Brief Report

Rapid Intraoperative Molecular Characterization of Glioma

Ganesh M. Shankar, MD, PhD; Joshua M. Francis, PhD; Mikael L. Rinne, MD, PhD; Shakti H. Ramkissoon, MD, PhD; Franklin W. Huang, MD, PhD; Andrew S. Venteicher, MD, PhD; Elliot H. Akama-Garren; Yun Jee Kang, BA; Nina Lelic, BS; James C. Kim, BS; Loreal E. Brown, BS; Sarah K. Charbonneau, BS; Alexandra J. Golby, MD; Chandra Sekhar Pedamallu, PhD; Mai P. Hoang, MD, PhD; Ryan J. Sullivan, MD; Andrew D. Cherniack, PhD; Levi A. Garraway, MD, PhD; Anat Stemmer-Rachamimov, MD; David A. Reardon, MD; Patrick Y. Wen, MD; Priscilla K. Brastianos, MD; Franklin W. Huang, MD, PhD; Andrew S. Venteicher, MD, PhD; Elliot H. Akama-Garren; Yun Jee Kang, BA; Nina Lelic, BS; Ganesh M. Shankar, MD, PhD; Joshua M. Francis, PhD; Mikael L. Rinne, MD, PhD; Shakti H. Ramkissoon, MD, PhD; Franklin W. Huang, MD, PhD; Andrew S. Venteicher, MD, PhD; Elliot H. Akama-Garren; Yun Jee Kang, BA; Nina Lelic, BS; James C. Kim, BS; Loreal E. Brown, BS; Sarah K. Charbonneau, BS; Alexandra J. Golby, MD; Chandra Sekhar Pedamallu, PhD; Mai P. Hoang, MD, PhD; Ryan J. Sullivan, MD; Andrew D. Cherniack, PhD; Levi A. Garraway, MD, PhD; Anat Stemmer-Rachamimov, MD; David A. Reardon, MD; Patrick Y. Wen, MD; Priscilla K. Brastianos, MD; William T. Curry, MD; Fred G. Barker II, MD; William C. Hahn, MD, PhD; Brian V. Nahed, MD; Keith L. Ligon, MD, PhD; David N. Louis, MD; Daniel P. Cahill, MD, PhD; Matthew Meyerson, MD, PhD

Methods

Tumor Specimens
Case records were reviewed, and glioma specimens were obtained under approval of the institutional review boards at Dana-Farber Cancer Institute and Massachusetts General Hospital. Samples were collected following informed, written consent. The records were reviewed under a protocol that allowed analysis of anonymized patient data collected following informed, written consent. There was no sex preference when obtaining specimens. For specimens with available sex information, the cohort was made up of 49% male and 51% female patients.

Rapid Genotyping Assay
We designed a quantitative polymerase chain reaction (PCR)-based method to detect cancer-specific mutations in specimens with low tumor density through (1) the inclusion of peptide nucleic acid (PNA) oligonucleotides that block amplification of wild-type alleles and (2) the incorporation of locked nucleic acid (LNA) into the detection probes to increase specific binding to the mutant allele (eMethods and eFigures 1 and 2 in the Supplement). Specifically, this assay was designed to detect IDH1 R132H/C/G/L/S and TERT promoter mutations on chromosome 5 at positions 1 295 228 and 1 295 250 based on human genome reference version 19, referred to as TERT C228T or TERT C250T (eTable 1 in the Supplement). We could detect serial dilutions representing 0.1% to 10.0% of positive control genomic extracts diluted in negative control extracts,
allowing for estimation of tumor purity (eFigures 3 and 4 in the Supplement).

Statistical Analysis
A 2-tailed, nonpaired t test was used to calculate the significance of the difference between the number of biopsy attempts required of diagnostic and nondiagnostic cases, with the estimated half-width of the 95% CI calculated using a t test distribution in Microsoft Excel. The 95% CIs for the reported sensitivity and specificity of the assay were calculated using the binomial test in the R statistical package (version 0.98.1091).

Results
To evaluate how intraoperative histologic analysis of low-cellularity tumors affects surgical decision making, we reviewed 72 cases of newly diagnosed WHO grade II diffuse gliomas treated at Brigham and Women’s Hospital from 2009 through 2014. In this series, biopsy specimens obtained in 28 (39%) of 72 cases could not be conclusively diagnosed as glioma on the intraoperative frozen specimen (Figure 5 in the Supplement). These inconclusive stereotactic biopsies required additional surgical sampling compared with cases with diagnostic biopsies (mean [SD] number of biopsies for conclusive glioma diagnosis, 3.08 [1.20] vs 1.13 [0.30]; P = .01) (eTable 3 in the Supplement). Because of the anticipated difficulty in establishing an intraoperative glioma diagnosis, surgeons may elect to perform a stereotactic biopsy before proceeding with a definitive resection in a separate procedure following final histologic confirmation.

At a Glance
- Intraoperative diagnosis of diffuse glioma is critical for distinguishing tumor from nonneoplastic mimics and defining the goals of neurosurgical resection.
- Intraoperative genotyping for recurrent mutations in IDH1 and the TERT promoter could characterize over 80% of diffuse gliomas.
- This assay was validated on 174 glioma specimens and was able to correctly identify WHO grades II and III glioma with 96% sensitivity (95% CI, 90%-99%) and 100% specificity (95% CI, 95%-100%).
- Glioma-defining mutations were identified in 13 of 14 previously inconclusive intraoperative stereotactic biopsy samples.
- It is possible to characterize glioma-defining mutations within 60 minutes during a live neurosurgical resection.
To augment intraoperative diagnosis, we developed an assay, OperaGen (for “operative genotyping”) to simultaneously genotype IDH1 and TERT promoter variants. We first evaluated OperaGen across a range of clinically annotated gliomas by comparing its molecular characterization with the histologic diagnosis of 80 archived formalin-fixed, paraffin-embedded glioma samples. OperaGen was able to detect every tumor with an IDH1 mutation (58 of 58 samples, Figure 1A). Concurrent IDH1 and TERT promoter mutations detected by OperaGen accurately identified oligodendrogliomas in 38 (95%) of 40 cases with known 1p/19q codeletion.5 In addition, isolated TERT promoter mutations were detected in 17 (77%) of 22 glioblastomas (GBMs), a frequency consistent with prior reports.6 The TERT promoter status was confirmed by next-generation sequencing (NGS) with coverage greater than 180,000× (eFigure 6 in the Supplement). In addition, patient survival correlated with a retrospective analysis of IDH1 and TERT promoter genotype status by OperaGen (eFigure 7 in the Supplement).

We next evaluated OperaGen in a cohort of frozen WHO grade II diffuse gliomas to determine whether this assay could clarify intraoperative diagnosis in these lower-cellularity tumors. Because of the high frequency of IDH1 mutations in these glioma subtypes, we designed VIC-conjugated oligonucleotide detection probes to capture additional IDH1 mutations otherwise not detected by immunohistochemical analysis found in up to 12% of IDH1-mutated gliomas.7 When multiplexed with the fluorescein-conjugated IDH1 R132H probe, these reactions allowed for simultaneous detection of glioma-specific IDH1 variants, discriminating the less frequent mutations from IDH1 R132H (eFigure 8 in the Supplement). With this design we were able to detect either IDH1 or TERT promoter mutations in 42 (95%) of 44 frozen diffuse glioma specimens (Figure 1B).

To explore the possible false-positive rate associated with OperaGen, we analyzed 50 frozen GBM specimens (eFigure 9 in the Supplement) and validated the results by high-depth sequencing. Separately, 14 non-glioma brain biopsy specimens tested negative for IDH1 and TERT promoter variants by OperaGen (eTable 2 in the Supplement). Both specificity and sensitivity of diagnosis were high for oligodendrogliomas, diffuse astrocytomas, oligoastrocytomas, and IDH1-mutant GBMs, where the strong relationship to IDH1 mutation in this aggregated series of 174 cases revealed 96% sensitivity (95% CI, 90%-99%) and 100% specificity (95% CI, 95%-100%) (eFigure 9 in the Supplement).
In contrast, sensitivity was lower for the diagnosis of GBM with isolated \textit{TERT} promoter mutations, consistent with reported rates in this tumor type and likely a reflection of alternative mechanisms of telomere maintenance.\(^8\)

We observed a higher rate of inconclusive intraoperative histology with stereotactic biopsies compared with resections (62\% vs 23\%, \textit{Figure 2A}). Because of its sensitivity, we asked whether OperaGen could identify glioma-specific mutations in limited tissue obtained from stereotactic biopsies. We identified 14 cases of glioma for which intraoperative pathology was inconclusive in spite of repeated biopsy sampling (eTable 3 in the \textit{Supplement}). In 93\% of these cases (13 of 14), we were able to identify \textit{TERT} promoter or \textit{IDH1} variants in the first available specimen (first or second biopsy core, \textit{Figure 2B}). As illustrated by these results, OperaGen could help establish a glioma diagnosis while minimizing the number of biopsies and the risks associated with additional passes.\(^9\)

We next demonstrated that this assay could be carried out during glioma resection, ruling out the presence of 0.1\% mutant allele fraction within 60 minutes (eFigures 3 and 10 in the \textit{Supplement}). We present 2 cases to demonstrate how this workflow could be applied in a clinical setting. The first case illustrates the ability to detect \textit{TERT C228T} in GBM (\textit{Figure 3A}). The second patient had a frozen section diagnosis of GBM; however, OperaGen detected an \textit{IDH1 R132H} mutation and no \textit{TERT} promoter mutation, which raised the possibility of a WHO grade II or III glioma (\textit{Figure 3B}). Indeed, the pathologic assessment from the permanent specimen ultimately established the tumor as an \textit{IDH1}-mutant WHO grade III anaplastic oligoastrocytoma. While intraoperative histologic analysis confirmed the lesion as glioma, further molecular classification as an \textit{IDH1}-mutant tumor could guide the decision to extend the surgical margin into adjacent nonenhancing tumor.\(^2,3\)

\textbf{Discussion}

Histologic assessment combined with molecular testing can provide a more accurate integrated diagnosis of brain tumors.\(^10\) In accord, research techniques have applied real-time PCR detection of \textit{IDH1} mutation\(^11\) or mass spectrometry to the intraoperative detection of 2-hydroxyglutarate, the metabolite of mutant \textit{IDH1}.\(^12\) OperaGen was designed to simultaneously detect distinguishing genomic features in both the \textit{TERT} promoter and \textit{IDH1} to sensitively identify diffuse glioma in an intraoperative timeframe. Optimal surgical intervention is shaped by the glioma subtype, since unlike primary GBM, \textit{IDH1}-mutant gliomas appear to benefit from complete resection of enhancing and nonenhancing disease.\(^2,3\)

While alternative techniques such as NGS can also detect somatic variants in low-purity tumors, the equipment, time, and analytical methods required for NGS preclude widespread clinical use and intraoperative application. Herein we have demonstrated an approach that can
identify tumor-specific alterations to a sensitivity of 0.1% mutant allele fraction within 60 minutes. We envision running this assay in parallel with frozen section analysis to augment cases where pathologic findings may be inconclusive. Though this testing may add time to these inconclusive cases, a definitive molecular diagnosis could reduce the instances of repeated biopsies, as highlighted in Figure 2, limiting the potential hazards associated with multiple sampling. Furthermore, the availability of intraoperative molecular information could obviate the need for staged craniotomies, thus reducing the risks associated with a second neurosurgical procedure. Before provisioning Operagen for clinical use, larger cohort studies will be needed to address false-positive and false-negative rates resulting from issues related to tissue quality or surgical sampling error. The ability to obtain intraoperative molecular information also expands the possibilities of administering direct intratumoral therapy or performing interstitial thermal ablation of glioma in a single procedure.13

Conclusions

The molecular genotyping approach described herein has potential application beyond the diagnosis of diffuse gliomas. TERT promoter mutations have been identified in a large number of other cancers5 and appear to distinguish tumor from non-neoplastic tissue. The principles of this assay could also be applied to other conditions associated with well-defined variants, such as KRAS, BRAF, and EGFR mutants to facilitate precise intraoperative marginal analysis.14,15 Ultimately, such translational genomic approaches are critical for improving point-of-care diagnosis in the era of precision cancer medicine.


