The Cancer Genome Atlas expression profiles of low-grade gliomas

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Differentiating between low-grade gliomas (LGGs) of astrocytic and oligodendroglial origin remains a major challenge in neurooncology. Here the authors analyzed The Cancer Genome Atlas (TCGA) profiles of LGGs with the goal of identifying distinct molecular characteristics that would afford accurate and reliable discrimination of astrocytic and oligodendroglial tumors. They found that 1) oligodendrogliomas are more likely to exhibit the glioma-CpG island methylator phenotype (G-CIMP), relative to low-grade astrocytomas; 2) relative to oligodendrogliomas, low-grade astrocytomas exhibit a higher expression of genes related to mitosis, replication, and inflammation; and 3) low-grade astrocytic tumors harbor microRNA profiles similar to those previously described for glioblastoma tumors. Orthogonal intersection of these molecular characteristics with existing molecular markers, such as IDH1 mutation, TP53 mutation, and 1p19q status, should facilitate accurate and reliable pathological diagnosis of LGGs. (<http://thejns.org/doi/abs/10.3171/2012.12.FOCUS12351>)

KEY WORDS • astrocytoma • oligodendroglioma • oligoastrocytoma • low-grade glioma

DIFFERENTIATING between LGGs of astrocytic and oligodendroglial origin remains a major challenge in neurooncology.^{8,23,28} Overcoming this challenge is paramount. Astrocytic and oligodendroglial tumors exhibit distinct natural histories, biologies, and therapeutic responses.^{14,33} As such, our ability to appropriately care for patients afflicted with LGGs and advance the standard of care greatly depends on accurate pathological diagnosis.

It is increasingly apparent that histological features are insufficient for consistently discriminating between the astrocytic and oligocytic LGG (Fig. 1).^{8,25} There is a general consensus in terms of the need for molecular tools in this regard.^{24,30} While great strides have been made toward the development of these tools with the discovery of TP53 mutations and 1p19q deletions, reliable molecular diagnosis remains elusive for the 30%–50% of LGGs that do not harbor these molecular markers.^{11,18}

We propose that the discrimination of astrocytic and oligocytic LGGs can be facilitated by comprehensive molecular analysis of these tumors. To this end, we analyzed the publicly available multiplatform molecular analysis of LGGs from TCGA.¹

Methods

Determination of G-CIMP Status

Methylation data related to glioblastoma and LGG

Abbreviations used in this paper: G-CIMP = glioma-CpG island methylator phenotype; GBM = glioblastoma multiforme; LGG = low-grade glioma; miRNA = microRNA; TCGA = The Cancer Genome Atlas.

were downloaded from TCGA data portal. The Level II data have been preprocessed and normalized per probe set by TCGA working group. Using the methods described by Noushmehr et al.,²⁰ we identified the top 1427 probes whose DNA methylation beta values varied most across the glioblastoma and LGG samples. The glioblastoma and LGG data sets were grouped and classified by k-medians clustering (number of clusters 3) of the selected probe sets. All clustering of the data and construction of the heatmaps were performed using the TM4 MultiExperiment Viewer (version 4.7.4, Dana-Farber Cancer Institute).²⁷

Determination of TCGA Subtype

Level III mRNA expression data were obtained from TCGA data portal for 27 LGG and 603 GBM specimens. Level III data consisted of expression calls for genes per sample following processing and normalization including log₂ transformation of the gene expression levels. Data from the LGG and GBM samples were compiled into a single data set, and relative gene expression values were calculated by subtracting from the gene estimate the mean expression values across patients and then dividing the difference by the standard deviation across all patients. Using the 840 gene signature reported by Verhaak et al.,³² we classified the tumors into proneural, neural, classical, and mesenchymal subtypes by k-medians clustering (number of clusters 4).

Differential Gene Expression Analysis

Level III mRNA expression data from Agilent Technologies G4502A-07 microarrays were obtained from

Fig. 1. Photomicrographs demonstrating common histopathologies of LGGs. **A:** Classic oligodendrogliomas, WHO Grade II, display uniform cellularity and uniformly round nuclei with small but distinct nucleoli that sit within perinuclear halos (the so-called fried-egg appearance). The infiltrative nature is suggested by myelinated axons coursing in the background of tumor cells. Thin “chicken-wire” vasculature is often seen (*arrow*). **B:** Low-grade diffuse (infiltrating) astrocytomas, WHO Grade II, are most commonly characterized by a fibrillary pattern, as shown here. Fibrillary astrocytomas have more than twice-normal cellularity attributable to pleomorphic astrocytes varying in size and shape. These cells often show nuclear elongation, angulation, hyperchromasia, pleomorphism, and enlargement. Again, the myelinated axons throughout the background confirm the tumor is infiltrating. **C:** Low-grade oligoastrocytomas, WHO Grade II, are controversial entities within neuropathology. Multiple patterns have been suggested to describe mixed oligoastrocytomas, and one suggested pattern is represented in this figure, with individual cells having both astrocytic and oligodendroglial features. Cells within this tumor have the nuclear elongation, angulation, and enlargement of an astrocytoma but are sitting within perinuclear halos like an oligodendroglioma. H & E.

TCGA data portal. The mean fold change analysis between the low-grade astrocytomas and oligodendrogliomas was performed on all genes in the full 17813 gene data set. Genes that demonstrated mean differential expression of at least 2-fold were kept as interesting genes for further analysis. Gene lists of interest were uploaded and analyzed using the online gene ontology tool DAVID 6.7 (National Institute of Allergy and Infectious Diseases, NIH, <http://david.abcc.ncifcrf.gov/>).^{9,10}

Level III miRNA expression data and their corresponding clinical data from 47 LGG samples were downloaded from TCGA data portal. The miRNA quantification data from each sample were combined into a single data set, and then mean fold change values for each gene were calculated by comparing astrocytomas with oligodendrogliomas. The miRNAs that demonstrated a mean fold change of at least 2 were kept as potentially interesting miRNAs for further analysis. The miRNAs were organized according to unsupervised hierarchical clustering, and the samples were grouped according to histological diagnosis.

Results

The G-CIMP Status

The G-CIMP status was determined for 24 LGGs (composing 11 oligodendrogliomas, 8 oligoastrocytomas, and 5 astrocytomas), 294 GBMs, and 2 matched normal specimens. Seventeen (70.8%) of the 24 LGGs were G-CIMP-positive tumors, compared with only 8.5% G-CIMP positivity among the 294 GBMs analyzed (Fig. 2 left). All oligodendrogliomas (11 of 11) exhibited G-CIMP positivity (Fig. 2 right). Only 1 of 5 astrocytomas was G-CIMP positive, whereas the distribution among oligoastrocytomas was more evenly divided, with 6 of 9 being G-CIMP positive. Both matched normal specimens were negative for the hypermethylation phenotype.

Analysis of mRNA

Twenty-seven LGG specimens were classified according to TCGA subtype by the expression pattern of the 840 gene signature defined by Verhaak et al.³² The LGGs analyzed included 8 oligodendrogliomas (4 WHO Grade II, 4 WHO Grade III), 9 oligoastrocytomas (2 WHO Grade II, 7 WHO Grade III), and 10 astrocytomas (1 WHO Grade II, 9 WHO Grade III). All but one of the characterized LGGs had gene expression characteristics of the proneural glioma subtype (Fig. 3 left). The lone exception was a WHO Grade II oligodendroglioma in a patient whose tumor’s molecular characteristics were consistent with the mesenchymal subtype. Notably, this lone patient with the mesenchymal oligodendroglioma died within 2 years of the surgical diagnosis, demonstrating a more aggressive course than is typically seen in oligodendroglioma tumors.

Differential gene expression analysis was then performed comparing oligodendrogliomas with astrocytomas (Fig. 3 right). The oligoastrocytomas were not included in this analysis since they represent a less descriptive subtype with overlapping histological components of the other 2 tumor types. Using a 2-fold mean differential expression cutoff, 338 genes were found to be upregulated and 283 genes were downregulated in the astrocytomas relative to the oligodendrogliomas. Gene ontology analysis of the 338 upregulated genes yielded gene ontology terms related primarily to mitotic functions and the inflammatory response, for example, mitosis, cell cycle phase, immune response, and inflammatory response (Table 1). The most significant cluster had an enrichment score of 8.64 and contained 54 of the 338 upregulated genes, many of which were mRNAs coding for inflammatory cell receptors, ligands, and major histocompatibility complexes. The cluster enrichment score for mitotic genes was 6.37, and this cluster consisted of 61 of the 338 upregulated genes.

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Fig. 2. Clustering of TCGA LGG and GBM tumors identifies G-CIMPs. After removing probe sets targeting the sex chromosomes, the 1427 Infinium DNA methylation probes, whose DNA methylation beta values varied the most across 294 TCGA GBM samples, were analyzed. The DNA methylation clusters are distinguished with a color code at the top panel: *pink*, G-CIMP-positive Cluster 1; *dark blue*, G-CIMP-negative Cluster 2; and *light blue*, G-CIMP-negative Cluster 3. **Left:** Heatmap of the DNA methylation data from 24 LGG samples combined with 294 GBM samples revealing the G-CIMP status after k-medians clustering (number of clusters 3) of the sample order. The level of DNA methylation (beta value) for each probe in each sample is represented by a color scale, with *red* indicating higher levels of methylation and *green* showing lower levels. Each sample is color labeled as described in the key for its tumor histology type (LGG vs GBM). **Right:** Heatmap of the 24 LGG samples clustered alone by k-medians clustering (number of clusters 3) revealing the G-CIMP status. Again, each sample is color labeled as described in the key for its tumor histology type (oligodendroglioma vs astrocytoma vs oligoastrocytoma).

Analysis of miRNA

We also compared miRNA expression differences between 23 oligodendrogliomas (12 WHO Grade II, 11 WHO Grade III) and 24 astrocytomas (2 WHO Grade II, 22 WHO Grade III) as obtained from TCGA microarray data (Fig. 4). Nine miRNAs were upregulated and

28 were downregulated more than 2-fold within the astrocytomas relative to the oligodendrogliomas (Table 2). The upregulated list included miRNAs known to be upregulated in GBMs as well as a variety of nonneural tumors such as miR-21.⁵ Downregulated miRNAs included those associated with neuronal differentiation, which are

Fig. 3. **Left:** Gene expression data identify the 4 TCGA subtypes. Using the 840 gene list defined by Verhaak et al., we ordered samples based on subtype predictions after k-medians clustering (number of clusters 4). Tumor types (GBM vs LGG) are distinguished with a color code at the top panel: *gray*, GBM; and *black*, LGG. The Cancer Genome Atlas subtypes are defined in the center panel: *red*, mesenchymal; *blue*, classical; *green*, neural; and *purple*, proneural. Heatmap showing the level of mRNA gene expression normalized for each gene using a color scale, with *red* representing higher levels of expression and *green* indicating lower levels. **Right:** Differential gene expression of astrocytomas and oligodendrogliomas. Direct comparison between the 2 tumor types revealing 621 mRNAs with a minimum mean fold change of 2. Tumor types (oligodendroglioma vs astrocytoma) are distinguished with a color code at the top panel: *orange*, astrocytoma; and *purple*, oligodendroglioma. Heatmap showing the level of mRNA gene expression normalized for each gene using a color scale, with *red* representing higher levels of expression and *green* indicating lower levels.

TABLE 1: Resulting gene ontology terms using the online tool DAVID for functional analysis of the 338 genes upregulated more than 2-fold in astrocytomas as compared with oligodendrogliomas

Gene Ontology Terms	Gene Count	p Value*	Corrected p Value†
Functional Cluster 1, Enrichment Score 8.64			
immune response	41	9.00E-12	8.40E-09
defense response	37	8.70E-11	2.30E-08
inflammatory response	25	1.80E-09	3.30E-07
response to wounding	29	1.20E-07	1.90E-05
inflammatory response	11	3.80E-07	3.10E-05
Functional Cluster 2, Enrichment Score 6.37			
M phase	29	2.40E-12	4.50E-09
mitosis	21	1.40E-11	4.50E-09
cell cycle phase	31	2.20E-11	1.40E-08
mitosis	23	2.70E-11	1.20E-08
nuclear division	23	2.70E-11	1.20E-08
M phase of mitotic cell cycle	23	3.90E-11	1.40E-08
organelle fission	23	6.00E-11	1.80E-08
cell cycle process	34	6.20E-10	1.40E-07
mitotic cell cycle	27	1.00E-09	2.10E-07
cell division	21	9.40E-09	1.50E-06
cell cycle	36	1.40E-07	2.10E-05
cell division	20	7.90E-07	1.10E-04
spindle	13	9.60E-06	2.60E-03
cell cycle	22	1.70E-05	7.80E-04
regulation of mitotic cell cycle	11	2.90E-04	1.50E-02
microtubule cytoskeleton	20	3.30E-03	7.20E-02
cytoskeleton	18	2.60E-02	3.80E-01
cytoskeletal part	23	1.00E-01	6.00E-01
cytoskeleton	30	1.60E-01	7.50E-01
intracellular non-membrane-bounded organelle	46	5.30E-01	9.70E-01
non-membrane-bounded organelle	46	5.30E-01	9.70E-01

* Significance of gene term enrichment by Fisher exact test. Values expressed in E notation.

† The p value is corrected for multiple testing by the Benjamini-Hochberg method. Values expressed in E notation.

known to be downregulated in higher-grade glial tumors such as miR-124 and miR-128.¹³

Discussion

Diagnostic uncertainty in discriminating between LGGs of oligocytic and astrocytic lineage remains a major challenge in neurooncology. Here, we analyzed TCGA data set in search of molecular biomarkers that could facilitate discrimination. The key findings of our study are as follows: 1) oligodendrogliomas are more likely to exhibit the G-CIMP phenotype relative to low-grade astrocytomas; 2) relative to oligodendrogliomas, low-grade astrocytomas exhibit a higher expression of genes related to mitosis, replication, and inflammation; and 3) low-grade astrocytic tumors harbor miRNA profiles similar to those previously described for glioblastoma tumors. Notably, such miRNA profiles were not observed in oligodendrogliomas. These molecular features may augment conventional histopathology in discriminating oligodendrogliomas from low-grade astrocytomas.

The G-CIMP phenotype was initially described by Noshmeh et al.²⁰ as a pattern of epigenetic modification that is found in a subset of glioblastomas. These tumors exhibit increased methylation of cytosine in CpG islands that are often found in the promoter regions of transcribed genes.³ It is thought that promoter methylation silences critical genes that mediate glioblastoma aggressiveness and therapeutic resistance. Consequently, G-CIMP-positive glioblastomas have a better prognosis than glioblastomas without the G-CIMP phenotype.²⁰ Our observation that oligodendrogliomas are more likely to harbor the G-CIMP phenotype is largely consistent with the more favorable prognosis of this LGG relative to low-grade astrocytic tumors. Five-year survival for patients with astrocytic LGGs is 56% compared with 74% in those with predominantly oligodendritic gliomas.³³ Some oligodendrogliomas with 1p/19q deletions are still slower growing tumors with even more indolent courses.²⁵

The increased expression of mRNAs related to mitosis and inflammation in low-grade astrocytomas relative to ol-

Fig. 4. Differential expression of miRNA from astrocytomas and oligodendrogliomas. Direct comparison between the 2 tumor types revealed 37 miRNAs with a minimum mean fold change of 2. Tumor types (oligodendroglioma vs astrocytoma) are distinguished with a color code at the top of the panel: *orange*, astrocytoma; and *purple*, oligodendroglioma. Heatmap demonstrating the miRNA gene expression normalized for each gene using a color scale, with *red* representing higher levels of expression and *green* indicating lower levels.

oligodendrogliomas is also consistent with the more aggressive clinical course of the astrocytomas.^{29,33} The increased expression of mRNAs related to mitosis probably mirrors increased rates of tumor proliferation.⁸ Increased expression of inflammatory cytokines in glioblastoma specimens has been associated with worsened overall survival.^{31,34,36} Emerging evidence suggests that the presence of inflammatory cytokines may enhance glioblastoma growth as well as suppress apoptotic and senescent processes within glioblastomas.^{6,36} The inflammatory markers upregulated in astrocytomas in the current analysis may reflect the increased number of microglia in astrocytic gliomas relative to oligodendrogliomas^{7,26} or the inherent expression of cytokines by astrocytic gliomas.^{12,19,35,36}

Aberrant miRNA expression is a universal theme during carcinogenesis.¹⁶ Moreover, the pattern of miRNA expression often marks the lineage of origin with regard to the tumor.^{15,17} In this context, it is gratifying that we uncovered distinct miRNA profiles in astrocytic and oligodendrocytic LGGs. Several of the miRNAs we found to be expressed at higher levels in the astrocytomas as compared with the oligodendrogliomas have been previously described as key contributors to glioblastoma formation, including miR-21.^{2,22} Conversely, many of the miRNAs downregulated in astrocytomas relative to oligodendrogliomas have been shown to be required in maintaining astrocytic differentiation, including miR-124⁴ and miR-128.²¹

Conclusions

Oligodendrogliomas and low-grade astrocytomas exhibit distinctly different molecular characteristics including G-CIMP status, mRNA expression, and miRNA profiles. Incorporation of these molecular platforms with existing molecular markers, such as IDH1 mutation, TP53 mutation, and 1p19q status, should facilitate accurate and reliable pathological diagnosis of LGGs.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

TABLE 2: Differentially expressed miRNAs in astrocytomas relative to oligodendrogliomas*

miRNA	Fold Change
hsa-miR-455	2.501695
hsa-miR-204	2.474307
hsa-miR-200a	2.417406
hsa-miR-21	2.313565
hsa-miR-937	2.312845
hsa-miR-1262	2.197591
hsa-miR-887	2.055209
hsa-miR-577	2.025004
hsa-miR-1251	2.015545
hsa-miR-128-2	0.489822
hsa-miR-451	0.482832
hsa-miR-126	0.479343
hsa-miR-1247	0.475773
hsa-miR-486	0.475747
hsa-miR-378	0.467384
hsa-miR-124-1	0.454637
hsa-miR-124-2	0.450175
hsa-miR-1274b	0.446444
hsa-miR-592	0.444133
hsa-miR-124-3	0.441731
hsa-miR-129-2	0.440461
hsa-miR-145	0.439702
hsa-miR-551b	0.433424
hsa-miR-320b-2	0.430294
hsa-miR-153-1	0.429561
hsa-miR-153-2	0.427691
hsa-miR-1224	0.411144
hsa-miR-129-1	0.400323
hsa-miR-139	0.390977
hsa-miR-133a-1	0.379083
hsa-miR-105-2	0.363826
hsa-miR-203	0.357636
hsa-miR-1-2	0.349742
hsa-miR-105-1	0.321588
hsa-miR-143	0.306174
hsa-miR-767	0.297406
hsa-miR-891a	0.212531

* hsa = *Homo sapiens*.

materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Carter, Chen. Acquisition of data: Muller. Analysis and interpretation of data: Gonda, Goyal, Chen. Drafting the article: Gonda, Cheung, Muller, Goyal. Critically revising the article: Gonda, Chen. Reviewed submitted version of manuscript: Goyal. Approved the final version of the manuscript on behalf of all authors: Gonda. Statistical analysis: Gonda. Study supervision: Carter, Chen.

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