**Candidate Pathways for Promoting Differentiation or Quiescence of Oligodendrocyte Progenitor-like Cells in Glioma**

Joseph D. Dougherty, Elena I. Fomchenko, Afua A. Akuffo, Eric Schmidt, Karim Y. Helmy, Elena Bazzoli, Cameron W. Brennan, Eric C. Holland, and Ana Milosevic

**Abstract**

Platelet-derived growth factor receptor alpha-positive oligodendrocyte progenitor cells (OPC) located within the mature central nervous system may remain quiescent, proliferate, or differentiate into oligodendrocytes. Human glioblastoma multiforme tumors often contain rapidly proliferating oligodendrocyte lineage transcription factor 2 (Olig2)-positive cells that resemble OPCs. In this study, we sought to identify candidate pathways that promote OPC differentiation or quiescence rather than proliferation. Gene expression profiling conducted in both normal murine OPCs and highly proliferative Olig2-positive glioma cells identified all the transcripts associated with the highly proliferative state of these cells and showed that among the various cell types found within the brain, Olig2-positive tumor cells are most similar to OPCs. We then subtracted OPC transcripts found in tumor samples from those found in normal brain samples and identified 28 OPC transcripts as candidates for promoting differentiation or quiescence. Systematic analysis of human glioma data revealed that these genes have similar expression profiles in human tumors and were significantly enriched in genomic deletions, suggesting an antiproliferative role. Treatment of primary murine glioblastoma cells with agonists of one candidate gene, Gpr17, resulted in a decreased number of neurospheres. Together, our findings show that comparison of the molecular phenotype of progenitor cells in tumors to the equivalent cells in the normal brain represents a novel approach for the identification of targeted therapies.

**Introduction**

Glioma is the most prevalent type of primary brain tumor in adults and prognosis, especially for the high-grade, stage IV glioblastoma multiforme (GBM), is dismal. Recently, work at the mRNA (1–3) and protein signaling levels (4) have converged upon similar classification schemes that identify distinct subgroups of high-grade glioma. These subgroups show differential gene expression profiles, signaling cascades, and response to treatment. In particular, one subgroup, referred to as proneural, is characterized by elevated expression of platelet-derived growth factor (PDGF), amplifications of PDGF receptor alpha (PDGFRα; ref. 4), and expression of oligodendrocyte lineage transcription factor 2 (Olig2; refs. 1, 2).

In the mature central nervous system (CNS), the PDGFRα and the chondroitin sulfate proteoglycan antigen (NG2) have been considered as markers primarily of the oligodendrocyte progenitor cells (OPC; refs. 5, 6). OPCs are sparsely and evenly distributed throughout white and gray matter. The adult OPC was thought to mainly serve as a repository for the generation of new mature oligodendrocytes (6) but may also serve as a neural stem cell (7). Although they are typically quiescent, they are more proliferative than any other population in the brain, with 1% to 5% actively cycling at any time (8). Regardless of their role, these cells are capable of at least 3 fates: they may terminally differentiate into oligodendrocytes, they may proliferate to produce additional progenitors, or they may remain quiescent.

We have previously generated a mouse model of glioma in which a Nestin (Nes)-positive neural stem cell can be induced to overproduce PDGFR-B (9, 10). The PDGFRα is activated by both homo- and heterodimers of A and B forms of PDGF (11). These mice generate aggressive gliomas resulting in a tumor mass composed of the highly proliferative cells of origin and cells derived from initially normal progenitors that are recruited to the tumor (9, 12–14). The presence of cells expressing Olig2 and PDGFRα in these tumors suggests that treatment strategies in the proneural subtype may be informed by knowledge of the signaling pathways that regulate the choice between proliferation, quiescence, and differentiation in these OPCs.

**Authors’ Affiliations:** 1Departments of Genetics and Psychiatry, Washington University, St. Louis, Missouri; 2Department of Neurosurgery, 3Brain Tumor Center, 4Department of Cancer Biology and Genetics, 5Department of Neurology, 6Department of Surgery, Memorial Sloan Kettering Cancer Center; and 7The GENSAT project, 8Laboratory of Molecular Biology, The Rockefeller University, New York, NY

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

**Corresponding Author:** Ana Milosevic, The Rockefeller University, 1230 York Ave, Box 286, NY 10065. Phone: 212-327-7286; Fax: 212-327-7888; E-mail: amilosevic@rockefeller.edu

**doi:** 10.1158/0008-5472.CAN-11-2632

©2012 American Association for Cancer Research.
Current glioma treatment, following resection of the tumor, is focused on targeting proliferating cells with temozolomide and radiotherapy (15). However, as previously noted (16), a complementary strategy would be to promote pathways for maintaining quiescence and/or driving terminal differentiation of the progenitors present in the tumor, as this may also serve to slow tumor growth. Prodifferentiation treatments may be particularly promising; although the tumor environment is likely selecting for mutations in genes that normally suppress cell-cycle progression and maintain quiescence, there may not be as much selective pressure on all prodifferentiation pathways.

We undertook a study in the PDGF-B–induced mouse model of glioblastoma to identify transcripts that could be important for the regulation of quiescence and differentiation in OPC-like cells. Applying our newly developed methodology for in vivo cell-specific translational profiling (17, 18), we identified all mRNAs specifically enriched in OPCs in normal mouse brain, including those likely to be important for proliferation, quiescence, and differentiation. We then contrasted this to the cell-specific translational profile of Olig2-positive cells in the mouse model of a proneural glioma, in which OPC-like cells are committed to proliferation at the expense of differentiation or quiescence. This permitted identification of candidate pathways that may serve as targets for promoting differentiation and quiescence in OPCs in mice. Examination of The Cancer Genome Atlas (TCGA) expression profiles of human gliomas established that analogous pathways are similarly regulated in human proneural GBM, suggesting their conservation as targets. Also consistent with an antiproliferative role, many of these targets show deletions in human GBM.

From this combined human and mouse screening, we have identified several candidate pathways for promoting quiescence and differentiation, which may serve as targets for complementary treatments.

Material and Methods

Full materials and methods are available online. All protocols involving animals were approved by the Rockefeller University and Memorial Sloan Kettering Cancer Center Institutional Animal Care and Use Committee.

New mouse lines

Bacterial artificial chromosomes (BAC) containing genes PDGFRα (RP23-55P22), Cnp1 (RP23-78L12), and Snap25 (RP23-290A18) were modified as described (17), to insert an EGFP-L10a fusion protein into the relevant translation start site.

Histology

Anatomy was carried out as described (17, 19). For immunofluorescence, samples were incubated with the cell-specific antibodies, and quantification was done on ×40 cortical fields imaged with confocal microscopy.

Profiling tumor model

Tumors were generated as described (9). Cells producing RCAS-h PDGF-B or RCAS-Cre virus were injected into the progeny of ink4a/arf−/− mice expressing tv-a receptor for RCAS under the Nes promoter, crossed to Olig2-Egfp::L10a mice. Telomeric repeat amplification protocol (TRAP) and microarray hybridizations were carried out as described (17, 18), except tumors were processed individually.

Figure 1. The Olig2::Egfp-L10a mouse permits study of oligodendrogliia. A1 and A2, DAB immunohistochemistry (IHC) for EGFP-L10a Olig2::Egfp-L10a mouse reveals a pattern consistent with expression in both OPCs and mature oligodendrocytes. B1–B3, confocal immunofluorescence for EGFP-L10a and Cnp1 confirms localization in mature oligodendrocytes (white arrowheads). C1–C3, confocal immunofluorescence for EGFP-L10a and Ng2 confirms localization in OPCs (white arrow). D, in normal brain, Olig2::Egfp-L10a translational profiles show enrichment of both Cnp1 and Cspg4 (Ng2) compared with total brain RNA. In glioma, Olig2::Egfp-L10a cells show a relative increase of Cspg4 and loss of Cnp1 RNA. All scale bars, 20 microns.
Microarray analysis

Microarray data were analyzed with Bioconductor module of the R statistical package, normalized as described (17, 20, 21) and deposited at GEO (GSE30626; ref. 22). To identify messages specific to each cell type specificity indices (pSI) were calculated as described (18). Transcripts with pSI < 0.05 were selected for further analysis.

Heat maps and hierarchical clustering were carried out in R. All color-coded scatter plots show only top 50 transcripts for each cell type, but all statistics were conducted on full lists (Supplementary Table S1). Differentiation or quiescence (DorQ) candidates were selected as those transcripts from the OPC list that were 2-fold higher in the average of the Olig2-Egfp-L10a normal cortex than the average of all 12 Olig2-Egfp-L10a tumor samples. Statistical comparisons were conducted with LIMMA module of Bioconductor.

For cross-species comparisons, human and mouse homologs were mapped by Gene Symbol. For DorQ candidates, mapping was confirmed by Blating mouse protein to human
genome (UCSC). Human microarray data were downloaded from TCGA and normalized as described (4).

Gene ontology analysis
For each cell type, all gene symbols for messages with a pSI < 0.05 were analyzed with BINGO (23). Biologic Process GO categories were evaluated to identify those with P < 0.01 using the hypergeometric test and Benjamini–Hochberg correction.

Neurosphere cell culture
Primary neurosphere cultures from tumors and wild-type mice were generated as described (24). Neurospheres were grown in 20 mg/mL EGF and 10 mg/mL bFGF, and uridine 5’-diphosphate sodium salt (UDP; 10–50 μmol/L), UDP glucose (100 μmol/L), and leukotriene D4 (LTD4; 100 nmol/L) were added on a daily basis. All data are average of cultures from 4 independent mouse tumors, counted in triplicate in wells of 100 to 1,000 spheres. Counts were normalized within each tumor to the number of spheres in 0 UDP condition.

Results
We recently developed the TRAP strategy, which allows profiling of all mRNAs bound to ribosomes in defined cell populations. This strategy entails using RAC transgenesis to express EGFP fused to the ribosomal protein L10a under the control of a “driver” gene specific to certain cell types in the brain. Thus, the cells of interest contain ribosomes with an EGFP tag enabling affinity purification of all ribosome-associated mRNA. We have generated and characterized bacTRAP mouse lines for a variety of cell types, including the Olig2-positive oligodendroglia (17).

Because Olig2 is frequently overexpressed in human glioma (25) and high levels of expression may particularly characterize the proneural subtype (2). We recently examined the expression of neural lineage markers, proliferation markers, and translational profile of Olig2::Egfp-L10a in our mouse model of glioma (12). This work revealed a severe perturbation of the translational profile of Olig2::Egfp-L10a cells. In a normal Olig2::Egfp-L10a mouse, both NG2-positive OPCs and 2’0,3’0-cyclic nucleotide 3’0-phosphodiesterase1 (Cnp1)-positive mature oligodendrocytes express EGFP-L10a (Fig. 1A–C; ref. 17). The tumor translational profiles for the Olig2::Egfp-L10a cells reveal a strong increase in translation for Cnp1 (the RNA corresponding to NG2) and decrease in Cnp1, relative to normal Olig2::Egfp-L10a cells (Fig. 1D). Although the data come from only 2 markers, it suggests the preponderance of Olig2::Egfp-L10a cells are in an immature state within the tumor. To investigate this thoroughly, we decided to identify the translational profile of
normal adult OPCs. With this data for comparison, we would then be able to determine whether Olig2::Egfp-L10a are in a more OPC-like state in the tumor, and if so, we could then examine how this state may differ from normal OPCs, as these differences may regulate cell fate choices and represent potential treatment strategies.

**Identification of the translational profile of the normal oligodendrocyte progenitors**

Identification of transcripts that are specific to OPCs requires not only knowledge of their profile but also the profiles of the other cell types. The brain contains 4 major classes of cells: neurons, astrocytes, and 2 types of oligodendroglia; mature oligodendrocytes and OPCs. We have previously generated and thoroughly characterized mouse lines that allow translational profiling from astrocytes (Aldh1L1::Egfp-L10a), mature oligodendrocytes (Cnp1::Egfp-L10a), and the pan-oligodendroglial line (Olig2::Egfp-L10a; ref. 17). However, to identify those mRNAs that are enriched in each of the major cell classes, we generated additional bacTRAP mouse lines targeting all neurons (Snap25::Egfp-L10a), as well as Pdgfraf expressing cells (Pdgfra::Egfp-L10a).

---

**Figure 3.** Analysis of Olig2::Egfp-L10a translational profiles in normal brains and tumors identifies candidates for differentiation and quiescence of OPCs. A, hierarchical clustering of Olig2::Egfp-L10a bacTRAP data from normal cortex reveals a profile intermediate to oligodendrocytes and OPCs, consistent with transgene expression in both populations. B, scatter plot of Olig2 bacTRAP microarray data (x-axis) compared with whole cortex microarray data (y-axis) for all transcripts. Olig2 shows clear enrichment of OPC (blue) and mature oligodendrocyte transcripts (green), and no enrichment of neuronal transcripts (red). C, average ratios (log2 scale) between Olig2::Egfp-L10a translational profiles in normal brain and whole cortex RNA for all transcripts of mature oligodendrocytes (green), OPCs (blue), and neuronal (red) lists. D, Heat map and hierarchical clustering of Olig2::Egfp-L10a translational profiles for 3 variations of the tumor model, PDGF++, PDGF-Cre, and recruited, show most similarity to normal OPC cells, though there are transcripts that distinguish them from OPCs (arrow).
To target neurons, we selected 2 genes as putative pan-neuronal drivers, complexin 1 (Cplx1) and synaptosomal-associated protein 25 (Snap25). Examination of multiple EGFP-L10a lines for both constructs revealed that Snap25 was the brighter and more reliable construct and thus selected for further analysis.

To target PDGF-responsive OPCs, we tested a BAC covering the Pdgfra gene. Multiple lines showed similar but not identical patterns of expression, targeting NG2-positive cells with varying intensity, but also subpopulations of neurons in some lines, as well as robust expression in the choroid plexus. For this study, we selected the line that targeted cortical NG2-positive OPCs most specifically. For each of the 4 cell classes, the lines used for further experiments were carefully characterized with confocal immunofluorescence to confirm accuracy of transgene expression and identity of each cell type (Fig. 2A–D).

For the newly generated lines, we quantified, from randomly collected confocal images of mouse cortex, the overlap of GFP with 4',6-diamidino-2-phenylindole (DAPI) and with each of 2 cell-specific markers. For Snap25, we labeled with pan-neuronal maker NeuN (Fig. 2A) or Tbr1 (not shown), a marker of deep layer neurons. In cortex, of 663 DAPI-positive cells counted, 57.6% were positive for GFP, consistent with the large proportion of neurons in this tissue. A total of 96.8% of GFP-positive cells were positive for NeuN, and 98.6% of NeuN-positive cells were positive for GFP. For Tbr1, of 592 Tbr1-positive cells counted, 99.8% were labeled with GFP. For Pdgfra, of 3,015 DAPI-positive cells counted, 8.2% were positive for GFP, consistent with the relative rarity of the OPC cells. A total of 88.8% of GFP-positive cells were labeled with NG2, and though receptors are often localized on processes far from the GFP-labeled cell bodies, obscuring counting, at least 51.4% of GFP-positive cells were clearly labeled with Pdgfra antibodies. Most of these cells distinctly had the morphology of OPCs, though both Ng2 and Pdgfra label pericytes as well. A subset of Pdgfra::EGFP-L10a cells also labeled with the proliferative marker Ki-67 (<2%), consistent with expectation for these cells (8).

For each new line, TRAP was conducted in triplicate on pooled cortices from 2 to 3 mice, and cell-specific polysomal mRNAs were queried with microarrays. Replicate arrays showed high reproducibility (minimum correlation = 0.978), though each cell type had a distinct profile (Fig. 2E). We used the specificity index statistic (21) to select for those transcripts significantly enriched in each cell population (Snap25, Add1L1, Cop1, and Pdgfra) relative to the other 3. These lists included known markers for each cell type (Supplementary Table S1, and Fig. 2F). The non-OPC cell types had significant over-representation of Gene Ontology (GO) categories consistent with their known roles in synaptic transmission (Neurons, Supplementary Fig. S2A), myelination (Oligodendrocytes, Supplementary Fig. S2B), or a role in lipid metabolism (Astrocytes, Supplementary Fig. S2C; ref. 26). As the OPC population is the most proliferative population in the cortex (8), the transcripts specific to OPCs showed a highly significant overrepresentation of categories related to the cell cycle (P < 1E-7, hypergeometric test with Benjamini–Hochberg correction; Supplementary Fig. S3), as well as in categories related to protein phosphorylation and biopolymer modification (P < 1E-3). For all cell types, there were also a variety of transcripts not currently annotated in known pathways. Overall, this evidence, along with presence of all known markers for OPCs on the list, suggests we have identified an OPC-specific profile, as well as generated a useful tool for assessment of these cells in vivo.

Characterization of Olig2-positive cells in mouse model of glioblastoma

We next applied our tools to characterize the oligodendrogial lineages in the context of a mouse model of GBM. Murine PDGF-driven GBM show high expression levels of Olig2 (Supplementary Fig. S1; refs. 12, 43). In a normal brain, the transgenic line Olig2::Egfp-L10a labels all oligodendroglial cells; both the NG2-positive OPCs and Cnp1-positive myelinating oligodendrocytes (Fig. 1A–C and ref. 17). Hierarchical clustering of TRAP microarray data positions the profile of these cells between that of mature oligodendrocytes and...
PDGFRA-positive OPCs (Fig. 3A). Comparison of the Olig2::Egfp-L10a profile to whole cortex reveals that it is enriched in both the OPCs and myelinating oligodendrocyte transcripts (Fig. 3B), consistent with it covering the entire lineage. First, we translationally profiled Ntv-a/ink4a/arf−/− Olig2::Egfp-L10a cells in primary tumors from our model (PDGF-B tumors). Hierarchical clustering revealed that these cells are most similar to OPCs of the normal brain. To test this observation, we repeated the analysis in 2 additional variations of our tumor model: primary tumors from this model with additional deletion of Pten (PDGF-Cre tumors), and in tumors induced by transplanting EGFP-L10a-negative tumor cells from PDGF-driven primary murine tumors into Ntv-a/ink4a/arf−/− Olig2::Egfp-L10a mice (recruited). Although this increases the variability among the tumor samples, all samples cluster most closely with OPCs (Fig. 3D). This provides systematic evidence that Olig2::Egfp-L10a cells are in a more OPC-like state in glioma.

The most parsimonious explanation of these findings is that the excess of PDGF-B ligand produced by the Nes-positive cell has driven the recruitment and expansion of the proliferating PDGFRA-positive cells, at the expense of quiescence or differentiation. To examine this, we plotted the translational profile of Olig2::Egfp-L10a cells in a normal mouse cortex versus Olig2::Egfp-L10a cells in tumors and labeled those transcripts that are specific to either OPCs or mature oligodendrocytes. On average, the tumor contains a clear enrichment of transcripts specific to OPCs and depletion of transcripts found in mature oligodendrocytes (Fig. 3E). Not surprisingly, the subset of OPC transcripts identified by GO as involved in cell cycle/cell proliferation, such as Melk (27) and Pbk (28), are highly enriched in the tumor samples, consistent with the Olig2::Egfp-L10a population being in a highly proliferative state inside of the tumor. However, surprisingly, there is also a subset of OPC transcripts that seem enriched in the normal brain.

Identification of differentiation or quiescence candidate transcripts in OPCs

In a normal brain, OPCs may proliferate, remain quiescent, or differentiate, but in the context of a tumor, they seem to proliferate at the expense of differentiation or quiescence. Comparison of the transcriptional profiles of Olig2::Egfp-L10a cells from normal brain and tumor pointed to a subset of 28 transcripts translated greater than 2-fold higher in normal brain (Fig. 3F, Table 1). We hypothesized that this subset contains those transcripts used in either differentiation or quiescence in oligodendroglia.

Careful examination of current literature supports this hypothesis. For example, Cdkn1c is required for the regulation of cell cycle and specification of OPCs (29), whereas Sema5a, an oligodendrocyte-specific semaphorin, plays a role in axon growth inhibition (30). G-coupled protein receptor Gpr17 has the role in myelination during development (31). Other DorQ candidate transcripts are known to be expressed in oligodendroglia, although their role is unclear. They include Rlbp (32) and C1qtnf2, whose mRNA expression pattern is consistent in part with the oligodendrocyte expression (33). Additional screening of the expression patterns of DorQ candidate transcripts in Allen Brain Atlas and Gene Expression Nervous System Atlas (GENSAT) confirmed that Gpr17, Rlbp1, Rlbp, Rtb, Bmp4, 2310031a18Rik, expression patterns are consistent with OPC localization. The presence of these transcripts in OPCs suggests that the DorQ candidates may have some relevance as complementary targets for treatment, if they are conserved in humans.

Differentiation and quiescence candidate transcripts show similar expression pattern in human glioma

As human proneural tumors are characterized by an abundance of PDGF-B and activation of PDGFRA signaling pathways, we sought to determine whether they contained a preponderance of OPC transcripts. The results, summarized in Supplementary Table S2, Supplementary Fig. S2, and Supplementary Fig. S4, showed that human proneural specific gene list has a highly significant enrichment of OPC transcripts. We carried out hierarchical clustering of the human gliomas using the OPC transcripts (excluding those categorized by GO as involved in cell cycle or DNA replication as enhanced cell cycle is common to all glioblastoma subtypes). Using just these transcripts, most of the proneural tumors clustered into a single large branch, suggesting that these OPC transcripts are sufficient to distinguish the proneural subtype (Supplementary Fig. S4B). On average, the non–cell cycle OPC transcripts are expressed at a higher relative level in proneural tumors (Supplementary Fig. S4C), though they are present at detectable levels in all subtypes. This suggests that part of what distinguishes the proneural subtype from the other types may be the relative abundance of OPC-like cells.

Although the analysis above showed that OPC transcripts in general have potential relevance to human glioma, we next examined the DorQ candidate subset of OPC transcripts specifically. We first analyzed the human TCGA dataset to determine whether these transcripts show the same pattern of expression that is present in the mouse tumors. Because the DorQ candidates were identified by using translational profiling data specifically for Olig2::Egfp-L10a cells, which is unavailable in the human samples, to generate an analysis analogous to human samples, we examined the expression of the DorQ candidates in total RNA profiles from mouse gliomas and normal mice (Supplementary Fig. S5A). With this method all OPC transcripts, including the DorQ candidates, are somewhat enriched in tumors compared with normal brain. However, the DorQ candidate transcripts are significantly less increased in tumors than the other OPC transcripts are (Supplementary Fig. S5D). We repeated this same analysis in human proneural tumors compared with normal human cortex, using the human homologs of these DorQ transcripts (Supplementary Fig. S5B). We find that, as with the mouse, the DorQ candidate transcripts are enriched, but significantly less so than the other OPC transcripts (P < 0.05; Supplementary Fig. SSD). Overall, the log_{2} fold change values of glioma/normal brain for all DorQ candidate transcripts is correlated at 0.62 (Pearsons correlation) across mouse and human comparisons, suggesting that the general pattern of expression of these pathways is conserved across mouse and human glioma (Supplementary Fig. S5C).
Differentiation and quiescence candidates are frequently deleted in human glioma

We next sought to determine whether the DorQ candidates were mutated in human glioma. Common source of genetic variation in the cancer genome are copy number variations (CNV), often resulting in deletions of tumor suppressor genes and amplifications of particular oncogenes (34). However, there are also a large number of apparently neutral CNVs that occur in glioma. Also, even if a certain gene is very commonly deleted in glioma, it does not necessarily implicate that gene in glioma biology; it could merely be adjacent to an important tumor suppressor. Nonetheless, if we consider these transcripts as a group, then the DorQ transcripts, particularly those that have a role in quiescence, or autocrine prodifferentiation pathways, should have a higher prevalence of deletions compared with duplications.

Thus we examined the CNVs for the DorQ candidates using the RAE algorithm for the set of human gliomas analyzed here (Fig. 4A; refs. 1, 35). Half of the DorQ genes showed a preponderance of deletions relative to duplications and 6 were deleted in nearly one quarter of GBM samples. On average, the 24 transcripts showed a 2.6 to 1 preponderance of deletions to duplications in human glioma, a result unlikely to be due to chance (P < .05, permutation testing, Fig. 4B) and consistent with the role of some of the DorQ candidates as tumor suppressors in human glioma through promotion of quiescence or differentiation.

Agonists of DorQ candidate Gpr17 can decrease clonogenicity of glioblastoma cells in vitro

Previously, others have tested perturbations of Bmp4 (36) and Rxrg (37) signaling for treatment of glioma and neuroblastoma, respectively. We sought to determine whether any of the other DorQ candidates identified here might also drive the glioma OPCs toward differentiation or quiescence. We decided to first test Gpr17 for 4 reasons. First, receptors make attractive targets for eventual treatment strategies, and in this case ligands have been recently characterized: Gpr17 is a G-Protein coupled receptor that responds to both the uracil nucleotides and cysteinyl–leukotrienes (38). Second, our survey of

<table>
<thead>
<tr>
<th>Mouse symbol</th>
<th>Entrez</th>
<th>Fold change</th>
<th>P</th>
<th>w/FDR</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1810041 L15Rik</td>
<td>72301</td>
<td>4.29</td>
<td>0.0004</td>
<td>0.0126</td>
<td>RIKEN cDNA 1810041L15 gene</td>
</tr>
<tr>
<td>2310031 A18Rik</td>
<td>69627</td>
<td>3.51</td>
<td>0.0120</td>
<td>0.1103</td>
<td>RIKEN cDNA 2310031A18 gene</td>
</tr>
<tr>
<td>9030409G11 Rik</td>
<td>71529</td>
<td>2.26</td>
<td>0.0013</td>
<td>0.0278</td>
<td>RIKEN cDNA 9030409G11 gene</td>
</tr>
<tr>
<td>9630013A20Rik</td>
<td>319903</td>
<td>20.05</td>
<td>0.0156</td>
<td>0.1305</td>
<td>RIKEN cDNA 9630013A20 gene</td>
</tr>
<tr>
<td>BC057371</td>
<td>194237</td>
<td>5.03</td>
<td>0.0003</td>
<td>0.0098</td>
<td></td>
</tr>
<tr>
<td>Bmp4</td>
<td>12159</td>
<td>9.96</td>
<td>0.0002</td>
<td>0.0077</td>
<td>Bone morphogenetic protein 4</td>
</tr>
<tr>
<td>C1q1</td>
<td>23829</td>
<td>3.69</td>
<td>0.1154</td>
<td>0.4434</td>
<td>Complement component 1, q subcomponent -like 1</td>
</tr>
<tr>
<td>C1qtnf2</td>
<td>69183</td>
<td>3.51</td>
<td>0.0043</td>
<td>0.0581</td>
<td>C1q and TNF-related protein 2</td>
</tr>
<tr>
<td>Calb2</td>
<td>12308</td>
<td>4.32</td>
<td>0.0015</td>
<td>0.0305</td>
<td>Calbindin 2</td>
</tr>
<tr>
<td>Caskin2</td>
<td>140721</td>
<td>2.91</td>
<td>0.0001</td>
<td>0.0061</td>
<td>CASK-interacting protein 2</td>
</tr>
<tr>
<td>Cdknc</td>
<td>12577</td>
<td>2.78</td>
<td>0.0588</td>
<td>0.2918</td>
<td>Cyclin -dependent kinase inhibitor 1C (P57)</td>
</tr>
<tr>
<td>Dct</td>
<td>13190</td>
<td>3.48</td>
<td>0.0425</td>
<td>0.2401</td>
<td>Dopachrome tautomerase</td>
</tr>
<tr>
<td>Gpr17</td>
<td>574402</td>
<td>2.80</td>
<td>0.0283</td>
<td>0.1688</td>
<td>G protein–coupled receptor 17</td>
</tr>
<tr>
<td>Kankl</td>
<td>107351</td>
<td>2.02</td>
<td>0.0035</td>
<td>0.0508</td>
<td>KN motif and ankyrin repeat domains 1</td>
</tr>
<tr>
<td>Knfl26a</td>
<td>668303</td>
<td>2.66</td>
<td>0.0566</td>
<td>0.2854</td>
<td>Kinesin family member 26A</td>
</tr>
<tr>
<td>Ladl</td>
<td>16763</td>
<td>43.22</td>
<td>0.0002</td>
<td>0.0097</td>
<td>Ladinin</td>
</tr>
<tr>
<td>Lim2s</td>
<td>225341</td>
<td>3.30</td>
<td>0.0016</td>
<td>0.0309</td>
<td>LIM and senescent cell antigen-like domains 2</td>
</tr>
<tr>
<td>Npm3-ps1</td>
<td>108176</td>
<td>3.35</td>
<td>0.0965</td>
<td>0.3969</td>
<td>Pseudogene</td>
</tr>
<tr>
<td>Rab32</td>
<td>67844</td>
<td>3.32</td>
<td>0.0111</td>
<td>0.1044</td>
<td>RAB32, member RAS oncogene family</td>
</tr>
<tr>
<td>Ras12</td>
<td>70784</td>
<td>3.33</td>
<td>0.0014</td>
<td>0.0286</td>
<td>RAS-like, family 12</td>
</tr>
<tr>
<td>Ras10</td>
<td>78748</td>
<td>7.10</td>
<td>0.0055</td>
<td>0.0664</td>
<td>Ras association domain family member 10</td>
</tr>
<tr>
<td>Rtbpl</td>
<td>19771</td>
<td>2.29</td>
<td>0.0160</td>
<td>0.1331</td>
<td>Retinaldehyde binding protein 1</td>
</tr>
<tr>
<td>Rnf43</td>
<td>207742</td>
<td>2.19</td>
<td>0.0008</td>
<td>0.0194</td>
<td>Ring finger protein 43</td>
</tr>
<tr>
<td>Rrxg</td>
<td>20183</td>
<td>2.71</td>
<td>0.0158</td>
<td>0.1318</td>
<td>Retinoid X receptor gamma</td>
</tr>
<tr>
<td>Sema5a</td>
<td>20356</td>
<td>2.11</td>
<td>0.0993</td>
<td>0.4039</td>
<td>Semaphorin 5A</td>
</tr>
<tr>
<td>Snx22</td>
<td>382083</td>
<td>2.17</td>
<td>0.0932</td>
<td>0.3873</td>
<td>Sorting nexin 22</td>
</tr>
<tr>
<td>Susd5</td>
<td>382111</td>
<td>3.59</td>
<td>0.2071</td>
<td>0.6356</td>
<td>Sushi domain containing 5</td>
</tr>
<tr>
<td>Trt</td>
<td>22139</td>
<td>8.69</td>
<td>0.0664</td>
<td>0.3124</td>
<td>Transhyretin</td>
</tr>
<tr>
<td>Ust</td>
<td>338362</td>
<td>2.84</td>
<td>0.0621</td>
<td>0.3019</td>
<td>Uronyl -2-sulfotransferase</td>
</tr>
</tbody>
</table>

NOTE: OPC genes with expression more than 2-fold higher in Olig2 cells from normal cortex than tumors. Includes average fold changes (linear scale) and LIMMA P value. More detail can be found in Supplementary Table S3.
translational profiles across a variety of neuronal and glial cell types suggested that Gpr17 is restricted to cells of the oligodendroglial lineage in the mature CNS (Fig. 5A; ref. 17), consistent with the Allen Brain Atlas, and Gpr17 knockout mice (31), suggesting treatments targeting this receptor will have few off-target effects, at least in the brain. Third, there is reasonably strong support for Gpr17 regulating the differentiation in oligodendrocytes, though there are unresolved differences in the direction of this regulation between the genetic and pharmacologic studies (31, 39). Finally, Gpr17 only rarely shows deletions in the human GBM samples (Fig. 4A), suggesting that this pathway may be intact but inactive in the tumor environment.

First, to confirm that Gpr17 has a pattern consistent with a DorQ candidate, we examined the Gpr17 protein expression in the cortex. DorQ candidates should overlap with at least a subset of Pdgfra cells. Figure 5A shows that a subset (11.5%; n = 148) of Pdgfra::eGFP-L10a expressed Gpr17 protein. Next, to address the effects of Gpr17 activation on glioma cells, we counted formation of primary neurospheres grown from fresh murine glioma cells in EGF/bFGF containing media, with or without the Gpr17 agonist UDP (Fig. 5B). At 2 to 3 days after plating, the number of cells in treated and untreated cultures was similar, indicating that UDP treatment did not have apparent toxicity at the concentrations used. However, treatment with 10 or 50 μmol/L UDP resulted in reduced formation of glioma spheres, suggesting that activation of Gpr17 can affect both the proliferation and self-renewal of murine glioma cells (Fig. 5B and C). We further expanded our findings to include the effects of additional agonists of both classes for Gpr17; UDP-glucose (100 μmol/L) and leukotriene D4 (LTD4; 100 nmol/L) on primary glioma sphere formation (Fig. 5B and C). Although the magnitude of the effects of these agents were variable across tumors, with a decrease ranging anywhere from 15% to 60% in primary neurosphere formation, each of the above compounds had a significant effect, consistent with activation of Gpr17 decreasing the proliferation and/or changing the potential of the sphere forming (stem cell-like) glioma cells. In contrast, neither UDP nor LTD4 had significant effects on the number of spheres formed from wild-type mouse cortices, when they were passaged into agonist-containing media (data not shown).

Intriguingly, exposure to UDP also results in a dramatic increase in the relative number of Olig2-expressing cells in neurospheres (red, Fig. 5D). This suggests that activation of Gpr17 favored the selective survival and/or expansion of Olig2

---

**Figure 4.** Differentiation or quiescence candidates have a preponderance of deletions in human glioma. A, output of RAE analysis on 204 human glioma samples analyzed for the 24 DorQ candidates with clear human homologues. Twelve of 24 of the genes have at least 2 times more deletions than duplications, and 6 of 24 are deleted in more than a quarter of the GBM samples. On average, these genes have a ratio of single deletions to duplications of 2.6 (black line), which occurs in less than 5% of permuted cases (gray line).
cells in vitro and altered the overall self-renewal and proliferation capacity of the glioma cells.

Discussion

Human gliomas show remarkable heterogeneity in cell composition, activated pathways, and gene CNVs. This heterogeneity poses an enormous challenge in finding effective treatments for this highly fatal tumor. An increasing number of mouse models help elucidate the basic processes of tumor initiation, growth, and maintenance, as well as serve as preclinical trials for selective glioma treatments (40). In particular, there is a previously proposed diagnostic concept of "GBM with oligodendroglial component" (41) for the subset of GBM displaying some oligodendrogial or oligodendrogloma-like component by histology. The most likely transcriptomic correspondent to this histologic definition is the proneural GBM; in addition to the shared cellular features, both have been shown to be more responsive to treatment than GBM in general (1, 2, 42). The features of the mouse model used in this study are most similar to those of this human proneural type of glioma, such as overexpression of PDGF-B, loss of Ink4a/Arf/pten tumor suppressor genes, and it displays a similar histologic phenotype to human tumors (12). In this model, the tumor-initiating cell is a PDGF-B–producing Nestin-positive stem cell that can recruit OPCs into the tumor environment, OPCs which can then develop sufficient mutations to be independently tumorigenic (12). A different mouse model has shown that OPCs can also generate a tumor by the cell autonomous expression of a constitutively active EGF receptor (43), though TCGA data suggest that human proneural tumors are not generally characterized by increased EGF signaling (1, 2). Nonetheless, this does suggest that both cell autonomous and non-cell autonomous mechanisms are capable of initiating tumors derived largely of OPC-like cells, which may respond to prodifferentiation OPC signals.

Figure 5. GPR17 agonists changed cellular properties and decreased clonogenicity in primary glioma cells. A, confocal immunofluorescence for EGFP-L10a and Gpr17 confirms localization in a subset of OPCs. B, phase contrast images of primary murine glioma spheres treated with 0 (No Tx), 10, or 50 µmol/L UDP. UDP treatment reduced sphere formation. C, counts of primary neurosphere formation is consistently decreased by Gpr17 agonists UDP, UDP-glu, LTD4: *, P < 0.05; ***, P < 0.001, paired t test. D, olig2 staining of untreated (No Tx) or 10 µmol/L UDP-treated sectioned murine glioma neurospheres. Red, Olig2; blue, DAPI.
Here we showed that translational profile of Olig2-positive cells in our model closely resembles the profile of OPC cells, and that OPC transcripts are sufficient to distinguish the human proneural glioma subtype. This confirmed the results from a study that used similar glioblastoma model with the pten/p53 deletion (44). Our data also confirmed the expression of stem and progenitor cell markers, as well as high expression of genes involved in cell cycle, in Olig2-positive cells in glioblastoma, consistent with numerous studies of human tumors and mouse models (25, 45–50). Our study went a step further generating the list of candidate transcripts, deleted often in human gliomas that may serve to promote the differentiation or quiescence of Olig2 cells in the tumor. Of course, future work will need to be done to establish which of these candidates are bona fide prodifferentiation or quiescence factors.

Gene expression and transcriptome analysis could provide valuable information about potential cell-specific therapy targets, and the expression profile of glioma cells has been extensively studied (reviewed in ref. 51). However, these analyses were carried out either on glioma cell lines or using the total mRNA from the tumor mass. The pitfall of this approach is that glioma cell lines do not possess the physiologic characteristics of the tumor cells in vivo and analysis based on the total mRNA extracted from the tumor does not reflect the differences in expression profile of specific cell subpopulations. In addition, this approach does not identify the actively translated pool of mRNAs. It has been shown that translational regulation plays an important role in tumorigenesis (52, 53). Thus, this study is the first that used the bacTRAP methodology to analyze the actively translated mRNAs in a cell-specific manner within the tumor.

The prevalence of hemideletions for DorQ genes in many human glioma samples suggested that they might serve as targets for glioma therapy. Bone morphogenetic factor 4 (Bmp4) and 4 other DorQ genes (Kank1, Lim5, Sirt3, and Rassfl10) are frequently epigenetically silenced or deleted in other cancer types, and/or are suggested to function as tumor suppressors (54–57). Several DorQ candidates were already tested either in vivo or in vitro (36, 58, 59). When we tested the activation of Gpr17 signaling in the murine GBM cells in vitro, our results suggested that Gpr17 agonists might drive the differentiation of highly proliferative, uncommitted tumor cells toward oligodendrogial fates. Similarly, Bmp4 has been tested in animal model of glioma and human glioblastoma cells (36) as well as in medulloblastoma (60). Cells isolated from human glioblastomas treated with Bmp4 showed decreased proliferation and increased differentiation in vitro, whereas in vivo treatment with Bmp4 blocked the tumor growth. Finally, agonists of the retinoid receptor gamma Rarg promote differentiation in human neuroblastoma (37) and glioblastoma (58) cell lines and have already shown some efficacy as part of combination treatments for thyroid cancer (59). In addition, many of the candidate DorQ transcripts have been detected in other types of tumors (54–57, 60–66), suggesting these candidates may have some relevance beyond GBM.

Human glioma tumors contain an abundance of cells expressing markers of glial progenitors and stem cells, suggesting that tumorigenesis involves expansion of a glial progenitor–like cell (67). The comparison of the translational profiles of tumors to OPCs and normal mature cells of the neuronal, oligodendroglial, and astrocyte lineage supports this hypothesis. The analysis confirmed the similarities of tumor cells with the immature PDGFRA-positive cells from the normal brain. It was also suggested that OPCs might serve as source of gliomas (43, 44, 67–69). Although this study could not provide the information about cell-of-origin for gliomas, the approach would be useful if normal immature glial and stem-like cell profiles are compared with glioma cells at different time points during tumorigenesis. The mouse line generated for this study (Pdgfra-Egfp-L10a) will provide reproducible access to OPCs for future translational profiling. However, beyond applications to glioma, this line should permit reproducible in vivo access for researchers interested in studies of the basic biology of these cells both in the normal mouse brain and experimental models of OPC-relevant diseases, such as multiple sclerosis or white matter stroke.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J.D. Dougherty, E.I. Fomchenko, A. Milosevic
Development of methodology: E.I. Fomchenko, E. Bazzoli
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.D. Dougherty, E.I. Fomchenko, E. Schmidt, K.Y. Helmy, E. Bazzoli, E.C. Holland, A. Milosevic, A.A. Akuffo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.D. Dougherty, E.I. Fomchenko, C.W. Brennan, E.C. Holland, A.A. Akuffo
Writing, review, and/or revision of the manuscript: J.D. Dougherty, E.I. Fomchenko, A. Milosevic, A.A. Akuffo
Study supervision: A. Milosevic

Acknowledgments
The authors thank Victoria Isakova and Daviana Martinez Osorio for their technical assistance, Elizabeth Griggs for image processing, and N. Ramanan and members of the N. Henrietta laboratory for their advice and support and also thank the RU Genomics Resource Center and the MSKCC Flow Cytometry Resource Center.

Grant Support
This work was supported by the Tri-Institutional Stem Cell Initiative award to A. Milosevic and E.C. Holland, the NIH (1R01NS067239-03 to J.D. Dougherty) and the Adelson Medical Research Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 15, 2011; revised July 2, 2012; accepted July 8, 2012; published OnlineFirst August 3, 2012.

References
2. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of


www.aacrjournals.org


4867

Published OnlineFirst August 3, 2012; DOI:10.1158/0008-5472.CAN-11-2632

Candidate Pathways for Promoting Differentiation in Glioma

glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98–110.


