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Clinicopathologic and FISH-Based Evaluation of CDKN2A/B and EGFR in IDH Mutant Astrocytomas

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ABSTRACT

Introduction: Isocitrate dehydrogenase(IDH)-mutant astrocytomas represent a biologically distinct but clinically heterogeneous lineage. In the World Health Organization Central Nervous System 5 (WHO, CNS) classification, CDKN2A/B homozygous deletion is a grade 4-defining alteration, while Epidermal growth factor receptor (EGFR) amplification is primarily associated with IDH-wildtype glioblastomas. This study evaluates the frequency and prognostic significance of these alterations in strictly defined IDH-mutant astrocytomas.

Methods: We analyzed 39 supratentorial IDH-mutant astrocytomas diagnosed between 2016 and 2020. CDKN2A/B deletion and EGFR amplification were assessed via routine fluorescence in situ hybridization (FISH) based testing and correlated with histologic parameters and clinical outcomes.

Results: CDKN2A/B deletion was identified in 61.5% of cases; however, high-level homozygous deletion (≥30% of nuclei) was present in only two cases. Epidermal growth factor receptor amplification was absent in all evaluable tumors. Neither WHO grade nor mitotic activity demonstrated a significant correlation with overall or disease-free survival. Notably, patients with high-level CDKN2A/B homozygous deletion remained alive without high-grade transformation at last follow-up.

Conclusion: These findings highlight the limited prognostic value of morphology within this molecular lineage and underscore the necessity of integrating molecular biomarkers into diagnostic frameworks. Larger multicenter cohorts with extended follow-up are essential to clarify the long-term prognostic significance of specific CDKN2A/B alterations.

Keywords: astrocytoma, CDKN2A/B deletion, EGFR amplification, FISH, prognosis, mitotic index

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INTRODUCTION

Diffuse gliomas represent the most common malignant primary tumors of the adult central nervous system and exhibit substantial biological and clinical heterogeneity (1). The discovery of isocitrate dehydrogenase (IDH) mutations fundamentally altered glioma taxonomy by revealing that tumors previously classified on the basis of morphology alone encompass distinct molecular lineages with divergent prognostic trajectories (1,2). IDH-mutant astrocytomas are defined by characteristic molecular features, most notably X-linked mutation (ATRX) loss and TP53 pathway alterations, which differentiate them from both 1p/19q-codeleted oligodendrogliomas and IDH-wildtype glioblastomas (3,4).

Despite this molecular homogeneity, clinical outcomes among patients with IDH-mutant astrocytomas remain variable. Traditional histopathologic grading criteria—cytologic atypia, mitotic activity, microvascular proliferation, and necrosis—were formulated prior to the advent of molecular subclassification and demonstrate limited prognostic precision within a lineage defined by uniform molecular drivers (5–7). Interobserver variability further diminishes the reproducibility of these criteria, contributing to misclassification and inconsistent prognostication (6,8,9). These limitations motivated the incorporation of CDKN2A/B homozygous deletion as a grade-4 defining molecular alteration in the WHO CNS5 classification, supported by large genomic datasets and meta-analyses demonstrating its strong association with adverse outcome (9–15).

Highlights

- **Mitotic count does not corelate with prognosis**
- **Tumor grade is an insufficient predictor of survival**
- **Astrocytomas can progress to higher grades**

Epidermal growth factor receptor amplification, by contrast, is a hallmark alteration of IDH-wildtype glioblastoma and is rarely encountered in IDH-mutant astrocytomas (16–19). In this context, EGFR status functions primarily as a lineage-confirming marker rather than a prognostic discriminator within IDH-mutant tumors.

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Although large-scale genomic studies have clarified the prognostic relevance of CDKN2A/B alterations, smaller fluorescence in situ hybridization (FISH)-based clinical cohorts frequently lack sufficient event rates to demonstrate early prognostic separation (20–22). Routine diagnostic data using standardized FISH interpretation criteria remain limited, yet such data are essential for understanding how these molecular markers perform in routine clinical settings. The present study aims to evaluate the frequency and prognostic implications of CDKN2A/B deletion and EGFR amplification in a single-center cohort of IDH-mutant astrocytomas, correlating molecular findings with histologic parameters and clinical outcomes.

METHODS

Thirty-nine adult patients diagnosed with supratentorial IDH-mutant astrocytomas between 2016 and 2020 were retrospectively identified from institutional archives. IDH1-R132 H immunohistochemistry was required for inclusion, and tumors with 1p/19q co-deletion were excluded to avoid oligodendroglial lineage contamination (23). X-linked mutation (ATRX) loss and p53 overexpression were recorded as canonical markers of astrocytic lineage (3,4). Histologic evaluation was performed according to WHO CNS5 criteria (2). Mitotic activity was quantified by counting mitotic figures across 10 consecutive high-power fields (2.37 mm^2 total area), following consensus recommendations designed to improve reproducibility (8,24).

Dual-color FISH using Abbott Vysis CDKN2A/CEP9 and EGFR/CEP7 probes was performed on formalin-fixed paraffin-embedded tissue sections. CDKN2A/B deletion was defined as a CDKN2A/CEP9 ratio below 0.8. Homozygous deletion was identified when tumor nuclei demonstrated complete loss of CDKN2A signals while retaining control centromeric signals, and cases with ≥30% homozygously deleted nuclei were classified as high-level homozygous deletion (20–22). Epidermal growth factor receptor amplification was defined as an EGFR/CEP7 ratio of ≥2.0 or the presence of tight signal clusters (17,18,22). A minimum of 100 non-overlapping tumor cell nuclei were evaluated per case. Clinical data, including age, sex, tumor location, treatment, and follow-up, were retrieved from medical records. Overall survival was calculated from the date of surgery to death or last follow-up, and disease-free survival from surgery to radiologic or histologic progression. Survival curves were generated using the Kaplan-Meier method, with comparisons performed using the log-rank test.

RESULTS

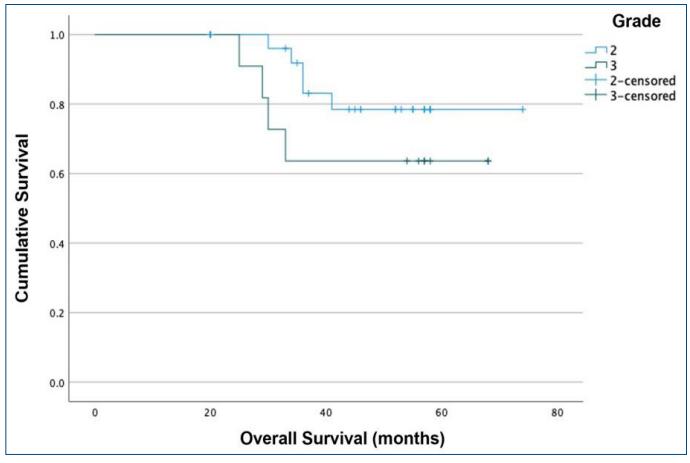
The median age at diagnosis was 38 years (range 18–55), and 61.5% of patients were male. Tumors most frequently involved the frontal and temporal lobes. Gemistocytic morphology was present in 38.5% of cases, and ATRX loss was observed in 74.3%, consistent with IDH-mutant astrocytic lineage features (3,4). Mitotic activity was uniformly low across the cohort: 53.8% of tumors demonstrated zero mitoses in 10 high-power fields, 33.3% showed one mitosis, and only 12.8% exhibited more than one mitotic figure. Neither mitotic index nor WHO grade correlated with overall survival (OS) or disease-free survival (DFS), consistent with prior evidence questioning the prognostic utility of conventional histologic grading within this molecular subtype (6–8,24,25) (Fig. 1 and 2).

CDKN2A/B deletion was detected in 24 of 39 tumors (61.5%). Seven cases demonstrated some degree of homozygous loss, whereas high-level homozygous deletion (≥30% of nuclei) was identified in two tumors. These two patients did not exhibit early aggressive clinical behavior and remained alive without high-grade transformation at a median follow-up of 52 months, although one developed a localized low-grade recurrence. Epidermal growth factor receptor amplification was not observed in any of the 27 tumors with adequate hybridization quality, aligning with current knowledge that EGFR amplification is characteristic of IDH-wildtype glioblastoma rather than IDH-mutant astrocytoma (16–19) (Fig. 3 and 4).

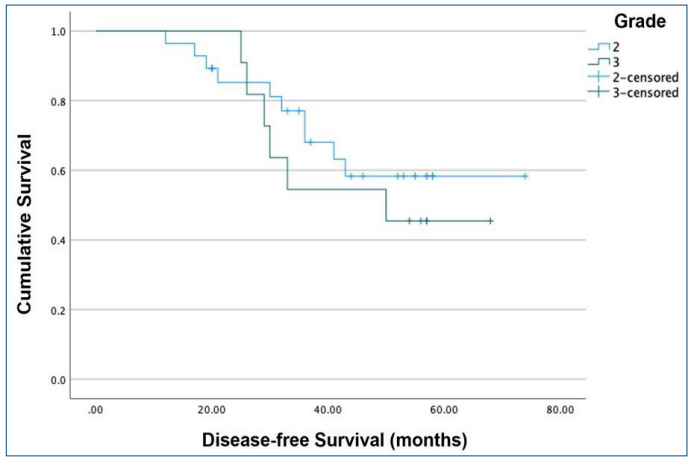
Two patients demonstrated radiologic and morphologic progression to WHO grade 4 during follow-up, yet both remained alive at last contact. The five-year overall survival rates for Grade 2 and Grade 3 tumors were 65.2% and 54.5%, respectively, with no statistically significant difference. These findings reinforce the limited discriminatory power of histologic grade in IDH-mutant astrocytomas (Fig. 5).

DISCUSSION

This study contributes additional evidence that traditional morphology-based prognostic markers—particularly WHO grade and mitotic index—offer limited prognostic resolution within IDH-mutant astrocytomas. Multiple interobserver and multi-institutional studies have documented substantial variability in the assessment of mitotic activity and histologic grade, which were originally developed for biologically heterogeneous pre-molecular tumor categories (5–8). Our findings, consistent with these prior observations, indicate that morphology alone cannot reliably stratify risk within a lineage unified by IDH mutation status.

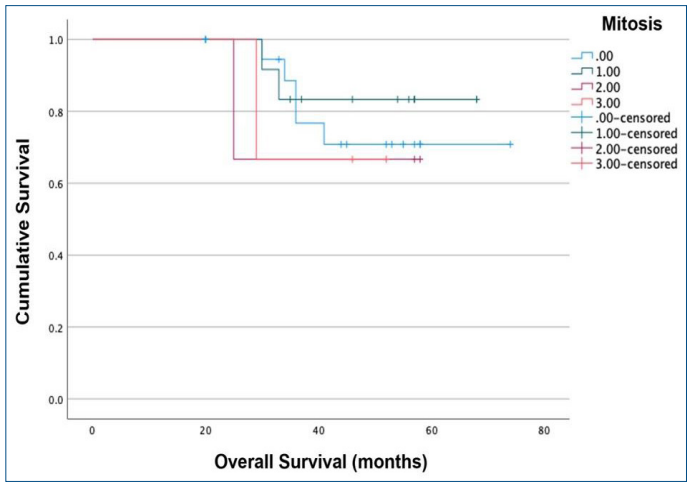


(a)

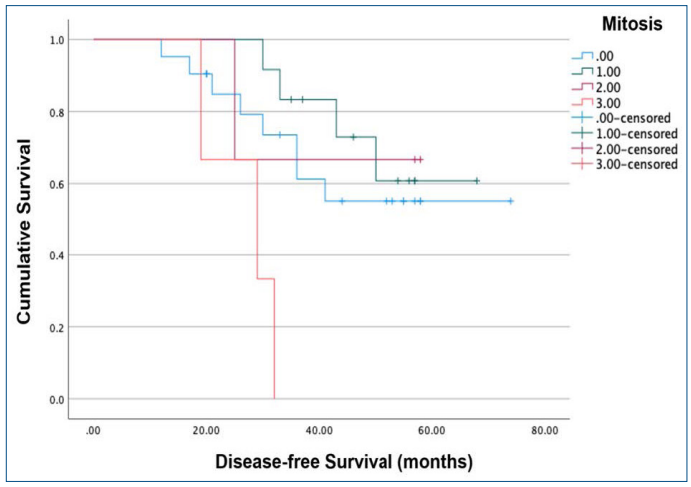


(b)

Figure 1. Tumor grade does not confer worse OS **(a)** or DFS **(b)** between Grade 2 and Grade 3 astrocytomas.



(a)



(b)

Figure 2. Mitoses per 10 high-power fields (HPFs, 2.37 mm²) do not confer worse OS **(a)** or DFS **(b)** between Grade 2 and Grade 3 astrocytomas.

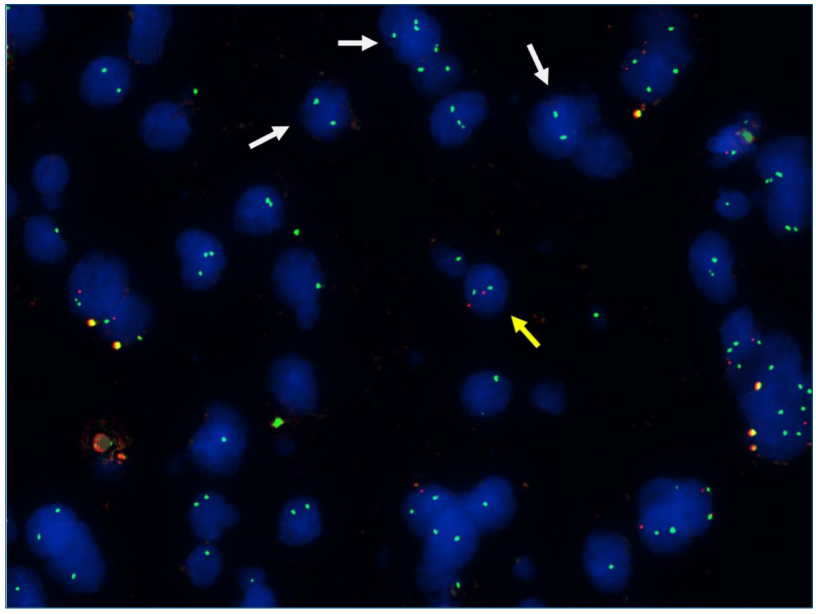
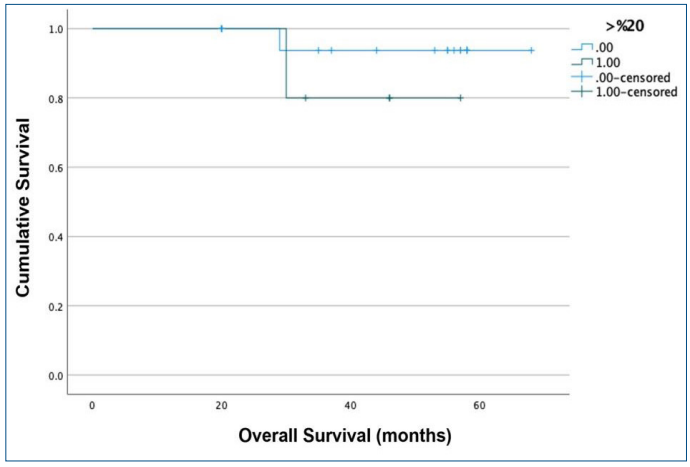
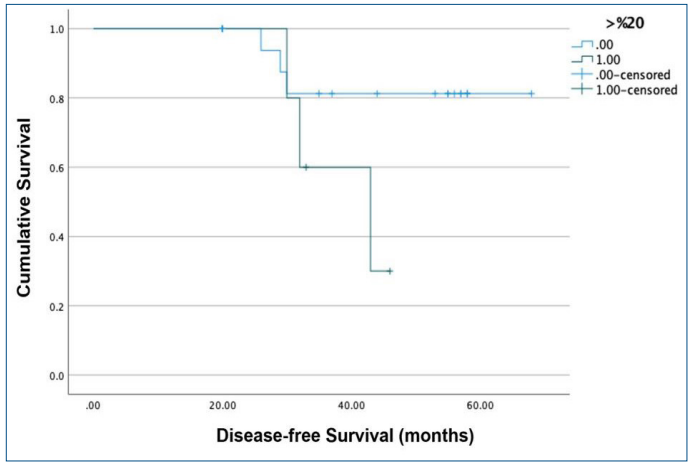


Figure 3. White arrows indicate homozygous CDKN2A/B deletions, yellow arrow indicates normal signals.



(a)



(b)

Figure 4. Hemizygous deletions in CDKN2A/B gene do not confer worse OS **(a)** or DFS **(b)** between Grade 2 and Grade 3 astrocytomas.

The absence of EGFR amplification in all analyzable tumors strongly supports the established view that EGFR-driven oncogenic signaling is a defining molecular feature of IDH-wildtype glioblastoma and is rarely, if ever, encountered in IDH-mutant astrocytomas (16–19). Its complete absence in this cohort reinforces the role of EGFR status as a lineage marker rather than a prognostic variable within IDH-mutant tumors.

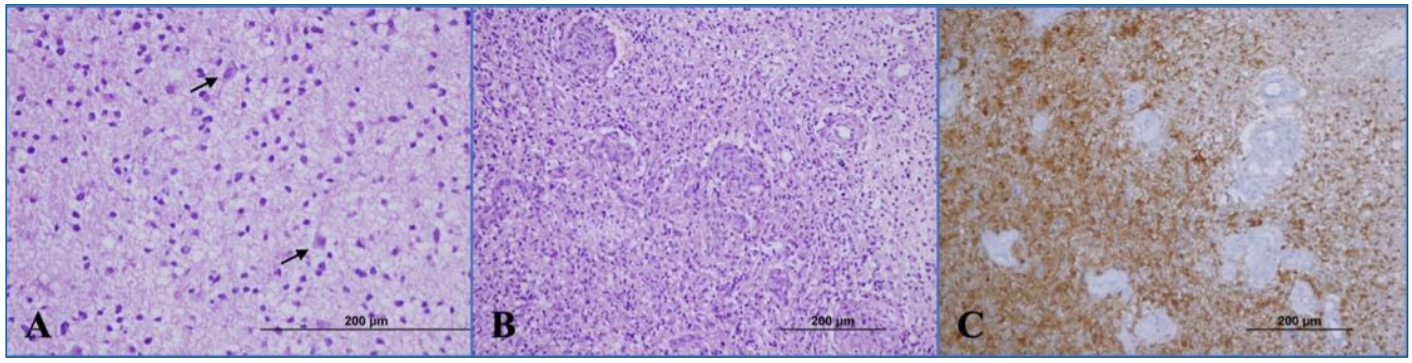


Figure 5. Histopathological features of tumor grade progression in an IDH-1 mutant astrocytoma **(a)**. Initial presentation of a Grade 2 astrocytoma characterized by mild cellularity without significant nuclear atypia or mitotic activity (H&E) **(b)**. Recurrent tumor at the 3-year follow up, demonstrating progression to a higher grade with prominent cytological atypia and microvascular proliferation (H&E). **(c)** Strong and diffuse cytoplasmic positivity for IDH-1 immunohistochemistry in the recurrent tumor cells, confirming the molecular lineage.

CDKN2A/B deletion was relatively common, whereas high-level homozygous deletion was rare, consistent with frequencies reported in prior FISH-based and sequencing-based studies (9,10,20,21,26). Large genomic datasets and recent meta-analyses consistently identify CDKN2A/B homozygous deletion as one of the strongest adverse prognostic biomarkers in IDH-mutant astrocytoma (9–15). However, early clinical outcomes in our cohort—including progression-free survival in both high-level homozygous-deleted cases—mirror findings from other small FISH-based studies, in which early follow-up may fail to capture long-term risk (20,21,26). This suggests that the deleterious impact of CDKN2A/B homozygous deletion may manifest predominantly during extended follow-up intervals.

Recent multicenter evidence has expanded understanding of CDKN2A/B alterations. Ippen et al. (2025) demonstrated that even hemizygous deletions independently worsen survival in IDH-mutant astrocytomas, suggesting a dose-dependent effect of CDKN2A/B copy-number reduction on tumor aggressiveness (27). In our series, hemizygous deletions were frequent but did not exhibit early adverse outcomes—likely due to limited sample size and follow-up. Nevertheless, these findings align with the concept that CDKN2A/B status behaves along a continuous prognostic spectrum, rather than as a binary alteration, and underscore the need for larger, genomically integrated datasets (9,27).

Additional molecular factors—including global copy-number burden, PDGFRA amplification, CDK4 alterations, and DNA methylation class—provide meaningful prognostic refinement (12–15,24,28). Thus, integrating molecular data offers superior prognostication compared with morphology alone. Despite the availability of sequencing-based approaches, FISH remains a robust and widely accessible tool in routine neuropathology practice.

Limitations

This study is limited by its single-center design and modest sample size, which restricts the ability to detect statistically significant associations, particularly for low-frequency events such as high-level CDKN2A/B homozygous deletion. Treatment heterogeneity was inherent to the retrospective design but did not introduce identifiable systematic bias. The absence of methylation profiling or genome-wide sequencing limits the capacity to explore interactions between CDKN2A/B alterations and broader genomic architecture. Although follow-up duration was sufficient to evaluate early and mid-term outcomes, longer longitudinal assessment will be necessary to fully characterize late divergence in survival curves, which is well-recognized in IDH-mutant gliomas (14,15,28).

As a result, in this cohort of IDH-mutant astrocytomas, traditional histologic indicators—including WHO grade and mitotic activity—did not reliably stratify clinical outcomes, underscoring their limited prognostic utility within this molecularly defined lineage. Epidermal growth factor receptor amplification was uniformly absent, further reinforcing its role as a hallmark of IDH-wildtype glioblastoma rather than a prognostic biomarker in IDH-mutant tumors (16–19). CDKN2A/B deletions were frequent, whereas high-level homozygous deletions were rare and did not exhibit early aggressive behavior. Emerging multicenter evidence, including recent data from Ippen et al. (27), suggests that CDKN2A/B alterations may exert a graded, rather than binary, biological impact, positioning CDKN2A/B status along a continuous prognostic spectrum (9,27). Together, these findings highlight the importance of integrating molecular biomarkers into diagnostic and prognostic frameworks. Larger multicenter studies incorporating methylation profiling and extended follow-up are essential to refine risk stratification and improve prognostic accuracy in IDH-mutant astrocytomas.

Ethics Committee Approval: This retrospective study was approved by the institutional ethics committee of Istanbul University, Istanbul Faculty of Medicine (issue number: 2022/476).

Informed Consent: Because of the study's retrospective nature and the anonymous use of patient information, ethics committee waived the requirement for informed consent.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- AYY, GÜ; Design- AYY, GÜ; Supervision- AYY; Resource- AYY; Materials- AYY; Data Collection and/or Processing- AYY; Analysis and/or Interpretation- AYY; Literature Search- AYY; Writing- AYY, SÇ; Critical Reviews- GÜ, BB, İD.

Conflict of Interest: The authors declared that there is no conflict of interest.

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