Of note, several of these patients had original diagnoses of either "oligodendroglioma" or "oligoastrocytoma"; however, with information on the 1p/19q, *ATRX*, and *TP53* status we were able to molecularly reclassify each of these cases as diffuse astrocytomas according to new WHO 2016 guidelines (1). We found that these rapidly progressing gliomas had significantly higher levels of CNA before progression to GBM (Figs. 2A and 3). There were also a number of more specific chromosomal alterations which were previously associated with secondary GBM that were altered in this group of histologically lower-grade, rapidly progressing astrocytomas, including losses at 9p, 10q, 13q, and gains at 4p12 and 12q14.1 containing genes known to be associated with GBM (Figs. 3C and 4) (32, 52). These findings indicate that chromosomal instability, as evidenced by an increased level of CNA, is a prognostic factor in *IDH*-mutant lower-grade gliomas, predicting a rapid recurrence and progression to GBM. This finding has implications for the management of these patients.

Increased CNA before histologic progression to GBM also suggests a possible mechanism for the poor outcomes in these tumors with otherwise favorable prognostic factors. CNA has been shown to be a factor in increasing malignant potential in tumors through the overall ratio of widespread amplification of DNA regions containing growth factor pathway components and cell cycle proteins, as well as widespread deletions in areas containing tumor suppressor genes (13, 53–56). *TP53*-inactivating mutations have been associated with increased levels of CNA and chromothripsis, a potential driving force of progression to higher-grade tumors (16, 57). However, since 13/14 of the rapidly progressing tumors and 14/16 of the conventional course control tumors included in this study harbored mutations in *TP53*, it is unlikely that *TP53* mutations are the explanation for the increased CNA levels or clinically aggressive behavior.

In contrast to the increased CNA levels, no consistent specific mutation differences were identified between the 2 groups (Fig. 5), and mutation load (point mutations per megabase) was not significantly altered in the rapidly progressing *IDH*-mutant astrocytoma group (Fig. 2B), suggesting that chromosomal instability rather than mutation load is the critical factor in the progression to GBM. The methylation pattern was also similar in both groups and consistent with *IDH*-mutant astrocytoma, as expected, which indicates that the downstream CpG island methylator phenotype (CIMP) caused by *IDH*-mutations is unchanged in the rapidly progressing group. A survey of 43 tumor suppressor genes with a known protective role in chromosomal stability identified a pathogenic mutation in 2 of the gliomas (14%) in the rapidly progressing *IDH*-mutant gliomas but none of the control *IDH*-mutant gliomas; further analysis revealed that none of the other 283 grade II–III gliomas in the TCGA had a pathogenic mutation in these 2 genes, *FANCB* and *APC*. The association of *FANCB* and *APC* mutations with chromosomal instability has been well established (45–49). Our results show that chromosomal instability can potentially negate the prognostically beneficial effect of *IDH* mutations in WHO II–III astrocytomas and suggest that increased CNA levels could serve as a biomarker for identifying *IDH*-mutant lower-grade astrocytomas at risk for rapid progression. The mechanism of the increased chromosomal instability remains unknown in the majority of the rapidly progressing *IDH*-mutant gliomas but in some cases it may be due to mutations in genes associated with chromosomal instability. While it was previously known that CNA was higher among *IDH1/2*-wildtype lower-grade gliomas and GBM irrespective of *IDH1/2* mutational status when compared with *IDH*-mutated lower-grade gliomas, the relationship between tumor progression and timing of this genomic instability has been unclear (12). In this report, we show that increased levels of CNA actually precede progression to GBM, at least in some cases with paradoxically rapid progression. This finding may provide an approach for identifying the lower-grade *IDH*-mutant astrocytomas at risk for a rapid progression and also suggests a mechanism underlying the progression.

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