Neuro-Oncology
23(9), 1423–1424, 2021 | doi:10.1093/neuonc/noab146 | Advance Access date 17 June 2021

Serum cell-free DNA epigenetic biomarkers aid glioma diagnostics and monitoring

Kevin C. Johnson and Roel G. W. Verhaak

The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut, USA (K.C.J., R.G.W.V.); Department of Neurosurgery, Amsterdam UMC, Vrije Universiteit, and Brain Tumor Centre, Cancer Center Amsterdam, Amsterdam, the Netherlands (R.G.W.V.)

Corresponding Author: Roel G. W. Verhaak, PhD, The Jackson Laboratory for Genomic Medicine, Farmington, CT 06032, USA (roel.verhaak@jax.org).

See the article by Sabedot et al, pp. 1494–1508.

A definitive diagnosis of diffuse glioma is aided by medical imaging and ultimately confirmed by pathological assessment of surgical specimens. However, invasive surgery can introduce undue risk to the patient in cases where imaging findings are ambiguous or anatomical location complicates tumor biopsy or resection. Liquid biopsies have emerged as a minimally invasive way to assist both initial diagnosis and monitor glioma evolution using biomarkers such as circulating tumor-derived cell-free DNA (cfDNA).[1–3] Gliomas have been shown to shed their nucleic acid content as cfDNA into body fluids, including cerebrospinal fluid[1,3–5] and blood (plasma[6] and serum[3]), thereby allowing for indirect genomic assessment of a tumor's molecular composition.

Prior studies have evaluated cfDNA's clinical utility largely via the detection of genetic changes, such as mutations or somatic copy number alterations, in cerebrospinal fluid.[1,3–5] This approach has demonstrated the feasibility of detecting and tracking genetic changes yet suffers from the drawback of a minimally invasive procedure (ie, lumbar puncture) and limitations in assay sensitivity due to a combination of cfDNA content, genetic alteration burden, and alteration clonality.[7] Complementary liquid biopsy molecular profiling efforts have focused on studying the epigenetic modifications of cfDNA in intracranial tumors[6] and other tumor types.[7,8] Epigenetic modifications such as DNA methylation located at cytosine followed by guanine dinucleotides (ie, CpGs) serve to encode cellular states. As a result, measurements of DNA methylation patterns can also provide information about the cell-of-origin for cfDNA. The cell-of-origin DNA methylation specificity is supported by the similarity between cfDNA methylation states and its matched tumor.[8] The discriminatory power of these analyses was recently demonstrated with plasma cfDNA DNA methylation successfully distinguishing amongst different intracranial tumor types with a similar cell-of-origin.[6]

In the current issue of *Neuro-Oncology*, Sabedot et al describe a noninvasive serum cfDNA collection approach followed by genome-wide DNA methylation assessment to study cfDNA epigenetic modifications in patients with glioma and non-glioma brain diseases (eg, meningioma, brain metastatic tumor, etc.).[9] Unlike a previous plasma cfDNA DNA methylation study that used methylated DNA immunoprecipitation followed by sequencing,[6] the platform used here was the same Illumina BeadChip DNA methylation technology used in large glioma cohorts that have previously defined methylation signatures associated with distinct clinical outcomes.[10,11] However, these previously identified glioma tissue signatures were not detectable in the serum cfDNA, which may reflect that serum cfDNA is a mixture of tumor and non-tumor cell cfDNA. Instead, the authors developed a novel cfDNA-specific DNA methylation signature that reflected the presence of glioma and associated immune differences in glioma patients (eg, elevated B cell signature). This signature's single CpG probe-based nature differs from the previously defined regional-based (300-bp) blood cfDNA signature,[6] but is similarly enriched at CpG dense genomic regions (eg, CpG islands).

To develop a sensitive classification method, the data were first split into a discovery set (glioma n = 38, non-glioma n = 42) and then a supervised epigenome-wide analysis was used to identify CpG sites where the methylation was similar between serum and matched tissue. The top differentially methylated CpGs in serum were determined by comparing patients with glioma with those harboring other central nervous system diseases. These serum-based DNA methylation markers were then used to construct a machine learning-based model able to distinguish serum samples belonging to glioma patients using a glioma-epigenetic-liquid biopsy (GeLB) score. The group confirmed these findings in a validation set (glioma n = 18, non-glioma n = 45) with high sensitivity (100%, 18/18) and specificity (97.78%, 44/45). A similar serum cfDNA epigenetic framework was also shown to be able to distinguish patients with IDH-mutant vs IDH-wild-type glioma. Together with prior publications,[6] the analyses presented here highlight that epigenetic fingerprints in cfDNA are capable of discriminating

between malignancies with similar origins, but different clinical trajectories.

The ability to non-invasively assess tumor DNA methylation profiles in serum is a potentially attractive approach for monitoring disease progression. The authors addressed this topic with a subset of patients (n = 33) where multiple samples were available and patients followed standard treatment regimens or, in a few cases, experimental agents. The GeLB score, an indicator for whether the serum sample was likely from a glioma patient, decreased at recurrence in a majority of patients for reasons not completely understood. While the GeLB score was developed using serum samples from patients with a primary glioma diagnosis, the general decrease in score suggests that treatment induces a DNA methylation shift in glioma cells similar to what has been observed in longitudinal glioma tissue sampling.[11] Despite the variability from baseline GeLB score, the authors did highlight specific cases where changes in GeLB score may reflect treatment resistance (ie, increases in GeLB score) or pseudoprogression (ie, decrease in GeLB score despite the radiographic suggestion of progression, histologically confirmed as necrosis or reactive tissue). The lack of cohort-level consistent longitudinal changes suggests that signatures used to predict initial disease may not be optimal for disease progression or that gliomas take diverse epigenetic evolutionary paths following therapy. While these analyses serve as a proof of principle that the serum cfDNA can measure dynamic DNA methylation states, larger studies are needed to refine signatures that predict patient response to treatment and progression.

The work from Sabedot et al[9] and Nassiri et al[6] provide convincing evidence that blood-derived cfDNA epigenetic signatures can help establish an initial diagnosis prior to surgical intervention. Clinical implementation of epigenetic liquid biopsies would provide complementary and additional diagnostic information to current modalities. However, key technical considerations need to be addressed prior to clinical adoption including standardized protocols for sample collection and cost-effective molecular profiling, robust deconvolution of non-tumor cfDNA signal, and more accurate therapeutic response assessments. Larger studies addressing these challenges will help successfully translate these early epigenetic liquid biopsy findings into improving brain tumor diagnostics and disease monitoring.

Conflict of interest statement. The text is the sole product of the authors and that no third party had input or gave support to its writing. R.G.W.V. is a cofounder of Boundless Bio, Inc.

References

1. Martinez-Ricarte F, Mayor R, Martínez-Sáez E, et al. Molecular diagnosis of diffuse gliomas through sequencing of cell-free circulating tumor DNA from cerebrospinal fluid. *Clin Cancer Res.* 2018;24;2812–2819.
2. Müller Bark J, Kulasinghe A, Chua B, Day BW, Punyadeera C. Circulating biomarkers in patients with glioblastoma. *Br J Cancer.* 2020;122(3):295–305.
3. Shi W, Lv C. Qi J, et al. Prognostic value of free DNA quantification in serum and cerebrospinal fluid in glioma patients. *J Mol Neurosci.* 2012;46:470–475.
4. Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. *Nature.* 2019;565:654–658.
5. Mouliere F, Mair R, Chandrananda D, et al. Detection of cell-free DNA fragmentation and copy number alterations in cerebrospinal fluid from glioma patients. *EMBO Mol Med.* 2018;10(12):e9323.
6. Nassiri F, Chakravarthy A, Feng S, et al. Detection and discrimination of intracranial tumors using plasma cell-free DNA methylomes. *Nat Med.* 2020;26:1044–1047.
7. van der Pol Y, Mouliere F. Toward the early detection of cancer by decoding the epigenetic and environmental fingerprints of cell-free DNA. *Cancer Cell.* 2019;36(4):350–368.
8. Lo YMD, Han DSC, Jiang P, Chiu RWK. Epigenetics, fragmentomics, and topology of cell-free DNA in liquid biopsies. *Science.* 2021;372(6538):eaaw3616.
9. Sabedot T, Malta T, Snyder J, et al. A serum-based DNA methylation assay provides accurate detection of glioma. *Neuro Oncol.* 2021;23(9):1494–1508.
10. Ceccarelli M, Barthel FP, Malta TM, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell.* 2016;164:550–563.
11. de Souza CF, Sabedot TS, Malta TM, et al. A distinct DNA methylation shift in a subset of glioma CpG island methylator phenotypes during tumor recurrence. *Cell Rep.* 2018;23:637–651.