Published in final edited form as:

Acta Neuropathol. 2011 April ; 121(4): 529–543. doi:10.1007/s00401-010-0773-z.

Genome-wide comparison of paired fresh frozen and formalin-fixed paraffin-embedded gliomas by custom BAC and oligonucleotide array comparative genomic hybridization: facilitating analysis of archival gliomas

Gayatry Mohapatra^{a,*}, David A. Engler^b, Kristen D. Starbuck^a, James C. Kim^a, Derek C. Bernay^a, George A. Scangas^a, Audrey Rousseau^a, Tracy T. Batchelor^{d,e}, Rebecca A. Betensky^c, and David N. Louis^{a,e,*}

^a Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

^b Department of Statistics, Brigham Young University, Provo, UT, USA

^c Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA

^d Stephen E. and Catherine Pappas Center for Neuro-Oncology, Massachusetts General Hospital, Boston, MA, USA

^e Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA

Abstract

Molecular genetic analysis of cancer is rapidly evolving as a result of improvement in genomic technologies and the growing applicability of such analyses to clinical oncology. Array based comparative genomic hybridization (aCGH) is a powerful tool for detecting DNA copy number alterations (CNA), particularly in solid tumors, and has been applied to the study of malignant gliomas. In the clinical setting, however, gliomas are often sampled by small biopsies and thus formalin-fixed paraffin-embedded (FFPE) blocks are often the only tissue available for genetic analysis, especially for rare types of gliomas. Moreover, the biological basis for the marked intratumoral heterogeneity in gliomas is most readily addressed in FFPE material. Therefore, for gliomas, the ability to use DNA from FFPE tissue is essential for both clinical and research applications. In this study, we have constructed a custom bacterial artificial chromosome (BAC) array and show excellent sensitivity and specificity for detecting CNAs in a panel of paired frozen and FFPE glioma samples. Our study demonstrates a high concordance rate between CNAs detected in FFPE compared to frozen DNA. We have also developed a method of labeling DNA from FFPE tissue that allows efficient hybridization to oligonucleotide arrays. This labeling technique was applied to a panel of biphasic anaplastic oligoastrocytomas (AOA) to identify genetic changes unique to each component. Together, results from these studies suggest that BAC and oligonucleotide aCGH are sensitive tools for detecting CNAs in FFPE DNA, and can enable genome-wide analysis of rare, small and/or histologically heterogeneous gliomas.

*Corresponding authors at: Massachusetts General Hospital, Tel.: 1 617 726 2966 Fax: 1 617 726 7533. gmohapatra@partners.org, dlouis@partners.org.

Introduction

Malignant gliomas are the most common primary brain tumors in adults. Despite multimodal therapy including extensive surgery, radiation and chemotherapy, the outcome remains poor (10). Adult diffuse gliomas are classified into astrocytomas, oligodendrogliomas and oligoastrocytomas and are graded into WHO grade II, WHO grade III and WHO grade IV, with the common glioblastoma (GBM) corresponding to grade IV (19). Molecular genetic analyses of malignant gliomas have already identified subgroups that correlate with clinical outcome (6,19,36,45). High throughput large scale studies, based on DNA copy number analysis, expression, methylation profiling and signal transduction pathways have also recognized molecular subclasses within GBM (2,3,22,31,42). When fully defined, genetic alterations associated with these subclasses will likely allow stratification of treatment groups (18,23,24).

DNA microarray-based comparative genomic hybridization (array CGH, aCGH) is a powerful tool to identify DNA copy number alterations across the genome (37,38). Array CGH is based on the principles of conventional CGH, which directly compares the genome of patient derived tumor (test) with reference (control) genome that is obtained from a pool of healthy individuals. The test and reference genomes are differentially labeled with fluorescent dyes and hybridized to the target DNA sequences simultaneously. Deviation of signal intensities after hybridization indicates losses and gains of DNA sequence copy number, allowing simultaneous detection of multiple DNA copy number alterations (CNA) throughout the genome (1).

Bacterial artificial chromosome (BAC) aCGH has been extensively used for studying various types of hematological malignancies and solid tumors, including gliomas (4,5,7,12,¹⁴,20,25,30,40). Most of these studies were performed using DNA from fresh frozen tissues and there have been relatively few reports in which formalin-fixed, paraffin-embedded (FFPE) DNA was used (8,13,26,33–35,41). The ability to use DNA from FFPE tumor tissues for aCGH is important for neuro-oncology since many malignant gliomas are biopsied stereotactically or as small samples, and the only tumor material typically available after routine pathological analysis of such cases is FFPE tissue. In addition, the ability to use FFPE tumors allows retrospective analysis of large numbers of clinically annotated samples and of rare types of gliomas. In addition, given the ability to examine histological details best in FFPE sections, elucidating the genetic features underlying the intratumoral heterogeneity in gliomas should be most readily achieved in FFPE material. In this study, we therefore designed and constructed a pan-genomic custom BAC array for glioma analysis and compared aCGH results from paired fresh frozen and FFPE oligodendrogliomas and GBMs.

We also evaluated oligonucleotide arrays for DNA copy number analysis because, although BAC array is a sensitive platform for detecting DNA copy number changes, it is time consuming, labor intensive and expensive and is beyond the reach of most individual laboratories to generate custom BAC arrays. With BAC arrays, it is also difficult to reach the levels of resolution and sensitivity that can be achieved with oligonucleotide platforms (46), and oligonucleotide arrays are readily available commercially. Therefore, a robust and reproducible method for labeling FFPE DNA for oligonucleotide aCGH would be of great practical consequence. To this end, we report a labeling protocol that was adapted for use with FFPE DNA and oligonucleotide arrays for aCGH.

Oligoastrocytoma (OA) is defined as a tumor composed of a “conspicuous mixture of two distinct neoplastic cell types morphologically resembling the tumour cells in oligodendroglioma and diffuse astrocytoma” (43,44). Biphasic and inter-mingled variants of

OA have been described. In the biphasic variant, juxtaposed but distinct areas of oligodendroglial and astrocytic differentiation are present, whereas in the intermingled variant, both components are intimately admixed. To demonstrate the utility of our labeling method for oligonucleotide aCGH from FFPE tissue, we therefore also investigated whether the oligodendroglial and astrocytic components of nine oligoastrocytic tumors have different genomic profiles. Our results show that a modified labeling method that does not involve amplification produces high quality aCGH data even with degraded FFPE DNA, and provides a practical model for the use of oligonucleotide array CGH for FFPE gliomas.

Materials and Methods

Cell lines and tumor samples

GBM lines A172 and U87 were purchased from ATCC. GBM lines SF126, SF188, and SF268 were obtained from the UCSF Brain Tumor Research Center tissue bank. Cells were plated in two 10 cm plates and cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin. Cells were grown until they were approximately 80% confluent. Cells from one plate were used for DNA extraction and the cells from the second plate were used to make slides for validation of aCGH data by FISH (28).

Paired fresh frozen tumor tissue and corresponding FFPE blocks for 24 oligodendrogliomas and 21 GBMs, and FFPE blocks for 9 biphasic anaplastic oligoastrocytomas (AOA), were procured from the Massachusetts General Hospital brain tumor tissue repository after appropriate human studies approval. The biphasic AOAs were selected based on the presence of juxtaposed but distinct areas of oligodendroglial and astrocytic components in the same tumor. Tumor samples were less than 5 years in age. For the frozen and FFPE paired samples, small pieces of frozen tissues were processed and H&E stained to confirm the presence of 70% or higher tumor cells. All H&E slides made from frozen tissue and FFPE blocks were reviewed by a neuropathologist and areas of tumor cells were demarcated and microdissected for DNA extraction. Cut slides and unused portions of the blocks were refrigerated prior to DNA extraction.

BAC array construction

A pan-genomic BAC array was constructed containing 4277 targets, with coverage of the human genome at an average resolution of 700 kb. The clones were selected from RP11, CTB, CTC, and CTD library for BAC clones and RP1, RP4 and RP5 libraries for PAC clones. Clones were selected from the National Center for Biotechnology Information Cancer Genome Anatomy Project and the University of California Santa Cruz genome build 16. BAC and PAC clones were purchased from Invitrogen Corp., Carlsbad, CA. The specificity of each clone was established by STS and/or EST-linked PCR and the chromosomal locations were confirmed by FISH. Supplementary Figure 1 shows the detailed map and description of the BAC array. Total human genomic DNA was used as a positive control; lambda DNA and printing buffer alone were used as negative controls. BAC clone selection, DNA extraction, mapping, target generation and printing were performed as described previously (26). All clones were printed in quadruplicate in spatially distinct sub-arrays.

DNA labeling and aCGH

Genomic DNA was isolated from 5 GBM cell lines, 45 paired fresh frozen and FFPE oligodendroglioma and GBM samples, 9 FFPE paired astrocytic and oligodendroglial samples, and normal whole blood from 4 anonymous donors using a routine protocol. Cell line DNA and fresh frozen tumor DNA were labeled following a published protocol (11).

FFPE DNA labeling and aCGH for the BAC array was performed following another published protocol (26).

For oligonucleotide aCGH, a modified nick translation protocol was used for labeling 9 biphasic AOA FFPE DNA samples from microdissected tissues. After DNA extraction, the integrity of the DNA was tested by running 2 µl on an agarose gel and by performing a standard PCR using 2 different primer sets with products ranging from 100–400 bp (41). The PCR along with the gel image helped gauge the size range of DNA fragments in each sample. Samples that produced robust PCR products had higher labeling efficiency. Briefly, for a 50 µl reaction, 2 micrograms of FFPE DNA, 5 µl of dNTP mixture in reaction buffer (0.2 mM dATP, dGTP, dTTP; 500 mM Tris/HCl, pH 7.8; 50 mM MgCl₂; 100 µM dithiothreitol; 100 µg/ml bovine serum albumin), 1 µl of Cy3-dCTP or Cy5-dCTP (1 ng/µl), 5 µl of 0.4 M U/µl DNase 1, 5 µl of 10 U/µl E. coli DNA polymerase 1 (Invitrogen) were combined and the volume was adjusted to 50 with distilled water. The reaction mixture was incubated for 10–12 hours at 15°C. Enzymes were inactivated by heating reaction at 85°C for 10 minutes. Fragment size was checked by running 3 µl of the labeled DNA by agarose gel electrophoresis. The size of the smear ranged from 100–400 bp. Equal amount of tumor and reference DNA were labeled and purified using PCR purification columns. Probes were precipitated and hybridized to Agilent 105K oligonucleotide arrays following a published protocol (11).

After hybridization and washing, array slides were scanned in Axon 4000B microarray scanner using GenePix Pro 4.0 software. Microarray images were analyzed and data points were generated using the GenePix Pro 4.0 software. Data were normalized by lowess (locally weighted least square regression) normalization method using Acuity 2.0 software (Axon Instruments, Inc., Union City, CA). Out of 4277 targets, 361 (8.4%) were flagged and excluded from analysis. The tiling path clones for 1q32 region were also excluded from this analysis leaving a total number of 3663 targets for analysis. The average normalized log₂ ratio of medians of signal intensities of tumor to reference was used for statistical analysis.

FISH analysis for aCGH data validation

To validate the CNAs detected by aCGH along 1p, 19q, 8, 11q and 12q, two-color FISH was performed on cell lines following previously reported protocol (27). For 1p36.3, PAC clone RP5-1092A11 mapping to the deleted region was labeled in Cy3-dUTP and the control clone RP11-181G12 mapping outside of the deletion was labeled in FITC-dUTP; for 19q13.3, BAC clone CTD-2571L23 was labeled in Cy3-dUTP and RP11-210G11 was labeled in FITC-dUTP; for 8q24.12, CTD-3056O22 was labeled in FITC-dUTP and RP11-11P7 mapping to 8p23.2 was labeled in Cy3-dUTP; for 11q23.3, RP11-770J1 was labeled in Cy3-dUTP and RP11-58K22 mapping to 11p11.2 was labeled in FITC-dUTP; and finally, for 12q24, CTD-2287P13 was labeled in Cy3-dUTP and RP11-87C12 mapping to 12q24.3 was labeled in FITC-dUTP.

Array CGH data analysis

DNA copy number alteration (CNA) was identified through dynamic thresholding of segmented aCGH data. Circular binary segmentation (CBS) was used to segment each hybridization into regions of common mean (32). For each hybridization, a scaled median absolute deviation (MAD) across all segments was then obtained. Markers assigned to segments with mean value greater than a scaled MAD were identified as gain. Likewise, markers corresponding to segments with mean value less than a scaled MAD were identified as loss. Selection of the scaling factor 1.95 for gains and losses was based on criteria outlined elsewhere (15).

To formally assess agreement between FFPE and frozen samples, sensitivity and specificity metrics were utilized. The CNAs measured from the frozen samples were considered to be the gold standard; sensitivity and specificity of the CNAs from the FFPE samples were measured relative to this standard. For each marker, FFPE sensitivity and specificity were separately obtained for three CNA event types: gain, loss, and no alteration. For example, sensitivity for gain at a given marker was estimated as the proportion of gains observed among the FFPE pairs of frozen samples that exhibited gain at that marker. Specificity for gain at a given marker was estimated as the proportion of non-gains observed among the FFPE pairs of frozen samples that did not exhibit gain at that marker.

Results

Measurement of BAC array detection sensitivity using normal lymphocyte and GBM cell line DNA

The sensitivity and the performance of targets on the BAC array were tested by hybridizing normal male and female lymphocyte DNAs in a gender mismatched manner. All targets produced expected log2 ratio close to 0 along the autosomes and a decrease or increase of X & Y chromosome copy numbers (Figure 1A). Clones that mapped to large polymorphisms were identified based on the database of genomic variants (47) and were eliminated from the analysis of tumor genomes.

To measure the detection sensitivity of the array, we used DNA from 5 GBM cell lines that were previously analyzed by conventional CGH (27). Figure 1B shows the genomic profiles of the cell lines. We detected all large genomic CNAs reported earlier for these lines. Array CGH detected many additional CNAs including high level amplifications and homozygous deletions that were not previously identified in these lines. A comparative account of the CNAs detected by conventional CGH and aCGH for these 5 cell lines is given in Table 1.

Five loci (two deletions along 1p and 19q and three amplifications along 8q, 11q and 12q) were selected for validation by FISH. Figure 2 displays the comparative analysis of aCGH and FISH results for these 5 loci. There was a perfect agreement between aCGH and FISH for all 5 loci.

BAC aCGH of paired frozen and FFPE gliomas

Chromosomal gains and losses detected by aCGH in each of the 45 frozen and FFPE paired samples are illustrated in Tables 2 and 3; and the frequency of CNAs are shown in Figure 3. As expected, loss of 1p and 19q were the most frequent CNAs in oligodendrogliomas. Of the 24 samples, 14/24 (58%) had combined loss of 1p/19q, 2/24 (8%) had combined partial loss of 1p/19q, and 5/24 (21%) had loss of 19q only. Other frequent CNAs include loss of 9q34.3 (33%), 4q32.3 (29%), 8q24.3 (29%), 9p21.3 (25%), 14q24.2 (21%), 10q23.2-q23.31 (17%); and gain of 8q24 (29%), 11q23-q25 (29%), 7q (25%). In GBM, combined loss of chromosome 10 and gain of chromosome 7 was observed in 17/21 (81%) tumors. Loss of 9p21.3 with or without homozygous deletion of *CDKN2A* and *CDKN2B* genes were observed in 15/21 (71%) tumors. Other deleted regions observed in more 25% of samples include 22q, 13q, 6q and 4q. More than 50% of GBM also showed gain of entire chromosome 19. Amplified genes in this panel of GBMs include *EGFR*, *PIK3C2B*, *GAB1*, *FGFR4*, *GLI*, *CDK4*, and *MDM2* (Table 3).

Complete concordance between frozen and FFPE samples in terms of loss, gain and no change was observed in 30/45 (67%) samples. In 10 cases, FFPE DNA failed to detect 1–3 CNAs compared to frozen DNA samples. In 5 cases, FFPE DNA detected 1–2 CNAs that were not present in the corresponding frozen DNA samples. Figure 4A and B show

examples of aCGH profiles of an oligodendroglioma that show complete concordance (4A) and one with disagreement (4B) between frozen and FFPE DNA.

Assessment of sensitivity and specificity of FFPE relative to the gold standard of frozen samples is displayed in Figure 4C. For each marker, the two top panels show the sensitivity and specificity relative to gain in the frozen sample, the two middle panels show the sensitivity and specificity relative to loss in the frozen sample, and the bottom panels show the sensitivity and specificity relative to no alteration in the frozen sample. For gain, 79 (2.2%) markers were below 95% sensitivity and 71 (1.9%) markers were below 95% specificity. For loss, 317 (8.7%) markers were below 95% sensitivity and 264 (7.2%) markers were below 95% specificity. For no change, 397 (10.8%) markers were below 95% sensitivity and 377 (10.3%) markers were below 95% specificity. In each of the plots, sensitivity and specificity based on fewer than five gold-standard (i.e., frozen) events are denoted in red. Overall, these results indicate excellent sensitivity and specificity using FFPE tissues.

Modified labeling procedure and oligonucleotide aCGH profiles of FFPE DNA

Figure 5A demonstrates an H&E-stained section of biphasic AOA-2 showing parts of the tumor with distinct oligodendroglial and astrocytic differentiation. Figure 5B illustrates aCGH profiles of paired astrocytic and oligodendrocytic components from 9 AOA FFPE DNA samples labeled by the modified method and hybridized to Agilent 105k oligonucleotide arrays. In all cases, gender was mismatched between the tumor and reference DNA. The modified labeling procedure produced uniform hybridization signals resulting in distinct CNAs with high signal-to-noise ratios. Note that the regions of the genome with normal copy number (i.e., along the baseline) appear smoother due to a significant reduction in the noise. Of the 9 AOAs, 4 had identical profiles in the astrocytic and oligodendroglial components (AOA-1, AOA-2, AOA-3 and AOA-6). The other 5 AOAs had differences between the astrocytic and oligodendroglial components along several regions in the genome (Figure 5b, red arrows). AOA-4 had gain of chromosome 7 in both components; gain of 8q21.13-q24.3 in the oligodendroglial component only; and gains of 10p, 10q11.22-q22.2, 19, and losses of 10q23.31-q26.3, 11p in the astrocytic component only. AOA-5 had gain of chromosomes 5, 7 including amplification of *EGFR*, 8, and 12; homozygous deletion of 9p21.3 including the *CDKN2A* and *CDKN2B* genes, losses of 10, 12q13.1, 14 and 22 in both components, whereas loss of chromosomes 6, 13 and 15 were confined only to the oligodendroglial component. In AOA-7, the oligodendroglial component showed losses of 2p25, 2q36.3-q37.3, 9p, 10q25.2-q26.3, and 18q; and gains of 7, and 11q; the astrocytic component showed gains of 1q21.1-q25.1, 7 including amplification of *MET*, 12p13.32-p13.33, 12q24.21-q24.33, 18p11.32, 19q12-q13.2, and losses of 1q25.2-q44, 3p12.3-p22.1, 3p25.3-p26.3, 9p including homozygous deletion of the *CDKN2A* and *CDKN2B* genes, 18p11.31-p11.31, and 19q13.31-q13.43. In AOA-8, both components showed loss of 9p and gain of 10p; the oligodendroglial component showed loss of 13q12-q31.3 and gains of 13q32.1-q34, 19 only; and the astrocytic component showed loss of 10q23.1-q26.3 only. Finally, in AOA-9, both components showed loss of 5p, 21 and gain of 8q; whereas the oligodendroglial component showed losses of 6p22.1-p25.3 and 6q22.31-q27 only.

Discussion

In this study, we designed and constructed a custom BAC array with emphasis on regions frequently altered in gliomas. First, we analyzed 5 GBM cell lines that are routinely used for *in vitro* studies and compared aCGH results to that of results identified by conventional metaphase CGH (27). We precisely identified all CNAs that were previously reported in these cell lines, but our results further showed that homozygous deletions were more

frequent than previously reported (17). *CDKN2A* and *CDKN2B* were most frequently homozygously deleted, with the region deleted in 4/5 cell lines. In addition, we identified homozygous deletions of *RB1* in SF126 and *CDKN2C* in U87 (Fig 2). Interstitial deletions along 1p and 19q were observed in A172 and U87 (Fig 2). Gain involving chromosome 7 was present in all 5 cell lines. In addition to the known amplification of *CMYC* in SF188, we identified amplification of several previously unknown oncogenes such as *MLL1* in SF126, SF188 and SF268; *CDK4* in SF188; and amplification of *MET* and *WNT2* in SF268. Finally, structural rearrangements involving chromosomes 1, 11, 13 and 19 were most commonly observed. As demonstrated, therefore, this custom BAC array offers a potentially important discovery tool for the study of malignant gliomas.

We next performed aCGH with this BAC array using DNA isolated from pairs of fresh frozen and FFPE gliomas. We found a high degree of concordance between genomic alterations detected in FFPE DNA compared to frozen DNA samples. Only 377 (10.3%) clones had sensitivity below 95% and 397 (10.8%) clones had specificity below 95% for gains, losses and no change regions combined. Of these clones, the lower sensitivity and specificity may merely be due to low frozen (i.e., gold standard) event counts; 30% of clones with sensitivity below 95% and 37% of clones with specificity below 95% corresponded to regions with fewer than five frozen events. Aside from sample size issues, there are several additional potential explanations for lower sensitivity and specificity among these clones. First, the great majority of these clones mapped to the pericentromeric regions of chromosomes 14 and 15 and telomeric regions of many chromosomes. Since these regions are repetitive in nature, the signal-to-noise ratio could be low due to incomplete repeat suppression during hybridization. The other clones with low sensitivity and specificity mapped at random through out the genome. A possible explanation for certain clones showing a high degree of variability could be high repeat content, regardless of the type of tumor DNA (frozen or FFPE) hybridized. Nonetheless, the high degree of concordance demonstrates that FFPE DNA can be reliably used for the study of malignant glioma copy number variation when such BAC arrays are available. For example, in addition to finding previously known CNAs in both oligodendrogliomas (14,39) and GBMs (22), we identified some novel amplified and deleted loci.

We then introduced a labeling protocol modified for generating probes from FFPE DNA and hybridization to enable use of high resolution oligonucleotide arrays. Since BAC clones have large inserts (ranging from 150–200 kb), the BAC array provides very high specificity. However, the sensitivity and resolution is compromised. The oligonucleotide arrays on the other hand provide very high resolution, but due to smaller probe size (60 bp) specificity can be compromised. During the isothermal labeling process, which allows synthesis of new DNA sequences, there is a bias towards representation of euchromatic versus heterochromatic DNA. In other words, depending on the repeat content of the sequence, the new DNA sequences are differentially synthesized while the DNA is being labeled. As a result of this bias in DNA synthesis, the heterochromatic regions of the genome are not uniformly represented in the probe. Since there is 35-fold increase in the resolution of the oligonucleotide array used for this study (21 kb for Agilent 105K array) compared to our BAC array (700 kb), the differential representation in the probe mixture produces a “wiggly” pattern. We overcame this phenomenon by labeling FFPE DNA using a method that does not allow synthesis of new strands of DNA while maintaining true representation of the tumor DNA and produces high quality uniform hybridization signal with accurate identification of CNAs. Using the modified labeling technique, we were thus able to show significant improvement in the signal-to-noise ratio, thereby reducing the number of possible false positives.

To demonstrate the investigative utility of this method, we examined the genomic profiles of 9 biphasic AOA samples, using DNA from microdissected tumor cells to determine if the oligodendroglial and astrocytic components within a given biphasic tumor displayed different genomic profiles. Notably, 4 of 9 biphasic AOAs showed identical profiles for both components but the other 5 samples had differences between the two components. Astrocytic components had many more CNAs compared to the oligodendroglial components. In 2 out of 5 cases, gain of chromosome 7 was present in both but amplification of *EGFR* and *MET* genes were confined to the astrocytic components. In one of the 5 cases, gain of 8q21.13-q24.3 was present only in the oligodendroglial component. These findings suggest that different components of an oligoastrocytoma may be derived from different clones during neoplastic progression. Different findings with regard to oligoastrocytoma clonality and cell of origin have been reported; some reports suggest a monoclonal origin (9,16), whereas others raise the possibility of different progenitor cell populations (21,29). Our results do not address the issue of cell of origin, but demonstrate that the histologically distinct regions within oligoastrocytomas may be associated with distinct genotypic changes. It remains to be shown, however, whether such alterations drive, or are a necessary consequence of, the distinct differentiation patterns.

In summary, the approach evaluated in this paper enables the combination of accessibility to archival FFPE tissues with the sensitivity of oligonucleotide arrays. As such, the approach should enable large-scale pan-genomic studies of small, rare and/or histologically heterogeneous malignant gliomas that are most optimally studied in FFPE material, as well as pan-genomic analyses of large numbers of clinically annotated archival cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH R21/R33 CA106695.

References

1. Bejjani BA, Shaffer LG. Clinical utility of contemporary molecular cytogenetics. *Annu Rev Genomics Hum Genet.* 2008; 9:71–86. [PubMed: 18949852]
2. Bredel M, Scholtens DM, Harsh GR, Bredel C, Chandler JP, Renfrow JJ, Yadav AK, Vogel H, Scheck AC, Tibshirani R, Sikic BI. A network model of a cooperative genetic landscape in brain tumors. *Jama.* 2009; 302:261–275. [PubMed: 19602686]
3. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A, Pedraza A, Holland E. Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PLoS One.* 2009; 4:e7752. [PubMed: 19915670]
4. Bussey KJ, Chin K, Lababidi S, Reimers M, Reinhold WC, Kuo WL, Gwady F, Ajay, Kouros-Mehr H, Fridlyand J, Jain A, Collins C, Nishizuka S, Tonon G, Roschke A, Gehlhaus K, Kirsch I, Scudiero DA, Gray JW, Weinstein JN. Integrating data on DNA copy number with gene expression levels and drug sensitivities in the NCI-60 cell line panel. *Mol Cancer Ther.* 2006; 5:853–867. [PubMed: 16648555]
5. Cai WW. Detection of DNA copy-number alterations in complex genomes using array comparative genomic hybridization. *Methods Mol Biol.* 2007; 381:105–119. [PubMed: 17984516]
6. Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, Silver JS, Stark PC, Macdonald DR, Ino Y, Ramsay DA, Louis DN. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst.* 1998; 90:1473–1479. [PubMed: 9776413]

7. Chin K, DeVries S, Fridlyand J, Spellman PT, Roydasgupta R, Kuo WL, Lapuk A, Neve RM, Qian Z, Ryder T, Chen F, Feiler H, Tokuyasu T, Kingsley C, Dairkee S, Meng Z, Chew K, Pinkel D, Jain A, Ljung BM, Esserman L, Albertson DG, Waldman FM, Gray JW. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell*. 2006; 10:529–541. [PubMed: 17157792]
8. Devries S, Nyante S, Korkola J, Segraves R, Nakao K, Moore D, Bae H, Wilhelm M, Hwang S, Waldman F. Array-based comparative genomic hybridization from formalin-fixed, paraffin-embedded breast tumors. *J Mol Diagn*. 2005; 7:65–71. [PubMed: 15681476]
9. Dong ZQ, Pang JC, Tong CY, Zhou LF, Ng HK. Clonality of oligoastrocytomas. *Hum Pathol*. 2002; 33:528–535. [PubMed: 12094379]
10. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Caveness WK. Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev*. 2007; 21:2683–2710. [PubMed: 17974913]
11. Gabeau-Lacet D, Engler D, Gupta S, Scangas GA, Betensky RA, Barker FG II, Loeffler JS, Louis DN, Mohapatra G. Genomic profiling of atypical meningiomas associates gain of 1q with poor clinical outcome. *J Neuropathol Exp Neurol*. 2009; 68:1155–1165. [PubMed: 19918127]
12. Jong K, Marchiori E, van der Vaart A, Chin SF, Carvalho B, Tijssen M, Eijk PP, van den Ijssel P, Grabsch H, Quirke P, Oudejans JJ, Meijer GA, Caldas C, Ylstra B. Cross-platform array comparative genomic hybridization meta-analysis separates hematopoietic and mesenchymal from epithelial tumors. *Oncogene*. 2007; 26:1499–1506. [PubMed: 16936777]
13. Joosse SA, van Beers EH, Nederlof PM. Automated array-CGH optimized for archival formalin-fixed, paraffin-embedded tumor material. *BMC Cancer*. 2007; 7:43. [PubMed: 17343727]
14. Kitange G, Misra A, Law M, Passe S, Kollmeyer TM, Maurer M, Ballman K, Feuerstein BG, Jenkins RB. Chromosomal imbalances detected by array comparative genomic hybridization in human oligodendrogliomas and mixed oligoastrocytomas. *Genes Chromosomes Cancer*. 2005; 42:68–77. [PubMed: 15472895]
15. Korkola JE, Heck S, Olshen AB, Reuter VE, Bosl GJ, Houldsworth J, Chaganti RS. In vivo differentiation and genomic evolution in adult male germ cell tumors. *Genes Chromosomes Cancer*. 2008; 47:43–55. [PubMed: 17943972]
16. Kraus JA, Koopmann J, Kaskel P, Maintz D, Brandner S, Schramm J, Louis DN, Wiestler OD, von Deimling A. Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendroglioma and oligoastrocytoma. *J Neuropathol Exp Neurol*. 1995; 54:91–95. [PubMed: 7815084]
17. Law ME, Templeton KL, Kitange G, Smith J, Misra A, Feuerstein BG, Jenkins RB. Molecular cytogenetic analysis of chromosomes 1 and 19 in glioma cell lines. *Cancer Genet Cytogenet*. 2005; 160:1–14. [PubMed: 15949564]
18. Liang Y, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO, Israel MA. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A*. 2005; 102:5814–5819. [PubMed: 15827123]
19. Louis DN, Ohgaki H, Wiestler OD, Caveness WK, Burger PC, Jouvett A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*. 2007; 114:97–109. [PubMed: 17618441]
20. Maciejewski JP, Tiu RV, O'Keefe C. Application of array-based whole genome scanning technologies as a cytogenetic tool in haematological malignancies. *Br J Haematol*. 2009; 146:479–488. [PubMed: 19563474]
21. Maintz D, Fiedler K, Koopmann J, Rollbrocker B, Nechev S, Lenartz D, Stangl AP, Louis DN, Schramm J, Wiestler OD, von Deimling A. Molecular genetic evidence for subtypes of oligoastrocytomas. *J Neuropathol Exp Neurol*. 1997; 56:1098–1104. [PubMed: 9329453]
22. The TCGA Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008; 455:1061–1068. [PubMed: 18772890]
23. Mischel PS, Cloughesy TF, Nelson SF. DNA-microarray analysis of brain cancer: molecular classification for therapy. *Nat Rev Neurosci*. 2004; 5:782–792. [PubMed: 15378038]

24. Mischel PS, Nelson SF, Cloughesy TF. Molecular analysis of glioblastoma: pathway profiling and its implications for patient therapy. *Cancer Biol Ther.* 2003; 2:242–247. [PubMed: 12878856]
25. Misra A, Pellarin M, Nigro J, Smirnov I, Moore D, Lamborn KR, Pinkel D, Albertson DG, Feuerstein BG. Array comparative genomic hybridization identifies genetic subgroups in grade 4 human astrocytoma. *Clin Cancer Res.* 2005; 11:2907–2918. [PubMed: 15837741]
26. Mohapatra G, Betensky RA, Miller ER, Carey B, Gaumont LD, Engler DA, Louis DN. Glioma test array for use with formalin-fixed, paraffin-embedded tissue: array comparative genomic hybridization correlates with loss of heterozygosity and fluorescence in situ hybridization. *J Mol Diagn.* 2006; 8:268–276. [PubMed: 16645215]
27. Mohapatra G, Kim DH, Feuerstein BG. Detection of multiple gains and losses of genetic material in ten glioma cell lines by comparative genomic hybridization. *Genes Chromosomes Cancer.* 1995; 13:86–93. [PubMed: 7542911]
28. Mohapatra G, Moore DH, Kim DH, Grewal L, Hyun WC, Waldman FM, Pinkel D, Feuerstein BG. Analyses of brain tumor cell lines confirm a simple model of relationships among fluorescence in situ hybridization, DNA index, and comparative genomic hybridization. *Genes Chromosomes Cancer.* 1997; 20:311–319. [PubMed: 9408746]
29. Mueller W, Hartmann C, Hoffmann A, Lanksch W, Kiwit J, Tonn J, Veelken J, Schramm J, Weller M, Wiestler OD, Louis DN, von Deimling A. Genetic signature of oligoastrocytomas correlates with tumor location and denotes distinct molecular subsets. *Am J Pathol.* 2002; 161:313–319. [PubMed: 12107116]
30. Nigro JM, Misra A, Zhang L, Smirnov I, Colman H, Griffin C, Ozburn N, Chen M, Pan E, Koul D, Yung WK, Feuerstein BG, Aldape KD. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. *Cancer Res.* 2005; 65:1678–1686. [PubMed: 15753362]
31. Nushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K. Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Glioma. *Cancer Cell.* 2010
32. Olshen AB, Venkatraman ES, Lucito R, Wigler M. Circular binary segmentation for the analysis of array-based DNA copy number data. *Biostatistics.* 2004; 5:557–572. [PubMed: 15475419]
33. Oosting J, Lips EH, van Eijk R, Eilers PH, Szuhai K, Wijmenga C, Morreau H, van Wezel T. High-resolution copy number analysis of paraffin-embedded archival tissue using SNP BeadArrays. *Genome Res.* 2007; 17:368–376. [PubMed: 17267813]
34. Paris PL, Albertson DG, Alers JC, Andaya A, Carroll P, Fridlyand J, Jain AN, Kamkar S, Kowbel D, Krijtenburg PJ, Pinkel D, Schroder FH, Vissers KJ, Watson VJ, Wildhagen MF, Collins C, Van Dekken H. High-resolution analysis of paraffin-embedded and formalin-fixed prostate tumors using comparative genomic hybridization to genomic microarrays. *Am J Pathol.* 2003; 162:763–770. [PubMed: 12598311]
35. Paris PL, Sridharan S, Scheffer A, Tsalenko A, Bruhn L, Collins C. High resolution oligonucleotide CGH using DNA from archived prostate tissue. *Prostate.* 2007; 67:1447–1455. [PubMed: 17654530]
36. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell.* 2006; 9:157–173. [PubMed: 16530701]
37. Pinkel D, Albertson DG. Comparative genomic hybridization. *Annu Rev Genomics Hum Genet.* 2005; 6:331–354. [PubMed: 16124865]
38. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet.* 1998; 20:207–211. [PubMed: 9771718]
39. Rossi MR, Gaile D, Laduca J, Matsui S, Conroy J, McQuaid D, Chervinsky D, Eddy R, Chen HS, Barnett GH, Nowak NJ, Cowell JK. Identification of consistent novel submegabase deletions in

- low-grade oligodendrogliomas using array-based comparative genomic hybridization. *Genes Chromosomes Cancer*. 2005; 44:85–96. [PubMed: 15940691]
40. Suzuki S, Moore DH 2nd, Ginzinger DG, Godfrey TE, Barclay J, Powell B, Pinkel D, Zaloudek C, Lu K, Mills G, Berchuck A, Gray JW. An approach to analysis of large-scale correlations between genome changes and clinical endpoints in ovarian cancer. *Cancer Res*. 2000; 60:5382–5385. [PubMed: 11034075]
 41. van Beers EH, Joosse SA, Ligtenberg MJ, Fles R, Hogervorst FB, Verhoef S, Nederlof PM. A multiplex PCR predictor for aCGH success of FFPE samples. *Br J Cancer*. 2006; 94:333–337. [PubMed: 16333309]
 42. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O’Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010; 17:98–110. [PubMed: 20129251]
 43. von Deimling, A.; Reifenberger, G.; Kros, JM.; Louis, DN.; Collins, VP. Anaplastic oligoastrocytoma. In: Kleihues, P.; Cavenee, WK., editors. *Pathology and genetics of tumours of the nervous system. World Health Organization Classification of Tumours*. Lyon: IARC Press; 2007. p. 66-67.
 44. von Deimling, A.; Reifenberger, G.; Kros, JM.; Louis, DN.; Collins, VP. Oligoastrocytoma. In: Kleihues, P.; Cavenee, WK., editors. *Pathology and genetics of tumours of the nervous system. World Health Organization Classification of Tumours*. Lyon: IARC Press; 2007. p. 63-65.
 45. von Deimling A, von Ammon K, Schoenfeld D, Wiestler OD, Seizinger BR, Louis DN. Subsets of glioblastoma multiforme defined by molecular genetic analysis. *Brain Pathol*. 1993; 3:19–26. [PubMed: 8269081]
 46. Ylstra B, van den Ijssel P, Carvalho B, Brakenhoff RH, Meijer GA. BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res*. 2006; 34:445–450. [PubMed: 16439806]
 47. Zhang J, Feuk L, Duggan GE, Khaja R, Scherer SW. Development of bioinformatics resources for display and analysis of copy number and other structural variants in the human genome. *Cytogenet Genome Res*. 2006; 115:205–214. [PubMed: 17124402]

Figure 1. Measurement of detection sensitivity of the BAC array

A) aCGH of normal male and female lymphocyte DNA. aCGH was performed using normal male and female lymphocyte DNA from 4 healthy donors that were used as reference for all tumors. The upper panel shows the profiles of male DNA labeled in Cy3-dCTP and female DNA labeled in Cy5-dCTP and lower panel shows the profiles of female DNA labeled in Cy3-dCTP and male DNA labeled in Cy5-dCTP, by random priming. Normalized log2 ratio of medians along the autosomes were close to 0, indicating normal DNA copy number, and along the X chromosome were -0.5 , and 1.5 indicating apparent loss or gain (from gender mismatch). **B)** aCGH profiles of DNA from GBM cell lines A172, SF126, SF188, SF268 and U87 used to measure the detection sensitivity/limits of the array. These cell lines were

previously analyzed using conventional CGH (27). Comparison of aCGH data with the conventional CGH data shows that all previously reported CNAs were identified (including known genes, labeled arrows) but with a higher degree of precision (Table 1). Several CNAs that were previously not reported were also detected (unlabeled arrows).

Figure 2. Validation of aCGH by FISH

A) To assess the detection limits of the array, FISH was performed using locus specific probes at 5 loci. aCGH detected a 3Mb interstitial deletion along 1p36 in U87 and loss of entire 1p in SF188. FISH was performed on the respective cells using RP5-1092A11 which maps within the 3 Mb deleted region was labeled in Cy3-dUTP and RP11-181G12 which maps upstream of the deletion was labeled in FITC-dUTP. In U87, 1 red and 2 green spots were detected, confirming the interstitial deletion, whereas in SF188 equal numbers of red and green spots were observed because all of 1p is deleted. **B)** aCGH showed a 1.4 Mb deletion in U87 and 7.2 Mb deletion (includes two adjacent interstitial deletions) in A172 on 19q13.3. FISH was performed using CTD-2571L23, which maps within the 1.4 Mb deleted region, labeled in Cy3-dUTP and RP11-210G11, which maps upstream of the smaller deletion but within the 7.2 Mb deleted region, labeled in FITC-dUTP. In U87, 1 red and 2 green spots were detected, confirming the interstitial deletion, whereas in A172 an equal number of red and green spots were detected, confirming that the 7.2 Mb deletion is occurring in a polysomic chromosome 19 background. **C)** aCGH detected the known amplification of *CMYC* at 8q24.12 in SF188. This was confirmed by FISH using *CMYC* specific BAC clone CTD-3056O22, labeled in FITC-dUTP and RP11-11P7, labeled in Cy3-dUTP, mapping to 8p23.2 as control. **D)** aCGH illustrates a previously unreported amplification on 12q14 in SF188. This was confirmed by FISH using BAC clone CTD-2287P13 labeled in Cy3-dUTP and RP11-87C2 labeled in FITC-dUTP. Further analysis using end-sequences from CTD-2287P13 indicated *CDK4*, *MARCH9* and *METTL1* genes to be contained within the BAC. **E)** aCGH highlights a highly rearranged chromosome 11 in SF126, and multiple amplifications in SF188 and SF268. SF268 has been reported to have 2 adjacent amplifications on 11q (27). Our results indicate that there are at least 5 high level amplifications in SF268 along 11q22-q24.1. Comparison of chromosome 11 profiles in these 3 cell lines defined a common region of amplification along 11q23.3 containing the *MLL1* gene. This was confirmed by FISH using a *MLL1* specific BAC clone RP11-770J1, labeled in Cy3-dUTP and RP11-58K22 on 11p11.2 labeled in FITC-dUTP as control.

Figure 3. Comparative analysis of aCGH profiles of paired frozen and FFPE samples

A) The two upper panels represent aCGH profiles of frozen and FFPE DNA for xT2500, with perfect concordance in CNAs between frozen and FFPE DNA for this sample. **B)** The two lower panels represent frozen and FFPE profiles for xT3491 DNA with small regions of disagreement indicated by arrows. **C)** Evaluation of sensitivity and specificity of CNAs detected in paired frozen and FFPE samples. The top two panels illustrate sensitivity and specificity relative to gain; the middle two panels illustrate sensitivity and specificity relative to loss; and the bottom panels illustrate sensitivity and specificity relative to no change in DNA copy number. The top line (1.0) indicates markers with perfect concordance, with points below 1.0 representing BACs with less concordance. Red points are sensitivity and specificity based on fewer than five gold-standard (i.e., frozen) events. The panels demonstrate excellent comparative sensitivity and specificity at the vast majority of loci (see text).

Figure 4. Frequency of gain and loss of DNA copy numbers in gliomas

A) Summary of CNAs in 24 oligodendrogliomas. **B)** Summary of CNAs in 21 GBMs. The X-axis represents the BAC clones from 1pter to 22qter. The Y axis represents the frequency of CNA, with gains in red above the baseline, and losses in green below the baseline.

Figure 5A

Figure 5B

Figure 5.

A) H&E staining of a biphasic AOA. Left panel shows oligodendroglial and right panel shows astrocytic differentiation (400X magnification). **B) Oligonucleotide aCGH profiles of biphasic AOAs from FFPE DNA.** Genomic profiles of AOAs 1–9 were generated using modified labeling protocol show superior quality hybridization due to uniform representation of tumor DNA in the probe mixture. Differences between the two components are shown in red arrows.

Table 1

Comparison of CNAs in GBM cell lines analyzed by CGH and aCGH

Cell line	Alterations detected by conventional CGH	Alterations detected by aCGH
A172	-1p22-p31.3, -1p33-p36, ++1q22-q24, -3p12-p13, +3q21-q29, -4q13, ++5q31.2-q32, +7p, +7q11, -8q12-q13, -9p, +9q, -10q25-q26, -11q14-q25, -12p13, -13, -14, -18q12.3-q23, ++19q13.4, -22, +Xp22-pter, -Xq21-q26, +Xq27-q28, -Y	-1p21.3-p31.1, -1p34.3-p36, +1q, ++1q22 (RAB25), +2, +3p22-p26, -3p12.3-p22.1, +3q, -4, -4q13.1-q21.21, +5, ++5q32-q33.2, -6, -6q26-q27, +7p, +7q11.12-q21.11, -7q31.33-q36, +8p, -8q13.1-q13.2, -8q, -9p, -9p21.3 (CDKN2A&B), +9q21.13-q31.3, -9q32, +9q33-q34.11, -9q34.12, +9q34.13-q34.3, -10q26, +11p, -11q12.1-q13.2, +11q13.3-q14.1, -11q14.2-q24, -12p12.3-p13, -13q12.12-q31.2, +13q31.3-q34, -14, +17, -18q12.3-q23, +19p, +19q13.1-q13.31, -19q13.32, -19q13.33, +19q13.4, +20, +21, -22, +Xp, +Xq12-q13, -Xq21-q27.2, +Xq27.3-q28
SF126	++1p36.2-p36.3, +5p, -5q11.2-q23.2, +7p, -9p21-p24, -10p, +11q12-q13, -11q23, -13, -18q21.3-q24, ++20q13.1-q13.3	++1p36.22-p36.3, -1q, +5p13.1-p15.3, -5p12, +7p, -9p21.3-p24, -9p21.3 (CDKN2A&B), +9q22.33, -9q34.3, -10p, +11q13.1-q13.4, -11q13.5, -11q14.3-q21, -11q22.3-q23.2, +11q23.3, -11q24.3, -13, -13q14.2(RB1), -18q21.32-q23, ++20q13.12-q13
SF188	-1p, +1q, -5, -6q12-q23.2, ++8q24, +10p, -10q, -11q12-q22, -13, -14, +16, +17p11.2-p12, -18, -19, +20, +X, +Y	-1p, +1q, -5, -6q13-q23.1, ++8q24.21 (MYC), -8q24.22-q24, +9q21.13-q21.13, +10p, -10q, -11q12.1-q23.3, ++11q23.3 (MLL1), -11q23.3-q24.3, +q24.3, -11q24.4, ++12q (CDK4), -13, -14, +16, -17p13.1-p13.3, +17p11.2-p12, -17q12, -18, -19, +20, +X, +Y
SF268	-2p24-p25, -2q14.2-q32.2, -3p11.2-p21.3, -3q, -4, -5q11.2-q23.2, -8p, -8q11.2-q23.2, -9p21-p24, -10, +11p, ++11q14-q21, +11q23, -13q31, -14q11.2-q24, ++14q31-q32, -16, +17q24-q25, -18q, +20, -22, -X	+1p, +1q, -2p25, +2p12-p24, +2q11.2-q14.3, -2q21-q31, +2q32-q37, +3p21.32-p26, -3p11.2-p21.2, -3q, -4, +5p, -5q11.2-q23.1, +5q23.2-q23.3, -5q31.1-q31.2, +5q31.2-q35, +6p, +6q12-q22.33, -6q23.2-q23.3, +6q24.1-q27, +7, ++7q31.2 (MET, WNT2), -8p, -8q11.21-q24.22, +8q24.12-q24.2, -9p21.3-p24, -9p21.3 (CDKN2A&B), -9q34.13-q34, -10, +11p, ++11q22.1-q22.3, ++11q22.3-q22, ++11q23.3, -11q24.3-q25, +12p, -12q, -13, -13q31.1-q32.1, -14, +14q31.13-q32.3, -16, +17, +18p11.2-p11.31, -18q, +20, +21q11.2-q21.3, -21q21-q22, -22, +X
U87	++1p13, -1p22, -6p, -6q12-q14, -6q23.2-q24.1, +7q32-q36, +9p12-p13, -9p21-p22, +9q, -11q14-q24, -12q21-q23, -13q12, -13q21, +13q22-q34, +14q21.2-q32, -16p13.2-p13.3, -18q, -20p, +20q, -X	-1p36, -1p32.3 (CDKN2C), -1p21.3-p31.1, +1p12-p21.2, +2q22.1-q33.3, +3, +4, +5, -6p21.1-p25, -6q12-q14.2, -6q16.3, -6q23.3-q24.2, +7q21.11-q21.3, +7q31.32-q36, +8q, +9p24, -9p21.3 (CDKN2A&B), +9q, +11q13.2-q13.3, -11q13.4-q24, -12q21.1-q23.1, -12q23.3, -13q12-q13.1, +13q13.2-q14.12, -13q14.2-q21, +13q21.3-q33, +14q22.2-q32, -16p13.2-p13.3, -18q12.2-q23, -19p13.1-p13.3, -19q13.3, -20p, +20q, -X

Table 2

Summary of CNAs in 24 paired frozen FFPE oligodendroglioma samples

Sample ID	Diagnosis	Primary/Recurrent	CNAs
xT71	O	P	-1p, -2q13-q37.3, -4q13.1-q35, -8q24.3, -9q34.13-q34.3, -12q24.3, -13q34, -14q24, -14q32, -16q23.1-q24.3, -19q
xT1067	OA	P	-4q12-q21.21, -4q34.1-q34.3, -6q13-q16.1, +7p21.1-p22, +8p, +8q, -10q25.1, +11q23.2-q25, -15q15.2-q22.31, -19q13.2
xT1668	O	P	-6q, +7q11.23-q36.3, +8q24.2, -8q24.3, -8q24.3, -9q, +11q22.2-q25, -12p, +19p, +20p
xT1941	OA	P	-1p, +3q23-q29, -4p16.3, +5p, -5q11.2-q14.3, +7p, +8q23.2-q24.2, -8q24.3, -9q33.3-q34.3, -10q25.2-q26.3, +11q13.4-q25, -12q13.11-q13.13, -13q34, -14q24-q32, -15q26.1-q26.3, +19p, -19q, -22q12.2-q13.33
xT2500	AO	P	-1p, -4p, -4q, +7p, +7q, -9p, +9q, -15, -19q
xT2812	OA	P	-1p, -1q32.2-q32.3, -2q11.2, -2q37, -6p21, -7q22.3, -8q24.3, +9p, -9q33.2-q34.3, -11p, -11q12.1-q14.1, +11q14.1-q25, -12q13.11-q13.2, -14q24, -14q32.12, -14q32.32-q32.33, -15q12-q15.2, -15q26.1-q26.3, -16p13.3, -19p12, -19q
xT2999	OA	P	-1p, +2p, +2q, +3p, +3q, +5p, +5q, +6p, +6q, +7p14-p22, +7q11.22-q22.1, +7q22.3-q36, +8q12.1-q24.2, -8q24.3, -9q34.13-q34.3, -14q32.2-q32.3, -16q11.2-q24.3, -19q, -22
xT3029	AO	P	-1p, -2p12, -14q11.2, -19q
xT3232	OA	P	+2p, -3q27.1-q29, -6q11.2-q16.1, +7p11.2-p15.3, +7p21.3-p22, +7q, +8q, -10p11.21-p11.22, -10q, +11q23.2-q25, -14q12-q21, +14q31-q32, +16p11.2-p13.2, +16p13.3, +16q, +17p11.2-p13.3, +17q24-q25, +21, +22q11.2-q11.23
xT3346	O	P	+11q14.1-q25, -13q34, -19q
xT3352	AO	P	-1p, -4p16.2-p16.3, -8q24.3, -9q34.13-q34.3, -11q23.2-q25, +12p12.3-p13.33, -13q34, -14q32, -15q12-q15.3, -16p13.3, -16q23.1-q24.3, -17p13.3, -19q, +20p, -22
xT3390	AO	P	-1p, -4q, -14q11.2-q32, -18, +19p, -19q
xT3395	OA	P	-1p36.3, +8q21.3-q24.3, +12p, -12q12, -14q24, -19q, +20p, -22
xT3409	OA	P	-1p36.1, -2q36.1-q37.3, +4q21.21-q25, -4q25-q35.3, -5q22.2-q35, -6q12-q15, +6q16.3-q23.2, -6q22.3-q27, +8q22.1-q24, -9p13.3-p24, -10q25.3-q26.3, +12p12.1-p13.33, -13q12-q21, -13q22.1-q31.2, +13q31.3-q34, -15q11.2-q22.31, +15q22.31-q26, +19p, -19q
xT3415	OA	R	-1p12-p36.21, 1p36.22-p36.3, +1q, -2p, -2q, -3p, -3q, -9p13.3-p24, -9q31.1-q33.2, -12q14.3, -18, -19q
xT3491	OA	P	+4q12 (<i>PDGFRA</i>)+7p, -8p, -9p21.3 (<i>CDKN2A</i> and <i>CDKN2B</i>), -10p, -10q, -11q13.2-q22.3, -17p11.2, -17p13.3, -17q11.2-q12, -19q12, -19q13.3, -22q11.23-q13.2
xT3533	O	P	-1p, -3p, -3q, -4p, -4q, -6q25.3-q27, -13q31.3-q34, -19q
xT3676	OA	P	+2p, -3p, -3q, -5p, -13, -19q
xT3740	O	R	-1p, -2p12, -2p13-p25.3, -2q, -3p21.3-p24.3, -3p25.3-p26.3, -4p, -4q, -5p, -5q11.2-q31.1, -6p12, -6p22.3-p25.3, -6q11.2-q21, -7p12.1-p12.3, -9p13.2-p21.3, -10q23.31, -11q14.5-q22, -13, -15q11.2-q13.2, -16p13.2-p13.3, +16p13.3, -18, -19q
xT3815	O	R	-1p34.3-p36.3+3q21.2-q29, +6p21.3-p25.3, +7q22.3-q36, -8q24.3, -9q34.13-q34.3, -10q25.3, -11p15.5, +12p, +12q12-q22, +17q21.3-q25, +18, -19q
xT3990	AO	P	-1p, -12q23.3-q24.2, -15, -19q
xT3993	AO	P	-1p, -4p, -4q12-q21.21, -4q28-q35, -8q24.3, -9q34.13-q34.3, -13q34, +17q12-q25, -19q

Sample ID	Diagnosis	Primary/Recurrent	CNAs
xT4238	OA	P	-1p, -1q, -2q37, -6q27, +7p, +7q, -9p, -9q22.2-q34.3, -18, -19q12, +19q12-q13.2, -19q13.2-q13.3
xT4757	OA	P	+2q22.1-q32.1, -8q24.3, -9q34.13, -11q13.3-q13.4, -19q

Table 3

Summary of CNAs in 21 paired frozen FFPE GBM samples

Sample ID	Diagnosis	Primary/Recurrent	CNAs
XT892	GBM	P	+3q21.3-q25.1, +3q26.1-q28, +7p, +7q, +EGFR, -9p13.2-p24, --CDKN2A&B, -10p, -10q, +19p, +19q, +20q, -22q13.2
XT1220	GBM	P	-1p13.3, -1p34.1-p36.3, +1q23.3-q24.2, -3p14.3-p21.32, +4q34.2-q34.3, -5q31.1-q31.3, -6p21.1-p21.32, -8q24.3, -9q33.3-q34.2, -10p11.2-p15.3, -10q21.2-q23.3, -10q24.1-q26.3, -11p15.5, -11q11.2-q14.1, -11q21-q24.2, +11q24.3-q25, +CDK4, -14q11.2-q12, -14q12, -14q24.1, -14q24.2-q24.3, -14q32, -15q12-q14, -15q25.3-q26, -16p12.3-p13.2, -16p13.3, -16q23.1-q24.3, -17p13.1-p13.3, -17q12-q21.32, -17q24.3-q25, -19p, -19q, -20p13, -21q22.3, -22q11.2-q13.31
XT1262	GBM	P	-1p36.3, +1q32 (PIK3C2B), -2q37.1-q37.3, -3p, -3q11.2-q27, -4p, -4q12-q21.21, +4q31.2 (GAB1), -5p12-p15.33, -5q11.2-q31.1, -5q33.1-q35.3, +7p, +EGFR, +7q, -8p, -8q, -9p, --CDKN2A&B, -9q33.1-q34.3, -10q21.1-q26.3, -11p15.3-p15.5, -11p11.2, -11q12.1-q22.1, -12q24.1, -14q11.2-q22.3, -14q24.2-q32, -15, -17p13.2-p13.3, -22
XT2838	GBM	P	-2p, -2q, -4p, -4q12-q21.21, -4q31.23-q34.3, -5p, -5q11.2-q23.2, -6p12-p21, -6p21.21-p25.3, -6q, +EGFR, +7q31.1-q31.2, -8p21.2-p23.2, -8q12.1-q24.12, -8q23.2-q24.1, -8q24.1-q24.3, -9p13-p24, -9q33.1-q34.12, -10p, -10q, +11p15.5, -11q13.4-q23.1, -12p, -12q, -13, -14, -15q15.1-q22.31, -15q26, -16p13.2-p13.3, -17p11.2-p13.3, -18p, -18q, -21q11.2-q21.3, -22q13.2-q13.33
XT3072	GBM	R	-1p34.1-p36.3, -2p11.2-p12, +2p12-p16.3, -2p24-p25.3, -2q11.2-q14.1, +2q14.1-q36.3, +3p11.2-p24.3, +3q11.2-q26.33, -3q26.33-q29, +4p12-p15.3, -4p16, +4q12-q35.1, +5p, +5q11.2-q23.2, -5q31.1, +6p22.3-p25.3, +7p, +7q, -8q24.3, -9q34.11-q34.3, -10q23.31-q26.3, -11p, -11q, +12p, +12q12-q13.11, -12q13.11-q13.13, +14q12, +14q24.3-q31.1, -14q32.12-q32.33, -15, -16p, -16q, -17p13.1-p13.3, +17q24, -17q24.3-q25, -18q23, -19q, +20q, -21q22.3, -22
XT3162	GBM	R	-2p, -2q, -4p16.2-p16.3, +5p, +5q11.2-q23.1, -5q31.1-q31.2, +7p, +7q, -9q33.3-q34.2, -10p, -10q, +12q14.1-q14.2, -13q21.1, +13q21.2-q32.3, -14q11.2, +14q12, -14q13.2-q32, -15, -16p13.3, -16q23.1-q24.3, +17p, +20p, +20q, -22
XT3278	GBM	P	-1p34.1-p36.3, -1q32.1-q32.2, -2p23.3-p25.1, +2p16.1-p16.3, -2q37.1-q37.3, -3q27.1, -3q29, -4p12-p16.3, -5q31.1-q35.3, -6p21.2-p21.32, -6q14.3-q27, +7p, +7q, -8q24.3, --CDKN2A&B, -9q33.3-q34.3, -10q22.1-q26.3, -11p, -11q23.2-q23.3, +12p, +12q12, -12q13.1-q13.3, -14q11.2, +14q12-q13.2, -14q23-q32, -15, -16p13.3, -16q13.3-q24.3, +17q22, +17q23.2-q25, -18q21.32-q23, -19q13.1-q13.4, +20p, +20q, -21q22.3, -22
XT3288	GBM	P	+2p, +2q, +7p, +EGFR, +7q, -9p21.1-p24, --CDKN2A&B, -10p, -10q, -17q24.3-q25, -22
XT3339	GBM	R	+1q, +3q11.2-q29, -4p16.3, +7p, +7q, -8q24.3, --CDKN2A&B, -9q34.11-q34.3, -10p, -10q11.21-q24.32, -12q13.11-q13.13, -13q34, -14q11.2, +14q12.1-q13.1, -16q23.1-q24.3, -17p13.1-p13.3, +19p, +19q, -22
XT3355	GBM	P	+1q21.1-q43, +2p, +2q11.2-q37.1, -3q29, -4p16.1-p16.3, -5q35.2-q35.3, -6p21, +7p, +7q, -8q24.3, -9q33.3-q34.3, -10p, -10q, -11p11.2, +11p12-p15.5, -11q11.2-q14.1, +12p11.2-p13.2, -12p13.2-p13.3, +12q12, -12q13.1-q13.3, +12q14-q23.3, -12q23.2-q24.33, +GLI, CDK4, MDM2, -14q11.2, +14q12-q23, +14q12, +16p, +16q, +17q22, +20p, -21q22.3
XT3546	GBM	P	+7p, +EGFR, +7q, -9p13.2-p24, --CDKN2A&B, -10p, -10q, +12p, +12q12, -13q34, +14q12, -22q13.1-q13.33
XT3655	GBM	P	+3q24, +7p, +7q, -9p21-p24, -10p, -10q, -11q, +12p, +12q12, -13q34, +14q12, +19q, -22q13.1-q13.33
XT3661	GBM	P	+1q, +7p, +EGFR, +7q, --CDKN2A&B, -10p, -10q, +12p, +12q, +19p, +19q, +21
XT3793	GBM	P	-2q37.1-q37.3, +3p, +3q, -4p16.1-p16.3, -4q22.1-q28.1, -4q34.3-q35.1, -6q23-q27, +7p, +EGFR, +7q, -8q24.3, --CDKN2A&B, -9q34.1-q34.3, -10p, -10q, -11p, -11q11.2-q13.4, +12p, +12q11.2-q12, -13, -14q11.2, -14q12, -14q24, -14q32, -15, -16p13.3, -16q23.1-q24.3, -17p13.1-p13.3, +19p, +19q, +20p, +20q, -22q12.2-q13.13
XT3885	GBM	P	+3q22.3-q26.32, +4p12-p16.2, -4p16.3, +4q11.2-q34.3, +5p, +5q11.2-q23.2, -5q31.1, +7p, +7q, -8q24.3, -10p, -10q, -13q34, +14q12, -16q23.1-q24.3, +19p, +19q
XT4203	GBM	P	-2p, -2q, -3p, -4p, -6p, -6q, +7p, +7q, -8q24.3, -9p13.2-p24, -9q33.3-q34.3, -10p, 10q, -11p, -11q11.2-q13.5, -13, -14q11.2, -14q23.3-q32, -16p13.3, -16q23.1-q24.3, -17p, -17q, +19p, +19q, -22

Sample ID	Diagnosis	Primary/Recurrent	CNAs
xT4327	GBM	P	+1p36.2, +7p, +7q, -8q24.3, -9p21-p24, --CDKN2A&B, -10p, -10q, -13, +14q12, -18q11.2-q21.1, +19q, -22q12.2-q13.33
xT466	GBM	P	+7p, ++EGFR, +7q, -8q24.3, -9p21.3-p23, --CDKN2A&B, -10p, -10q, +19p, +19q
xT4254	GBM	P	-2p11.2-p12, -2q37.1-q37.3, +3q11.2-q29, -3q29, -4p, -4q, +7p, +7q, +8p, +8q, --CDKN2A&B, -10p, -10q, -11p11.2, -11q11.2-q13.4, -13, -14, -16p, -16q, -17p13.1-p13.3, +19p, +19q, -22
xT885	GBM	P	+1p36.2, -4q34.3, ++5q35.2 (EGFR4), +7p11.2-p12, -10q25.1-q26.3, +11p11.2-p15.3, +11q14.1-q25, ++GLI, CDK4, MDM2, +13q21.1-q21.2, +16p12.3-p13.13, +16p13.3, +17q25.1-q25.2, +19p, +19q
xT4723	GBM	P	-1p34.1-p36.1, -1p36.2-p36.3, -2q37.1-q37.3, -5q31.1-q31.3, -5q35.2-q35.3, -6p21.1-p21.32, +7p, ++EGFR, +7q, -8q24.3, --CDKN2A&B, -10p, -10q, -11q, -14q32.2-q32.3, -15q25.2, -17p11.2-p13.3, +19p, +19q, +20p, +20q, -22q12.2-q12.3

Table 4

Summary of CNAs identified in biphasic AOAs

Tumor ID	Oligodendroglial Component	Astrocytic Component	Both Components
AOA-1	none	none	-2q36.1-q37.3, +13.2-q25, -4q26-q35.2, +5q13.3-q15, -5q21-q35.3, +6q14-q23.2, -6q23.3-q27, -7q11.21-q21.11, +8q2.3-q24.3, -9p13.3-p24.3, -10q25.3-q26.3, +12p13.3, -13q13.2-q21.33, -13q22.2-q31.2, +13q31.3-q34, -15q13-q22.1, +15q22.2-q26.1, +15q26.3, +19p, -19q
AOA-2	none	none	+1p12-p34.2, -1p34.3-p36.3, +1q, -6p12.1-p12.3, +6p21.1-p25.3, -6q, +7, ++ <i>EGFR</i> , -9p13.3-p24.3, +9q33.4-q34.3, -10, -13, +19, -22
AOA-3	none	none	-19q, -21
AOA-4	+8q21.13-q24.3	+10p, +10q11.22-q23.1, -10q23.31-q26.3, -11p, +19	+7
AOA-5	-6, -13, -15		+5, +7, ++ <i>EGFR</i> , +8, -- <i>CDKN2A&B</i> , -10, -12q13.1, -14, -22
AOA-6	none	none	-1p, -19q
AOA-7	-2p25, -2q36.3-q37.3, -10q25.2-q26.3, +11q, -18q	+1q21.1-q25.1, -1q25.2-q44, -3p12.3-p22.1, -3p25.3-p26.3, + <i>MET</i> , -- <i>CDKN2A&B</i> , +12p13.32-p13.33, +12q24.21-q24.33, +18p11.31-p11.31, +19q12-q13.2, -19q13.31-q13.43	+7, -9p
AOA-8	-13q12-q31.3, +13q32.1-q34, +19	-10q23.1-q26.3	-9p, +10p
AOA-9	-6p22.1-p25.3, -6q22.31-q27	none	+5p, +8q, -21