Clinical and molecular characteristics of congenital glioblastoma

Margaret E. Macy, Diane K. Birks, Valerie N. Barton, Michael H. Chan, Andrew M. Donson, B.K. Kleinschmidt-DeMasters, Lynne T. Bemis, Michael H. Handler, and Nicholas K. Foreman

Department of Pediatrics (M.E.M., V.N.B., M.H.C., A.M.D., N.K.F.), Department of Pathology (B.K.K.-D.), Department of Neurology (B.K.K.-D.), Department of Neurosurgery (D.K.B., B.K.K.-D., M.H.H.), and Department of Medical Oncology, Anschutz Medical Campus, University of Colorado, Denver (L.T.B.); and Children’s Hospital Colorado, Aurora, Colorado (M.E.M., D.K.B., V.N.B., M.H.C., A.M.D., M.H.H., N.K.F.)

Congenital glioblastoma (cGBM) is an uncommon tumor of infancy with a reported variable but often poor cure rate, even with intensive therapy. Five patients with cGBMs, arising de novo and not in familial tumor predisposition kindreds, were studied for histological and biological features, using Affymetrix microarray. Tumors were large, often associated with hemorrhage, extended into the thalamus, and often bulged into the ventricles. One patient died acutely from bleeding at the time of operation. The 4 surviving patients underwent surgery (1 gross total resection, 3 subtotal resections or biopsies) and moderate intensity chemotherapy without radiation, and remain progression-free at a median time of 36 months (range, 30–110 months). Affymetrix microarrays measured gene expression on the 3 cGBMs from which frozen tissue was available. Unsupervised hierarchical clustering of cGBMs versus 168 other central nervous system tumors demonstrated that cGBMs clustered most closely with other high-grade gliomas. Gene expression profiles of cGBMs were compared with non-congenital pediatric and adult GBMs. cGBMs demonstrated marked similarity to both pediatric and adult GBMs, with only 31 differentially expressed genes identified (false discovery rate, <0.05). Unique molecular features of cGBMs included over-expression of multiple genes involved in glucose metabolism and tissue hypoxia. cGBMs show histological and biological overlap with pediatric and adult GBMs but appear to have a more favorable outcome, with good response to moderate intensity chemotherapy with only subtotal resection or biopsy. Further study may determine whether identified gene expression differences contribute to the improved survival seen in these tumors.

Keywords: brain tumor, gene expression, glioblastoma, infant, microarray.

Congenital brain tumors account for <2% of all pediatric brain tumors, typically presenting within the first 2 months of life. Congenital brain tumors are usually supratentorial (in contrast to the infratentorial predilection of pediatric brain tumors) and, most often, are teratomas, astrocytomas, primitive neuroectodermal tumors, choroid plexus papillomas, or glioblastomas (GBMs). Congenital glioblastoma (cGBM) is among the rarest type of congenital brain tumor, with <50 cases reported in the literature. Histologically, these tumors are similar to other GBMs and present as hypercellular, infiltrative glial tumors with necrosis and/or pseudopali-sading necrosis, vascular proliferation, and increased mitotic activity and MIB-1 rate. Infants with these tumors can be stillborn or extremely ill at birth and have, in some cases, been reported to have a poor prognosis, with a mean survival of approximately 2 months among untreated patients. A feature that may contribute to very poor prognosis in a subset of these tumors is the tendency to be associated with frequent bleeding or intracranial hemorrhage resulting in a high mortality within the first week of life. However, there have been reports of patients existing with limited or no treatment. These reports indicate that cGBMs have more unpredictable and, perhaps, more favorable clinical course than GBMs in older children or adults. Two recent studies identified long-term
survivors treated with surgery and/or chemotherapy, with an estimated 24–33-month median survival, suggesting that, if the infants are able to tolerate birth and initial surgery or biopsy, this diagnosis is not as bleak as was formerly perceived. Infants with cGBMs have achieved long-term survival and cure with a variety of regimens; however, these are often aggressive regimens using multiple chemotherapeutic agents. All 8 infants aged ≤24 months with GBMs treated in the CCG 945 study of the “8-in-1” chemotherapy regimen showed clinical progression. Four of these patients with GBM were aged ≤6 months and could be considered to have had cGBMs.

In adult GBMs, alterations in the expression of epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), phosphatase and tensin homolog (PTEN), INK4a/ARF, Isocitrate dehydrogenase 1(IDH1), and TP53 have been identified. Pediatric GBMs also have mutations in TP53, with nuclear P53 over-expression; however, both are less frequent in children <3 years of age. They also have a lower incidence of PTEN deletions and EGFR mutations, supporting the notion that pediatric and adult GBMs have distinct molecular pathways of tumorigenesis. Genetic differences between congenital, pediatric, and adult GBMs have been studied in a limited number of cases, but no genome-wide comparisons of pediatric GBMs that include cGBMs have been reported to date. The most thorough study of cGBMs was published by Brat et al., who characterized 6 cGBM examples for clinicopathologic and genetic features; none of their cases were associated with any known germline mutation or familial syndromes. They found that 2 of 6 cGBMs showed 10q deletion, but none of the 6 possessed EGFR amplification or TP53 mutation, despite the fact that 5 of 6 showed nuclear P53 immunoreactivity in 15%–30% of tumor cells. Winters and colleagues found that 2 of the 3 cGBMs they studied had p53 accumulation and had prolonged survival, differing from reported nuclear TP53 expression and associated poor prognosis in non-congenital pediatric GBMs identified by Pollack et al.

Because of the limited clinicopathologic and genetic information on cGBMs, we report on 5 patients who received a diagnosis of cGBM at our institution from 2002 through 2010. Four of these patients survived to be successfully treated with surgery (1 gross total resection, 3 subtotal resections or biopsy only) and a moderately intense chemotherapy regimen. All 4 remain alive without evidence of disease, to date. In this report, we show for the first time that cGBMs have a gene signature that, although similar, has some distinct differences from both pediatric and adult GBMs. These findings represent a fundamental beginning step to understanding the biology of cGBMs and further suggest that a nihilistic treatment approach is not warranted in cGBMs. Indeed, we demonstrate that, even with a moderately intense chemotherapy treatment plan, long-term survival can be expected, provided the infant survives the immediate postbirth and postoperative period.

Methods and Materials

Patient Characteristics and Tumor Specimens

Cases were identified via a retrospective database review of all pediatric patients with brain tumor diagnosed at our institution from 2002 through 2010. A total of 5 cGBMs were identified for this study. One of the 5 patients had undergone initial surgery at an outside institution but then was referred to our hospital for further treatment. The remaining 4 patients received their surgical procedures at Children’s Hospital Colorado from 2002 through 2010. Medical charts were reviewed for age at presentation, clinical presentation, sex, type of surgery performed, outcome of surgery, imaging studies, tumor location and histology, chemotherapy regimens, patient outcome, and survival. These data were reviewed under the approval of our Institutional Review Board (COMIRB # 05-149).

For light microscopy, tumor sections were cut at 4 microns and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed for glial fibrillary acidic protein (GFAP; polyclonal, 1:2500 dilution, no antigen retrieval, Dako Corporation), MIB-1 (monoclonal, 1:400 dilution, antigen retrieval, Dako Corporation), synaptophysin (monoclonal, 1:50 dilution, no antigen retrieval, Biogenex), and p53 (monoclonal, prediluted, antigen retrieval, Ventana). For 3 of the 4 patients operated at our institution (patients 1, 2, and 5), tumor samples were collected at the time of surgery and snap frozen in liquid nitrogen, according to internal review board approval (COMIRB protocol #95-500). Patient characteristics are summarized in Table 1. An additional 12 pediatric GBMs (pGBMs), 6 adult primary GBMs (aGBMs), and 159 other central nervous system (CNS) tumors collected from Children’s Hospital Colorado or University Hospital at time of surgery in a similar manner served as comparison examples for comparative gene expression studies.

Genetic Analysis

Standard cytogenetic culture-based characterization was performed on 3–4-day independent primary in situ cultures and 4-day suspension cultures of brain tumor specimens from 3 of 4 cases operated at our institution (patients 1, 2, and 5).

Fluorescence In Situ Hybridization

Two patients (patients 2, 5) were tested via fluorescence in situ hybridization studies (FISH). A dual-color chromosome enumeration assay for interphase cells was performed on formalin-fixed, paraffin-embedded tissue that was pretreated with proteinase K and hybridized with Vysis (Abbott Laboratories) tricolor probe for the chromosome 4q12 region, including the FIP1L1, CHIC2, and PDGFRα genes. Further details of FISH analysis have been previously described elsewhere.
Histone H3.3 Sequencing

Direct sequencing of the histone H3.3 gene on patients 1, 2, 4, and 5 was conducted after PCR amplification of genomic DNA from snap frozen tissue if available or formalin-fixed paraffin (patient 4). DNA was extracted using a DNeasy kit (Qiagen) according to the manufacturer’s directions. The PCR primers were derived from this sequence obtained from GenBank NM_002107.4. The forward primer was 5′-AAC TGG CTA CAA AAG CCG CTC TCA-3′ and the reverse primer was 5′-CGA GGT CTC CTT AGA CCT CCA GGT AAG A-3′.

Direct sequencing was conducted at the University of Colorado Cancer Center Sequence Core.

Gene Expression Profiling by Microarray

Gene expression of the GBM samples was measured using Affymetrix HG-U133 Plus 2 GeneChip microarrays (Affymetrix). All samples were processed and hybridized in small batches (4–8 samples at a time), approximately corresponding to time of acquisition. Standardized procedures were used to decrease the possible variability between runs. RNA was extracted from each sample using an RNeasy or DNA/RNA AllPrep kit (Qiagen) according to the manufacturer’s directions. Five micrograms of RNA was then reverse-transcribed using a T7-(dT) 24 oligomer and Superscript II Reverse Transcriptase (Invitrogen). The resulting cDNA was converted to cRNA, fragmented and labeled using the Enzo BioArray HighYield RNA transcript labeling kit (Enzo Life Sciences). For 1 pediatric GBM sample, limited amount of starting RNA precluded use of the Superscript II/Enzo protocol. Instead, 400 nanograms of RNA from this sample was processed using the Ambion MessageAmp Premier RNA Amplification Kit (Applied Biosystems), according to manufacturer’s directions. RNA quality for all samples was verified using the Nano Assay Protocol for the 2100 Bioanalyzer (Agilent) at 2 time points: after initial extraction of the RNA from the tumor sample, and following preparation of the RNA for chip hybridization. The fragmented, labeled RNA was hybridized to the HG-U133 Plus 2 GeneChips following manufacturer’s instructions.

Gene Expression Microarray Data Analysis

Analysis of the gene expression microarray data was performed in R (http://www.r-project.org/), using packages publicly available through Bioconductor (http://www.bioconductor.org/). As a first step, the scanned data (171 CEL files) were background corrected and normalized using the gcRMA algorithm, resulting in log2 gene expression values for this specific analysis. All subsequent analyses used these normalized values for input. CEL files and normalized data have been deposited in GEO (accession number GSE32374).

Hierarchical clustering was performed using the normalized gene expression microarray data from the 3 cGBMs and 168 other CNS tumors. Genes with expression or variability in the bottom 25% across all samples were omitted because of their noninformative nature. Distances based on Spearman correlations were calculated for input to an agglomerative algorithm using average linkage, as implemented in the Bioconductor hclust function.

Differential gene expression between congenital and other GBM subgroups was calculated using the Bioconductor limma function. Data were filtered prior to input to eliminate probesets not expressed in any samples or that showed only limited variance across samples. The limma function performs pair-wise comparisons between a target group and each of the other user-defined groups in the dataset. It employs an Empirical Bayes approach to calculate a moderated t statistic and calculating a False Discovery Rate (FDR) that accounts for multiple testing both in and among groups.

Functional annotation of differentially expressed genes was performed with the NIH Database for Annotation, Visualization, and Integrated Discovery (DAVID) Web tool (http://david.abcc.ncifcrf.gov/), using Biological Process Gene Ontology (GO) terms and PANTHER Biological Process terms. Functional network analysis and disease associations were examined using Ingenuity Pathways Analysis (Ingenuity Systems; http://www.ingenuity.com), which uses an extensive database of knowledge derived from published data. Gene set enrichment analysis (GSEA) was used to examine enrichment of genes in predefined reference sets that are based on biological knowledge. Unlike other approaches that examine only genes meeting a
predetermined cutoff, this tool computes an aggregate score for all genes in the reference set, based on their relative ranking in the data.

Previous research with adult GBMs has identified molecular subclasses among these tumors, grouping them into mesenchymal and pro-neural phenotypes. To ascertain how cGBMs compared with these subclasses, principal components analysis (PCA) was performed with the GBMs in our dataset, using the probe sets that were at least 3-fold over-expressed for mesenchymal, proliferative, or proneural GBM molecular subclasses as defined by Phillips et al. Pediatric and adult GBM samples were assigned to molecular subclasses based on the highest mean Z-score in all probe sets for each category.

Results

Patient Characteristics and Treatment

For the 5 patients with cGBMs identified, the mean age at diagnosis was 6.6 weeks (range, 6 days–3 months) (Table 1). All patients underwent operations; gross total resection could only be achieved in 1 patient in 2 operations, with the other 4 patients having biopsy (1 patient) or subtotal resections (3 patients). Patient 2 died during surgery because of extensive intra-operative hemorrhage. The 4 surviving patients were treated with carboplatin (8 mg/kg × 2 days) and etoposide (3 mg/kg/day × 3 days) every 21 days for a range of 6–10 cycles. The therapy was well tolerated, with minimal complications. This regimen was initially chosen for its relatively low toxicity profile in infants. After completion of the study, all 4 patients were alive and disease-free 30–110 months (median, 36 months) after the completion of therapy. No patient received radiation.

Histological Features of cGBMs

Histologically, all 5 cGBM samples were characterized by moderately hypercellular glial tumor with relative cellular monotony in terms of size and shape at low power (Fig. 1A and B), absence of significant pleomorphism seen best on squash preparations (Fig. 1C), hemorrhage, and increased vascular density (Fig. 1D), sometimes with vascular thrombosis and microvascular proliferation (Fig. 1E). Necrosis was present in all 5 cases (Table 2), either zonal (3 cases) (Fig. 1F) or pseudopalisading (2 cases) necrosis (Fig. 1G). Very acute necrosis with nuclear and neutrophilic debris was seen in one case (patient 4). Hemosiderin pigment deposition was focal in 2 cases and extensive in a third case (Fig. 1H), indicating more subacute to chronic bleeding into tumor (Table 2). This case also manifested encrustation of elastic fibers by calcium-containing basophilic mineralization, known as Gamma-Gandy body formation (Fig. 1H), further verifying chronic repetitive bleeding into the tumor. This suggests a chronicity of bleeding within the tumor, indicating that the tumor may have been present for a considerable period.

Mitotic figures were easily discerned in all tumors, except in the smallest biopsy (patient 2), where definite mitoses were identified but more difficult to identify because of sample size. Mitotic activity was brisk, reaching 26/10 high-power fields (HPFs) on patient 1, 17–32/10 HPFs on patient 2, 3–4/10 HPFs on the small biopsies of patient 3, 8/10 HPFs on patient 4 (in non-hemorrhagic areas of tumor), and 10/10 HPFs on patient 5. With the exception of patient 3, whose biopsy was extremely small, cases manifested an elevated MIB-1 cell cycling rate ranging from 15% to 30% (see Table 2).

These findings were similar to that of the large detailed series published by Brat et al. In their study, a wide range of mitotic indices (7–42/10 HPF) and MIB-1 rates (10%–60%) were similarly identified.

Because of the rarity of cGBMs, careful attention was given to histologically excluding other tumor types, especially choroid plexus papilloma/carcinoma and CNS supratentorial primitive neuroectodermal tumor (ie, PNET). All 5 tumors possessed cells with fibrillary, eosinophilic cytoplasm and showed GFAP-immunoreactivity (Fig. 1I). The histological features and GFAP-immunostaining excluded choroid plexus papilloma/carcinoma. None of the tumors contained small blue cell nuclear features suggestive of PNET and synaptophysin immunostaining was negative in all 5 cases. Neurofilament immunostaining had been additionally performed on patients 3 and 4 and was negative. In the cases in which sufficiently large tissue specimens were available for accurate assessment (patients 1, 2, 4, 5), infiltrating tumor cells were identified at the perimiter of the lesion. Unlike some low-grade gliomas, microcyst formation was not present in any tumor.

The smallest biopsy (patient 3) manifested focally increased reticulin staining and suggested a possible gliosarcoma; however, the relatively low MIB-1 rate on this case (Table 2), coupled with small sample size and absence of a true admixed, marmoreal pattern of interdigitation between glial and mesenchymal elements made it difficult to render a confident diagnosis of gliosarcoma, Gliosarcomas have been reported as congenital tumors42 with similar outcomes to classic cGBMs with reported survivors with chemotherapy and surgery alone. In the World Health Organization 2007 criteria, gliosarcoma is considered to be a variant of GBM rather than a separate entity.44

Thus, all 5 tumors paralleled those in previous reports of cGBMs and fulfilled World Health Organization criteria for diagnosis of GBM based on the presence of 3 or 4 of the following criteria: nuclear abnormalities, multiple (and numerous) mitotic figures, and the presence of necrosis, either pseudopalisading (patients 1 and 2) or zonal (patients 3–5). In addition, patient 2 had microvascular proliferation.

Genetic Results

Cytogenetic studies on patient 5 revealed a tetraploid clone in 6 of 12 metaphase spreads; the remaining 6 cells contained a normal diploid karyotype. Studies
from patient 2 revealed no detectable clonal abnormalities of chromosome number or structure. Patient 3 had undergone surgery at an outside hospital and no cytogenetic studies were performed. Patient 4 had no studies. The specimen from patient 1 revealed a loss of Y in 5 of 20 metaphases, with one cell showing loss of a copy of

Fig. 1. Histopathological characteristics of congenital GBMs (cGBMs). (A) Tumors were moderately hypercellular glial tumors with a relatively monotonous tumor cell population; note mitotic activity (arrow). Hematoxylin and eosin (H&E), 600×. (B) A second cGBM manifesting a more spindled appearance, but, again, relative monotony in terms of cell size and shape. H&E, 400×. (C) Squash preparation highlights the absence of significant pleomorphism or prominent nucleoli in the tumor cells. Note the delicate fibrillar eosinophilic cytoplasm in the cells. H&E, 600×. (D) Most tumors manifested increased vascular density, sometimes consisting of delicate, arcuate vasculature without thrombosis or microvascular proliferation (arrows). H&E, 200×. (E) Two of the cGBMs showed focal microvascular proliferation and vascular thromboses. H&E, 400×. (F) Tumors were deep-seated and located adjacent to ventricle, abutting the ependymal lining (top), and this example also demonstrates extensive zonal necrosis (bottom). H&E, 400×. (G) Pseudopalisading necrosis and vascular thrombosis (arrow) were identified in several cGBMs. H&E, 200×. (H) Hemosiderin pigment deposition (lower right) was extensive in one case, indicating subacute to chronic bleeding into tumor. This case also manifested Gamma-Gandy body formation (arrows), verifying chronic repetitive bleeding into the tumor. H&E, 400×. (I) Immunoreactivity for glial fibrillary acidic protein (GFAP) was present in all cases. Immunostaining for GFAP with light hematoxylin counterstain, 600×.
chromosome 17; the remaining fourteen cells contained a normal karyotype (Table 2).

It was not possible to determine whether the diploid clones detected as the only growth in 1 of these cultures, and half the populations in the other 2 patients (which were in combination with tetraploid or near tetraploid clones) were actually diploid tumor versus overgrowth by normal cells.

FISH was negative for EGFR amplification in both patients in whom it was tested; PTEN loss was not detected in the one patient for whom the test was informative. None of the 4 patient samples tested had the recently described K27M or G34R mutations in the histone 3 variants H3F3A, which encodes the histone H3.46,47

**Microarray Results**

Global gene expression was measured using Affymetrix microarrays for the 3 cGBMs, which had frozen tumor tissue available. Hierarchical clustering was performed to examine the relationship of these cGBMs to other pediatric CNS tumors based on gene expression. A dataset of 171 CNS tumors, which included the 3 cGBMs, was used as input to the clustering. The resulting dendrogram consisted of 5 major branches segregated broadly into choroid plexus tumors, PNET/medulloblastoma tumors, AT/RTs, ependymomas, and gliomas. The cGBMs were located on the glioma branch with their gene expression most closely resembling other high-grade glioma tumors (Fig. 2).

To evaluate further the similarity of cGBMs to other GBMs at a molecular level, the cGBMs were compared to 12 pediatric noncongenital (pGBMs) and 6 primary adult GBMs (aGBMs) to identify differentially expressed genes. Using a false discovery rate (FDR) cutoff of 0.05, only 21 genes were significantly different in cGBM, compared with pGBM, and 24 genes were different compared with aGBM (Supplementary material, Table S1). To put this into perspective, the same cohort of pGBMs was compared to 3 randomly selected samples from other tumor categories. The resulting differences were as follows: 14 differentially expressed genes in pGBMs versus aGBMs, 133 versus diffuse intrinsic pontine gliomas, 300 versus pilocytic astrocytomas, 741 versus gangliogliomas, and 1000–2500 genes each for AT/RT, ependymoma, PNET, and medulloblastoma. Thus, our cGBM samples clearly showed a gene signature highly consistent with other GBMs.

Overall, a total of 31 genes, 28 up-regulated and 3 down-regulated, were significant in comparison to either pGBMs or aGBMs, with 14 genes common to both comparisons, and the remaining 17 showing a strong trend towards significance in the other comparison.
Expression of genes commonly amplified or deleted in pGBMs or aGBMs was examined to see whether similar expression was seen in the cGBMs. Seven pediatric and adult GBMs had been evaluated by FISH for amplification of EGFR. The 2 samples with positive amplifications also showed high gene expression levels (>5000), whereas those with no amplification showed lower levels (<1000) (Fig. 3A). EGFR expression was uniformly low in the cGBMs, consistent with negative amplification seen by FISH in 2 of the cGBM patients (Table 2). Some pGBMs showed substantially higher expression of PDGFRα, consistent with findings of amplification in the pediatric group, as reported by other studies.39,48,49 Expression for cGBMs was generally lower, and similar to that seen in the aGBMs (Fig. 3B). Copy number analysis by FISH indicated no amplification in the 2 cGBMs samples tested. No down-regulation of PTEN or up-regulation of MYCN was suggested from the cGBM gene expression data.

To determine how the cGBM samples would fall into molecular subclasses as defined for adult GBMs (mesenchymal, proliferative and proneural38) and to compare our findings with those previously reported by others,39,40 we performed principal components analysis of our congenital, pediatric, and adult GBMs, using a robust set of genes identified by other researchers to distinguish between the different subclasses.38 The cGBMs grouped most closely with the mesenchymal subclass, although they also showed more proliferative and proneural features than mesenchymal GBMs in children or adults (Fig. 4). The mesenchymal subclass of GBM is characterized by extensive angiogenesis.38 The PCA results are consistent with the histopathology observed in the cGBMs, which noted extensive vascularization of these tumors.

Assessment of Gene Expression Results Based on Patient Age and Anatomical Location

Additional analysis was performed to determine whether the 31 genes differentially expressed in the cGBMs were likely to be attributable to either patient age or anatomical location, rather than diagnosis.

To determine whether any of the significant genes were a function of the younger age of patients with
cGBM, expression for these 31 genes was evaluated in 14 pairs of tumor samples matched for diagnosis, but differing in age, with the younger age ≤ 11 months and a mean older age of 93 months. The diagnoses covered a broad range of tumor types to avoid bias and consisted of choroid plexus (2), craniopharyngioma (1), ependymoma (2), ganglioglioma (1), anaplastic astrocytoma (1), medulloblastoma (1), pilocytic astrocytoma (2), pilomyxoid astrocytoma (3), and primitive neuroectodermal tumor (1). Only GRK5 showed a significant difference (paired t test, P < .05) between young and old, indicating that the differential expression of this gene in cGBMs might be attributable to age differences rather than diagnosis. As all cGBMs were supratentorial, we also examined whether the differentially expressed genes could be attributable to location bias. Therefore, the cGBMs were compared with 3 pGBMs from similar supratentorial locations for these genes. Only CHST11 showed a small, nonsignificant difference in expression when compared with location matched samples (1.1 fold, P = .73), suggesting that differences in this gene may be attributable to location rather than diagnosis. However, there may be confounding effects because of to age or location that were not detectable because of the overall small sample number.

**Gene Expression in cGBMs as it Relates to Biological Processes**

To gain insight to the functional significance of cGBM biological differences, the 31 genes differentially expressed were analyzed for enrichment in biological processes using the bioinformatics tool DAVID (Supplementary material, Table S2). Signal transduction was significantly enriched (P < .002), with 16 genes (52%) included in this process. Receptor tyrosine kinase function was also enriched (P < .01) with 4 genes, RET, RASGRF2, EFNA5, and ALK. Processes related to homophilic cell adhesion and to glycosylation were also enriched (P < .05). Ingenuity pathway analysis (IPA) was used to examine interaction networks and disease associations within the differentially expressed genes. No significant networks were detected; however, 12 (39%) of 31 genes were found to be associated with glucose metabolism disorder (P < .001). Among these were CRH, RET, ALK, and FOXP1 (data not shown).

Geneset enrichment analysis (GSEA) was used to evaluate enrichment among gene sets known to be involved in development and cancer. Unlike the previous tools which use an unranked list of only the significant genes, GSEA uses the relative expression of all genes to determine enrichment when compared with predefined sets of genes with common processes. One gene set was enriched among cGBMs compared to the other GBMs, “P53HypoxiaPathway” (P < .05) (Supplementary material, Fig. S1). Nine genes in cGBMS contributed to this enrichment, including TP53.

**Discussion**

In this study, we report 5 patients with congenital GBMs, all of whom presented within the first 3 months of life. All were located in deep gray matter adjacent to ventricles, and 3 of the patients had extension of their large parenchymal tumors into the intraventricular space.

Only 1 of the 4 patients who survived their operations had a complete resection of the tumor; however, all have had survival much greater than would be expected in an older patient. There has been increasing evidence that the extent of resection of pediatric high-grade gliomas strongly and independently correlates to survival.\(^\text{50-52}\) In glioblastoma in adults, similar conclusions have been drawn.\(^\text{53,54}\) This has led to a perspective among pediatric neurosurgeons that “the demonstration of a survival advantage provided by radical resection should prompt neurosurgeons to treat malignant pediatric astrocytomas with aggressive surgical resection.”\(^\text{52}\)

Indeed, it was this perspective which led us to attempt to resect the apparent glioblastoma in the child who did not survive her operation. Our experience suggests that to the contrary, in this particular group of children, the diagnosis of congenital glioblastoma does not warrant an operation with more than mean risk.

Alternative diagnoses of choroid plexus papilloma/carcinoma, PNET, and teratoma were excluded by histological and immunohistochemical features. An interesting feature of the cGBMs was the markedly increased tissue bleeding, which is consistent with many reports of tumor-associated hemorrhage in these tumors.\(^\text{55-57}\) In addition, several manifested evidence of remote bleeding, with hemosiderin pigment deposition and even Gamma-Gandy body formation, a feature seen in tumors that have been present for sufficient length of time to generate repetitive bleeding episodes.\(^\text{41}\) Similar to previous observations, we noted significant p53 immunohistochemical expression, and absence of EGFR amplification, where tested.\(^\text{4,10}\) Nuclear p53 was seen in 15%–30% of nuclei in 4 of 5 cases (Table 2), yielding a strong expression categorization when using the scoring system of Brat et al.\(^\text{18}\)
The nuclear monotony and absence of pleomorphism in our cases also paralleled the findings of Brat et al. Classic culture cytogenetic studies on one patient demonstrated a diploid population only (Table 2). In 2 of our 3 patients on which it was performed, a diploid clone was seen in combination with aneuploidy in a near tetraploid context (patient 1), while in the other, tetraploidy was seen in combination with diploidy (patient 5). Curiously, another subtype of GBM that exhibits significant nuclear monotony is the small cell astrocytoma/GBM described by Perry et al.; however, this tumor type contains diploid populations throughout the tumor by FISH. The difference in ploidy between these 2 tumors that both share ostensibly similar histological monotony is an interesting observation in our study of cGBMs, but of uncertain significance.

Molecularly, hierarchical clustering analysis showed that the gene expression in the 3 assessable cGBMs was quite similar to each other and was also very comparable to other high-grade gliomas, both adult and pediatric. Comparison of cGBMs to pediatric and adult GBMs found only 31 genes differentially expressed between them. This is analogous in scale to the differences seen between pediatric GBMs compared to adult GBMs. Because of the small number of samples tested, there may be additional differences or similarities which could not be detected. Similar to pediatric GBMs, no amplification of EGFR was observed and EGFR gene expression was very low in all 3 cGBM samples measured. Similarly, no PDGFRα amplification was seen in the 2 cGBMs successfully evaluated by FISH (data not shown), and PDGFRα gene expression levels were much lower than those seen in a subset of patients with pGBM, suggesting that amplification of PDGFRα may be unusual in cGBMs, unlike pGBMs. However, because of our sample size, we cannot rule out that this may occur in cGBMs at a low frequency, similar to pGBMs.

With respect to GBM molecular subclasses recently identified by Philips et al., the 3 cGBMs again group together, falling in the mesenchymal subgroup, but with more proliferative and proneural features than adult mesenchymal GBMs. As the mesenchymal category of GBM is characterized by extensive angiogenesis, the PCA results are consistent with the extensive vascularization observed in these cGBM tumors. Of note, the mesenchymal subtype is more aggressive and is associated with decreased survival among adults with GBMs, which is in direct contrast to these very young infants with excellent survival. Paugh et al.’s analysis of pediatric GBMs also found that a subset of pGBMs was similar to aGBMs in the mesenchymal subgroup, and of interest, one of their tumors was also in an infant.

cGBMs, although similar both histologically and molecularly to other GBMs, in our small series, appear to have a much better prognosis with surgery and moderate intensity chemotherapy, suggesting that the few gene expression differences might confer a better prognosis for these tumors. Because of the small number of patient samples, these findings need further validation with a larger number of cGBM patient samples. Functional analysis revealed that >50% of the differing genes were involved in signal transduction, including 4 receptor tyrosine kinases. In other molecular subclassification of GBMs, a variety of mutated or overexpressed tyrosine kinases have been found and felt to be involved in the oncogenesis of the tumors. Glucose metabolism is another area which functional analysis demonstrated differences in expression; in our cGBMs, 39% of the differing genes were involved in glucose metabolism. Glucose metabolism pathways have been implicated in the prognosis of aGBMs. IDH1 mutations, most commonly found in adult patients <60 years of age, have been shown to portend an improved prognosis over wild-type IDH1 GBMs. IDH1 was not one of the differentially expressed genes; however, these data suggest that glucose metabolism pathways, although poorly understood, are potentially important in the pathogenesis of GBMs. Corticotropin releasing hormone (CRH) showed the largest difference in gene expression (>300-fold higher) in cGBMs than pGBMs or aGBMs, which may suggest a reason for the involvement of glucose metabolism pathways.

Genes differentially expressed in cGBMs were enriched for the P53 hypoxia pathway, and the cGBMs showed increased expression of p53 at the mRNA level, compared with other GBMs. On the basis of previous studies by Pollack et al., increased P53 expression or TP53 mutation results in worse outcome. Again, the expression pattern in cGBM appears to be contradictory for this subtype. Of interest, adult patients with IDH1 mutations have a high frequency of TP53 mutations, with low frequency of other mutations, yet have an improved prognosis. None of our patients tested had the recently described histone H3.3 mutations found in pGBMs and diffuse pontine gliomas. Although our sample size is small, with only 4 patients tested, and we did not test for other alterations in the chromatin remodeling complex; this may further support that cGBMs are biologically different from their pediatric and adult counterparts.

These data, derived from a small sample set, demonstrate that indicators of poor prognoses in other GBMs do not appear to apply to this small population of infants with congenital GBMs. Further work is needed to identify the molecular signatures that make this population so unique, perhaps through whole genome sequencing or comparative genome hybridization analysis. This study, while providing insight into a rare tumor, is limited because of the small sample size. As such, a larger study of both the biology and a similar chemotherapy regimen in infants with cGBMs is necessary to further validate and solidify both the biology and chemotherapy results discussed here.

In conclusion, cGBMs differ from pediatric and adult GBMs in the expression of only a limited number of genes. cGBMs in some instances may have a significantly better prognosis than pediatric or adult GBMs, provided that the infant survives birth and can tolerate surgery and be treated with chemotherapy. In our series, patients with subtotal resection or biopsy also did well, suggesting that with this moderate chemotherapy, aggressive surgery is not necessary and may cause more morbidity in this fragile population. Moreover, these tumors are
highly vascular and prone to bleeding, further supporting a conservative neurosurgical approach. From our experience, patients with cGBMs seem to respond to even moderately intense chemotherapy regimens and do not require stem cell rescue or dose-dense chemotherapy regimens for a response. The discovery of differential expression in cGBMs, although based on a small sample set, is the first step in elucidating why cGBMs have an improved prognosis and may, conversely, provide insight into the worse prognosis of pediatric and adult GBMs.

Supplementary Material

Supplementary material is available at Neuro-Oncology Journal online (http://neuro-oncology.oxfordjournals.org/).

Acknowledgments

We thank the Morgan Adams Foundation for financial support of this project.

Conflict of interest statement. None declared.

Funding

This work was supported by the National Institutes of Health (K12 CA086913-08 to M.M.) and the Morgan Adams Foundation.

References

