Letter to the Editor

Targeted copy number analysis outperforms histologic grading in predicting patient survival for WHO grades II/III IDH-mutant astrocytomas

Since the division of World Health Organization (WHO) grades II/III astrocytomas by isocitrate dehydrogenase (IDH) mutational status, historical histologic grading schemes no longer provide prognostic information for IDH-mutant astrocytoma, which comprises the majority of these tumors.^{1,2} As an alternative to histologic grading, our group,³ and others,⁴ have demonstrated that a combination of whole chromosome and gene level somatic copy number alterations (SCNAs) portend survival. Using data from The Cancer Genome Atlas (TCGA), our group has previously shown that IDH-mutant astrocytomas can be stratified into 3 distinct prognostic SCNA subtypes (determined by cyclin-dependent kinase inhibitor 2A [*CDKN2A*] deletion, *CDK4* amplification, and loss of either whole chromosome 14 or loss of 14q), which are independent of WHO grade (II–IV): M1 (worst survival), M2 (intermediate survival), and M3 (best survival) (Fig. 1).³ While these SCNA subtypes were validated in a large cohort of glioblastoma patients from the German Glioma Network,³ they have not been validated in lower-grade WHO II/III IDH-mutant astrocytomas. Given the underwhelming performance of histologic grading of IDH-mutant astrocytomas, we sought to validate our prognostic SCNAs in an institutional cohort of WHO grades II/III IDH-mutant astrocytomas.

Institutional file review captured a cohort of newly diagnosed WHO grades II/III diffuse gliomas resected between 2000 and 2010 ($n = 178$) (human subject use approved by the University of Washington institutional review board). Inclusion criteria required patients to have at least 5 years of clinical follow-up, if not recurrence or death earlier ($n = 109$). Extracted DNA underwent genome-wide methylation analysis using the InfiniumR EPIC Methylation Array ($n = 66$). IDAT files were analyzed (<https://www.molecularneuropathology.org>) to classify diffuse gliomas into one of the 3 following categories: astrocytoma IDH-wildtype, WHO grades II/III ($n = 7$); astrocytoma IDH-mutant, WHO grades II/III ($n = 34$); and oligodendroglioma

IDH-mutant and 1p/19q-codeleted, WHO grades II/III ($n = 25$).⁵ IDH-mutant astrocytomas were further analyzed by WHO histologic grading and proposed SCNA molecular subtypes. In this cohort, and consistent with previous reports,² histologic grading was not prognostic for progression-free survival (PFS; $P = 0.349$) or overall survival (OS; $P = 0.139$) (Fig. 1). However, evaluation of SCNA subtypes M1–M3 provided prognostic information for recurrence-free survival ($P = 0.0044$) and OS ($P < 0.0001$) in this institutional cohort, similar to what was observed in the dataset from TCGA (Fig. 1). SCNA subtypes were further broken down by their constitutive components. *CDKN2A* deletion was associated with worse OS ($P = 0.0136$), but only trended for PFS ($P = 0.146$) (Fig. 1). Amplification of *CDK4* did not show a difference for either PFS ($P = 0.722$) or OS ($P = 0.587$). Loss of chromosome 14 demonstrated worse PFS ($P = 0.0044$), but only trended for OS ($P = 0.237$) (Fig. 1). Overall, the combination of *CDKN2A* deletion, *CDK4* amplification, and chromosome 14 loss provides useful prognostic information in WHO grades II/III IDH-mutant astrocytomas, while histologic grading is not prognostic.

As diffuse glioma classification is refined in the era of molecular diagnostics, grading will likely incorporate molecular features beyond IDH mutational status. Stratifying IDH-mutant astrocytomas by SCNAs such as *CDKN2A* deletion, with or without additional genetic/chromosomal markers, may serve as the basis for molecular grading. Determining SCNAs may also be more consistent and reproducible for identifying aggressive IDH-mutant astrocytomas than grading by mitotic activity, thereby reducing the interobserver variability in neuropathological diagnosis and clinical risk stratification. SCNA subtyping of IDH-mutant astrocytomas may also be important for clinical trial enrollment. Our group has previously shown that IDH-wildtype glioblastoma SCNA subtypes are unevenly distributed in medical and surgical trial cohorts relative to large general population cohorts, having implications for trial enrollment.⁶ Therefore, it may be necessary to incorporate SCNA analysis into clinical trials for IDH-mutant astrocytoma to ensure even distribution of molecular subtypes across treatment arms in order to allow generalizability of clinical trial results and determine subtype-specific therapeutic strategies.

Keywords

astrocytoma | isocitrate dehydrogenase | IDH | molecular profiling | biomarkers

Fig. 1 Prognostic value of copy number alterations for IDH-mutant diffuse astrocytomas. (A) Algorithm for SCNA subtype derivation. SCNA subtypes are defined by 3 genetic loci: amplification of *CDK4* (a *CDK4*), homozygous deletion of *CDKN2A* (del *CDKN2A*), and loss of chromosome 14 (lossChr14): M1 = a *CDK4* and/or del *CDKN2A* + lossChr14; M2 = a *CDK4* and/or del *CDKN2A* + no lossChr14 or no a *CDK4* or del *CDKN2A* + lossChr14; M3 = no a *CDK4* + no del *CDKN2A* + no lossChr14. (B) Overall survival (OS) of IDH-mutant diffuse astrocytomas (WHO grades II and III) by SCNA subtypes in TCGA dataset. (C) Kaplan-Meier survival curves for University of Washington (UW) cohort of IDH-mutant astrocytomas. Progression-free survival (PFS) and OS by WHO histologic grading, SCNA subtype, CDKN2A homozygous deletion status, and loss of chromosome 14 status. All *P*-values were determined using Cox proportional hazards regression.

Funding

Research reported in this publication was supported by a Seattle Translation Tumor Research (<http://www.sttrcancer.org>) Precision Medicine Grant awarded to P.J.C. Normal brain control tissue used in this study was obtained from the University of Washington Neuropathology Core, which is supported by the Alzheimer's Disease Research Center (NIH AG05136) and Adult Changes in Thought Study (NIH AG006781).

Conflict of interest statement. The authors declare that there are no conflicts of interest.

Authorship statement. PJC and ECH designed the study. Data collection was performed by PJC. PJC analyzed and interpreted data. PJC and ECH participated in drafting, revising, and approving the manuscript.

Patrick J. Cimino and Eric C. Holland

Department of Pathology, Division of Neuropathology, University of Washington School of Medicine, Seattle, Washington (P.J.C.); Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington (E.C.H.)

Corresponding Authors: Patrick J. Cimino, MD, PhD, Department of Pathology, Division of Neuropathology, University of Washington, 325 9th Avenue, Box 359791, Seattle, WA, 98104 (pjcc@uw.edu); Eric C. Holland, MD, PhD, Division of Human Biology, and Solid Tumor and Translational Research, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N., Mailstop C3-168, Seattle, WA, 98109 (eholland@fredhutch.org).

References

1. von Deimling A, Ono T, Shirahata M, Louis DN. Grading of diffuse astrocytic gliomas: a review of studies before and after the advent of IDH testing. *Semin Neurol*. 2018;38(1):19–23.

2. Olar A, Wani KM, Alfaro-Munoz KD, et al. IDH mutation status and role of WHO grade and mitotic index in overall survival in grade II-III diffuse gliomas. *Acta Neuropathol.* 2015;129(4):585–596.
3. Cimino PJ, Zager M, McFerrin L, et al. Multidimensional scaling of diffuse gliomas: application to the 2016 World Health Organization classification system with prognostically relevant molecular subtype discovery. *Acta Neuropathol Commun.* 2017; 5(1):39.
4. Shirahata M, Ono T, Stichel D, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. *Acta Neuropathol.* 2018;136(1):153–166.
5. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature.* 2018;555(7697):469–474.
6. Cimino PJ, McFerrin L, Wirsching HG, et al. Copy number profiling across glioblastoma populations has implications for clinical trial design. *Neuro Oncol.* 2018;20(10):1368–1373.