From Banding to BAM Files
Genomics Informs Diagnosis and Precision Medicine for Brain Tumors

Adrian M. Dubuc, PhD

KEYWORDS

• Central nervous system (CNS) • Brain tumors • Genomics • Epigenomics
• Next-generation sequencing • Cytogenetics

Key points

- Diagnosis of tumors of the central nervous system often necessitates evaluation of genomic features.
- Pediatric and adult brain tumors are characterized by distinct patterns of single nucleotide variants, copy-number alterations, epigenomic alterations, and/or structural rearrangements.
- No single genomic/epigenomic assay is currently suitable for all brain tumors; however, combinatorial use of cytogenetic and molecular methods can improve diagnosis and therapeutic management.

ABSTRACT

Tumors of the central nervous system (CNS) have been historically classified according to their morphologic and immunohistochemical features. In 2016, updates to the classification of tumors of the CNS by the World Health Organization revolutionized this paradigm. For the first time, genomic findings, whether whole-arm chromosomal aberrations or single nucleotide variants, represent a necessary and critical component of diagnosis, contributing or superseding histologic findings. These updates stem from decades of technical innovation and genomic discovery. During this time, there has been a dramatic expansion and evolution in clinical genomic assays for these tumors, informing diagnosis and guiding therapeutic management.

OVERVIEW

Tumors of the central nervous system (CNS) are highly heterogeneous in nature, demonstrating a diverse disease course from clinically benign to highly aggressive.[1] Patient stratification and disease management are predicated on accurate diagnosis. Although morphologic and immunohistochemical evaluation has long been espoused as a means of tumor classification, advances from the "omics" era have shed light on the inherent limitations of this approach.[2] Today, it is appreciated that tumors with similar morphologic appearance may demonstrate unique molecular features, highlighting their distinct genomic or epigenomic cause. In 2016, the World Health Organization (WHO) updated the classification of many CNS entities, for the first time incorporating genotypic features to the phenotypic diagnostic criteria.[3] These changes were implemented to more accurately define disease entities, in turn leading to improved patient management. Notably, these molecular markers include pathognomonic single nucleotide variants (SNVs), copy-number alterations, and structural rearrangements. Because there currently does not exist a single assay that can simultaneously and robustly interrogate this panoply of clinically significant genomic alterations,

The author has no sources of funding to disclose. The author declares no conflict of interest.
Department of Pathology, Harvard Medical School, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA
E-mail address: adubuc@bwh.harvard.edu

Surgical Pathology 13 (2020) 343–347
https://doi.org/10.1016/j.path.2020.02.007

clinicians are required to understand the inherent strengths and weaknesses of the current modalities of testing to inform their ordering practices.

Clinical laboratory genetics has traditionally been divided into 2 broad categories: (1) cytogenetics, focused on the numerical and structural evaluation of chromosomes[4]; and (2) molecular genetics, evaluating the nature and structure of genes.[5] This divide was predicated on the divergence of both techniques and expertise required to perform analyses. Cytogenetics necessitated cell culture and microscopy techniques,[6] whereas molecular genetics emphasized polymerase chain reaction–based approaches.[5] Technological advances in laboratory medicine, largely driven by advances in sequence-based approaches, have forcefully begun to blur the once distinct boundaries between these fields.[7] Although laboratory genomics continues to evolve toward a single discipline, current approaches for cancer diagnostics remain largely centered on evaluating either cytogenetic or molecular genetic alterations. In the present review, the importance and limitations of cytogenetic and molecular genetic assays are described, highlighting their current and future potential.

EVOLUTION IN CYTOGENOMICS

Study of chromosome form and structure is intrinsically tied to lessons learned from karyotyping. In this modality of testing, cells are cultured in vitro, arrested in metaphase, and subsequently, chromosome are banded, permitting visual appreciation of both copy number and structural variation at a single-cell level.[7] For brain tumors, the use of karyotyping (first in a research setting) identified recurrent pattern of copy-number alterations, most often losses, associated with specific disease entities.[4,8] Many disease-specific chromosomal profiles identified during this era, including polysomy of chromosome 7 with concomitant loss of 9p and chromosome 10 described in gliomas, still hold true today.[4] Moreover, cytogenetics afforded us with a mechanistic understanding of oncogene activation or tumor suppressor gene disruption.[9] By example, the identification of small acentric extrachromosomal fragments, namely double minutes, as well as homogenous staining regions, is now well understood to represent a means through which oncogene amplification occurs,[9] a phenomenon well established and highly prevalent in glioblastoma associated with *EGFR* amplification.[10] Similarly, cytogenetic approaches can easily resolve changes to ploidy, including whole-genome doubling now thought to represent an independent predictor of poor prognosis across brain tumor entities,[11] or haploidy, associated with giant-cell glioblastoma.[4] Karyotyping, however, is plagued by significant limitations. Resolution of chromosome studies by standard Giemsa trypsin G-banding can only detect alterations greater than ~7 to 10 Mb. Moreover, and perhaps more significantly, karyotyping requires actively diving cells. As improvements in surgical methodologies have resulted in appropriately smaller biopsies, fresh tissue is often limited, and even when obtained, a normal result does not exclude the possibility of a neoplastic proliferation because outgrowth of normal tissue is not uncommon.[12] Despite its limitations, karyotyping is still used as a diagnostic assay in several clinical laboratories; however, its utility is rapidly diminishing and is increasingly being replaced by microarray and sequence-based approaches.

To overcome some of the inherent limitations of karyotyping, newer cytogenetic techniques, namely, fluorescence in situ hybridization (FISH), emerged. FISH studies use fluorescently labeled DNA probes, typically 150 to 500 kb in length, that bind DNA, to assess the copy number or structure of a specific genomic locus, or limited number of loci.[13] FISH studies can be performed on interphase nuclei and thus do not require cells to be actively dividing and can be adapted for both fresh or formalin-fixed paraffin embedded (FFPE) material. FISH studies are ideally suited for clinical scenarios in which a differential diagnosis necessitated the detection or exclusion of a specific structural rearrangement, such as BRAF rearrangement associated with pilocytic astrocytomas. Results can be achieved in 3 to 7 days from paraffin material, or within the 1 to 2 days for fresh tissue. Notably, most paraffin FISH studies are performed on 5-μm sections. When performed in this manner, the morphology and tissue architecture are retained. Areas of specific interest can thus be specifically evaluated.[13,14] Much like karyotyping, FISH provides single-cell analysis, thereby capturing intratumoral heterogeneity, evident in glioblastomas with EGFRvIII variant.[15] However, the targeted nature of FISH studies can lead to disconcerting and well-documented false positives. Most notably, confirmation of 1p/19q whole-arm co-deletion typically involved FISH-based assessment. The most common commercially available probe set used clinically evaluates the ratio of 1p to 1q versus the ratio of 19p to 19q to assess whole-arm co-deletion.[14] This indirect measure is problematic as IDH-mutant astrocytomas can, rarely, display subtelomeric deletions leading to false-positive 1p/19q deletion results using FISH

studies.[16] As a result of this diagnostic pitfall, the WHO in fact recommends assays that can confirm the presence of whole-arm deletion for the diagnostic confirmation of oligodendroglioma.[3]

As a means of evaluating the genome-wide copy-number landscape, chromosomal microarray (CMA) is ideally suited. This assay involves DNA extraction and subsequent fragmentation. DNA molecules are labeled and hybridized onto a solid matrix.[7] The amount of labeled DNA that hybridizes to a specific probe (ie, feature) of the microarray generates a proportional signal, which can be normalized to a reference and subsequently converted into copy-number state.[7] The ability to accurately assess genome-wide copy-number aberrations can provide important and necessary support for diagnosis of gliomas, clearly delineating copy-number profiles associated with oligodendrogliomas versus those detected in astrocytomas and primary glioblastomas.[17,18] Similarly, for pediatric tumors such as medulloblastoma, a copy-number profile can support molecular subclassification, with admittedly variable success ranging from 47% to 79% of cases.[19] Technological improvements in both the probe density and the array design of CMAs have resulted in marked changes. With increased density, CMAs can now readily detect alterations often as small as ~50 kb, and optimized design facilitates use of DNA extracted from FFPE material.[20] CMA does not have the ability to identify evidence of balanced rearrangements[20]; however, intragenic copy-number alterations may be suggestive of unbalanced rearrangements. By example, the presence of 240-kb deletion on 6q22.1 partially encompassing the 5′ region of *ROS1* is now known to correlate an in-frame GOPC-ROS1 fusion, which may be responsive to targeted inhibition,[21,22] while a 1.9 Mb gain on 7q34 partially *BRAF* is known to be pathognomonic of KIAA1549-BRAF fusion.[19] CMA results are necessarily more complex than other cytogenetic testing, in part because of the increased resolution, which may impact the turn-around time in a clinical setting. Recently, guidelines have been described in an effort to achieve consistency in the manner in which CMAs results are reported.[23]

FROM SINGLE NUCLEOTIDE VARIANTS TO WHOLE-EXOME SEQUENCING

The rapid evolution of clinical laboratory genomics is perhaps no more evident than in the review of changes to sequencing approaches. Sanger-based methods, in which DNA replications occur through use of dideoxynucleotides that cause chain termination, were traditionally used for the detection of gene-level alterations, most often in the form of SNVs.[24] Through capillary electrophoresis, fragments are sorted by length, and the underlying DNA sequence was obtained.[24] Sanger sequencing remains the gold standard in part because of the quality of data and the length of sequencing reads.[25] Rapidly, new approaches for sequencing were developed, including, but not limited to, pyrosequencing. Through pyrosequencing, genomic loci, often mutational hotspots, could be rapidly and cost-effectively evaluated.[14]

More recently, next-generation sequencing (NGS) approaches revolutionized the ease through which genomic information could be gathered. Gigabases of data could rapidly and cost-effectively be generated, which led to increased understanding of the underlying genomic complexity of many tumor types. Today, a vast array of both commercial and custom NGS approaches exists, which has been deployed clinically. These assays show tremendous versatility. They can be RNA or DNA based, amplicon derived versus capture based designed primarily for relatively rapid (3–7 day) analysis of SNVs and short insertion/deletions (indels) to whole-exome sequencing approaches. Although there is no single approach that has been universally adopted, many laboratories offer targeted panels with 150 to 500 genes that are known to be clinically important across many cancer types. Although this technology has tremendous capabilities, NGS has created a new bottleneck, often requiring extensive bioinformatics support, including generation of knowledge bases to facilitate rapid clinical interpretation. Beyond the single nucleotide alterations exists the potential to further extract mutational signature and copy-number aberrations from the data that are generated. Since the discovery of checkpoint inhibitor efficacy against multiple tumor types with mismatch repair deficiency,[26] assessment of increased tumor mutational burden and mutational signatures associated with inactivation of DNA repair machinery is becoming increasingly desired for treatment planning.[27] Furthermore, despite the limited genomic coverage of targeted platforms, the copy-number data extracted has the potential to be equivalent to that from CMA, potentially obviating dedicated copy-number analysis in most instances.[28]

EPIGENOMICS AS AN EMERGING DIAGNOSTIC TOOL

Recently, epigenetics, and specifically, DNA methylation patterns have been described as a robust means of confirming or supporting the histopathology diagnosis of CNS tumors.[29] DNA methylation patterns observed in these tumors are a reflection of its cellular origin, state of differentiation, and subsequent somatic alterations.[30] Methylation profiling is currently performed on both fresh and FFPE-derived specimens, in which extracted DNA is bisulfite converted and subsequently hybridized onto a microarray platform.[31] Bisulfite conversion deaminates unmethylated cytosines, resulting in conversion to uracil, whereas methylated residues are protected.[29] Profiling of large cohorts of tumors coupled with machine learning approaches was used to develop an epigenomic classifier, whose use was concordant with histopathology diagnosis in 88% of 1104 cases evaluated.[29] Notably, in the remaining 12% of cases, methylation-based studies resulted in revision of the histopathology diagnosis. Although this approach has the potential to clarify unusual cases, much of this work stems from a single-institutional experience and has not been widely adopted as a routine diagnostic tool in North America.

SUMMARY

Given the plurality of various testing modes that exist, and the absence of a uniform standard diagnostic approach, disease-specific testing algorithms have the potential to both confirm histopathology diagnosis when needed and identify possible actionable alterations for personalized medicine approaches. As molecular technologies and bioinformatics capabilities continue to improve, it is possible that a single, stand-alone assay will someday be able to deliver all clinically relevant genomic alterations for a given disease. For the time being, however, proper assay selection must necessarily be guided by clinical suspicion and treatment planning requirements.

REFERENCES

1. Archer TC, Sengupta S, Pomeroy SL. Brain cancer genomics and epigenomics. Handb Clin Neurol 2018. https://doi.org/10.1016/B978-0-444-64076-5.00050-8, 1st edition.
2. Van Den Bent MJ, Weller M, Wen PY, et al. A clinical perspective on the 2016 WHO brain tumor classification and routine molecular diagnostics. Neuro Oncol 2017;19:614–24.
3. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016;131:803–20.
4. Bigner SH, Mark J, Friedman HS, et al. Structural chromosomal abnormalities in human medulloblastoma. Cancer Genet Cytogenet 1988;30:91–101. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3422050.
5. Katsanis SH, Katsanis N. Molecular Genetic Testing and The Future of Clinical Genomics. Nat Rev Genet 2013;14(6):415–26. Available at: https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fpubmed.ncbi.nlm.nih.gov%2F23681062%2F&data=02%7C01%7CJ.Surendrakumar%40elsevier.com%7C3d2286b260054f383aac08d7eacbd0c5%7C9274ee3f94254109a27f9fb15c10675d%7C0%7C0%7C637236034187789808&sdata=4f6m0myoo8%2FQiuSmJpSq96ybY-Dae62tGIAPtGGdi5g4%3D&reserved=0.
6. Wan TSK. Cancer cytogenetics: methodology revisited. Ann Lab Med 2014;34:413–25.
7. Speicher MR, Carter NP. The new cytogenetics: blurring the boundaries with molecular biology. Nat Rev Genet 2005;6:782–92.
8. Biegel JA. Cytogenetics and molecular genetics of childhood brain tumors. Neuro Oncol 2004;1:139–51.
9. Albertson DG, Collins C, McCormick F, et al. Chromosome aberrations in solid tumors. Nat Genet 2003;34:369–76.
10. Turner KM, Deshpande V, Beyter D, et al. Extrachromosomal Oncogene Amplification Drives Tumor Evolution and Genetic Heterogeneity. Nat Publ Gr 2017;543:122–5. Available at: https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fpubmed.ncbi.nlm.nih.gov%2F28178237%2F&data=02%7C01%7CJ.Surendrakumar%40elsevier.com%7C3d2286b260054f383aac08d7eacbd0c5%7C9274ee3f94254109a27f9fb15c10675d%7C0%7C0%7C637236034187789808&sdata=z1JhxXHgSviEbfkx8gr2%2BwvW9hOsq%2FVRYtzlrOCzfZE%3D&reserved=0.
11. Bielski CM, Zehir A, Penson AV, et al. Genome doubling shapes the evolution and prognosis of advanced cancers. Nat Genet 2018;50:1189–95.
12. Smith SC, Warren LM, Cooley LD. Maintaining a methods database to optimize solid tumor tissue culture: review of a 15-year database from a single institution. Cancer Genet 2019;233–234:96–101.
13. Horbinski C, Miller CR, Perry A. Gone FISHing: clinical lessons learned in brain tumor molecular diagnostics over the last decade. Brain Pathol 2011;21:57–73.

14. Park SH, Won J, Kim SI, et al. Molecular testing of brain tumor. J Pathol Transl Med 2017;51:205–23.
15. Francis JM, Zhang CZ, Maire CL, et al. EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing. Cancer Discov 2014;4:956–71.
16. Leeper HE, Caron AA, Decker PA, et al. IDH mutation, 1p19q codeletion and ATRX loss in WHO grade II gliomas. Oncotarget 2015;6:30295–305.
17. Suzuki H, Aoki K, Chiba K, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nat Genet 2015;47:458–68.
18. Buckner J, Giannini C, Eckel-Passow J, et al. Management of diffuse low-grade gliomas in adults–use of molecular diagnostics. Nat Rev Neurol 2017;13:340–51.
19. Dubuc AM, Ligon AH. From Prognostication to Personalized Medicine: Classification of Tumors of the Central Nervous System (CNS) Using Chromosomal Microarrays. Curr Genet Med Rep 2017;5:117–24.
20. Neill SG, Hauenstein J, Li MM, et al. Copy number assessment in the genomic analysis of CNS neoplasia: an evidence-based review from the Cancer Genomics Consortium (CGC) Working Group on Primary CNS Tumors. Cancer Genet 2020. https://doi.org/10.1016/j.cancergen.2020.02.004.
21. Davare MA, Henderson JJ, Agarwal A, et al. Rare but recurrent ROS1 fusions resulting from chromosome 6q22 microdeletions are targetable oncogenes in glioma. Clin Cancer Res 2018;24:6471–82.
22. Kiehna EN, Arnush MR, Tamrazi B, et al. Novel GOPC(FIG)-ROS1 fusion in a pediatric high-grade glioma survivor. J Neurosurg Pediatr 2017;20:51–5.
23. Mikhail FM, Biegel JA, Cooley LD, et al. Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). Genet Med 2019;21:1903–15.
24. Shendure J, Balasubramanian S, Church GM, et al. DNA sequencing at 40: past, present and future. Nature 2017;550:345–53.
25. Rizzo JM, Buck MJ. Key principles and clinical applications of "next-generation" DNA sequencing. Cancer Prev Res (Phila) 2012;5:887–900.
26. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372(26):2509–20.
27. Campbell BB, Light N, Fabrizio D, et al. Comprehensive analysis of hypermutation in human cancer. Cell 2017;171(5):1042–56.
28. Kerkhof J, Schenkel LC, Reilly J, et al. Clinical validation of copy number variant detection from targeted next-generation sequencing panels. J Mol Diagn 2017;19(6):905–20.
29. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. Nature 2018;555:469–74.
30. Jaunmuktane Z, Capper D, Jones DTW, et al. Methylation array profiling of adult brain tumours: diagnostic outcomes in a large, single centre. Acta Neuropathol Commun 2019;7:24.
31. Hovestadt V, Remke M, Kool M, et al. Robust molecular subgrouping and copy-number profiling of medulloblastoma from small amounts of archival tumour material using high-density DNA methylation arrays. Acta Neuropathol 2013;125:913–6.