

In silico Molecular Target Validation Demonstrates Transforming Growth Factor Beta 2 is Strongly Expressed in Pediatric Diffuse Intrinsic Pontine Glioma and Glioblastoma Multiforme

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ABSTRACT

Aim: The purpose of the present study was to perform a comprehensive analysis of transforming growth factorbeta 2 (TGFβ2) gene expression in pediatric diffuse intrinsic pontine glioma (DIPG) and glioblastoma multiforme (GBM). Materials and Methods: We performed a meta-analysis of TGF^β2 gene expression for primary tumor specimens from 29 pediatric DIPG and 82 pediatric GBM (p-GBM) patients in the publicly available archived datasets GSE26576, GSE19578, GSE32374, GSE34824, and GSE49822. **Results:** Our data provide unprecedented evidence that TGF^β₂ is expressed at high levels in pediatric DIPG as well as p-GBM. Three TGFβ2 probe sets exhibited increased levels of expression in DIPG patients (n = 29): Mean fold difference for Probeset 228121 at was 2.48 (linear contrast $P = 3.40 \times 10^{-4}$); Probeset 220407 s at was 2.00 (linear contrast P = 0.006); and Probeset 209909 s at was 1.81 (linear contrast P = 0.0185). One of the TGFB2 probe sets was also exhibited the highest level of mean expression in DIPG patients; TGFB2 228121 at $(\text{mean} = 9.25 \pm 0.18 \log, \text{robust multiarray analysis [RMA]})$ and YAP1 224894 at $(\text{mean} = 8.55 \pm 0.19 \log, \text{RMA})$. Similar results were obtained for p-GBM patients (n = 82). Three probe sets for TGF β 2 significantly upregulated >2-fold in p-GBM patients (Probesets: 209909_s_at [fold change = 4.03, $P = 1.78 \times 10^{-5}$]; 228121_at [fold change = 3.57, $P = 8.81 \times 10^{-5}$]; and 220407 s at [fold change = 2.73, P = 0.0019]). The three probe sets with highest expression levels in p-GBM were TGFB2 228121 at (mean = 9.24 ± 0.15), TGFB3 209747 at (mean = 6.99 ± 0.086), and TGFB1 203085 s at (mean = 6.80 ± 0.16). Conclusions: Our findings indicate that TGFB2 gene product may serve as a target for immunotherapy in pediatric DIPG and GBM. Our findings also significantly expand the current knowledge of TGF β 2 expression in brain tumors and provide new evidence that TGF^β2 gene and its interactome are expressed in pediatric DIPG and GBM at significantly higher levels than in normal tissues or low-grade gliomas. This previously unknown differential expression profile uniquely indicates that TGF^β2 would be an attractive molecular target for the treatment of pediatric DIPG and GBM.

Key words: Chemotherapy resistance, diffuse intrinsic pontine glioma, glioblastoma, glioma, immunotherapy, transforming growth factor-beta 2 gene

INTRODUCTION

entral nervous system tumors including malignant brain and brainstem tumors are the most common solid tumors in children and adolescents up to the age of 19 years and the most common cause of cancer death.^[1-4] Despite modern aggressive multimodality therapy, the outcome for children with high-grade gliomas (HGGs) including glioblastoma multiforme (GBM) or diffuse intrinsic pontine glioma (DIPG) remains quite poor, and new therapeutic interventions are required to improve disease control.^[5-18]

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DIPG is a group of very aggressive brain tumors affecting the brainstem and representing the second most common malignant brain tumor of childhood.^[6,8,9] DIPGs are inoperable due to their anatomic location and almost invariably fatal.^[6,7] Radiation therapy (RT) provides only temporary symptom relief with no overall survival (OS) benefits.^[6] Conventional chemotherapy drugs, including temozolomide, are not effective and do not have an established role in the management of DIPG.^[7-9,14-17] Over the past decade, no significant progress has been made in the treatment of DIPG and the prognosis remains dismal, with a mean OS of 9-12 months from the time of diagnosis, a median survival time of approximately 10 months, and a 2-year OS rate of <10%.[6,8-17] Five-year survival is <3% and long-term survivors have evidence of substantial cognitive impairment, likely as a consequence of RT^[6] While GBM is the most common and lethal primary brain tumor in adults,^[1-4] it represents only a small minority of primary brain tumors in children.^[1,3] Like DIPG, pediatric GBM (p-GBM) is a devastating disease in children with substantial morbidity and mortality and no effective standard therapy.^[6] OS in p-GBM varies from 10 to 73 months and the median survival in p-GBM ranges from 13 to 73 months with a 5-year survival of <20%.[5,6,19] Furthermore, there is no standard treatment for recurrent or refractory (R/R) progressive DIPG or p-GBM after the failure of RT and no salvage regimen has been shown to extend survival.^[5-7,19] Therefore, there is an urgent need for therapeutic innovations for the treatment of R/R DIPG and p-GBM, as reflected by multiple treatment modalities being evaluated in early neurooncology clinical trials.[8-10,17-22]

The transforming growth factor (TGF)-beta superfamily comprises three isoforms, TGF-beta 1 (TGF\u00b31), TGF\u00b32, and TGFB3. TGFB binds to the serine/threonine kinase TGF β receptor 2 (TGF β R2), which leads to the formation of a heterotetrameric complex between the serine/threonine kinases TGFBR1 and TGFBR2.[23-29] The constitutive active TGFBR2 phosphorylates TGFBR1, which, in turn, recruits phosphorylates and activates Smad2 and Smad3 activating a Smad-dependent signal transduction pathway which results in activation of a signature transcriptome. $TGF\beta$ can also stimulate Smad-independent signaling through activation of the phosphatidylinositol 3-kinase-Akt pathway and other serine kinases. TGFB can promote tumor growth and metastasis by facilitating the epithelial-mesenchymal transition which supports tumor invasion and dissemination by releasing tumor cells into the surrounding environment and promoting their motility, chemoattraction, migration, and invasiveness [23,25,26,29] TGFB has been implicated in (i) treatment resistance to targeted therapeutics, chemotherapy as well as immune checkpoint inhibitors, (ii) metastasis, (iii) disease progression, and (iv) poor survival. TGFB expression in solid tumors has been associated with poor prognosis as well as resistance to RT, targeted therapeutics, and chemotherapy.^[23-29] In addition, TGF β controls the tumor microenvironment (TME) to restrain antitumor immunity by restricting T-cell infiltration and suppressing both the innate and adaptive immune system elements. TGF β -mediated inhibition of functional maturation of natural killer (NK) cells results in a high number of immature NK cells and eventually impaired recognition and clearance of tumor cells.^[30,31] TGF β also plays a role in tumor tolerance by recruiting Treg cells toward the primary tumor site as a means of immune evasion. Inhibition of TGF β 2 in tumor tissue has the potential to reverse the tumor-induced immune suppression while inhibiting tumor growth and invasion.

Amplified activity of TGFβ-Smad signaling pathway may contribute to the malignant phenotype and poor prognosis of GBM in adult patients in part by enhancing tumor growth, invasion, and angiogenesis. Glioblastoma-initiating cells (GICs) are shown responsible for the initiation and recurrence of tumors. Notably, TGFB induces the selfrenewal capacity of GICs.[32-34] Notably, GBM is characterized by a T-cell exhaustion signature and pronounced T-cell hyporesponsiveness of the TME and TGF β 2 has been implicated as a key contributor to the immunosuppressive landscape of TME in HGGs, especially during the later stages of disease progression. For the reasons detailed above, TGF β has emerged as an attractive target for the therapeutic intervention of glioblastoma.[35-47] OT101, a TGFB2-specific phosphorothioate antisense oligodeoxynucleotide (ASO), is a first-in-class RNA therapeutic designed to abrogate the immunosuppressive actions of TGF β 2. OT101 is intended to reduce the level of TGF β 2 protein in HGGs, including GBM, and thereby delay the progression of disease. OT101 has orphan drug status for the treatment of HGG and has been shown to (i) reduce TGF^{β2} production/secretion, (ii) inhibit proliferation as well as invasive migration, and (iii) enhance sensitivity to lymphokine-activated killer cell-mediated cytotoxicity of TGF_β2-expressing human HGG cells.^[18,19] The feasibility of intratumoral application of OT101 for the treatment of R/R HGG patients was first shown in Phase I clinical trials that also provided early activity signal patients.^[18,19] The preliminary findings of Phase II study NCT00431561 have provided the proof of concept that OT101 can be administered intratumorally through convection-enhanced delivery (CED) for up to 6 months with a favorable safety profile and results in early disease control at 6 months at a rate comparable to that achieved with temozolomide.^[20] OT101 exhibits clinically meaningful single-agent activity and induces durable complete and partial responses in R/R adult HGG patients including young adults with GBM or anaplastic astrocytomas.[20,48] Here, we provide unprecedented evidence that TGFB2 messenger RNA (mRNA), the molecular target of OT101, is expressed at very high levels in both pediatric DIPG and p-GBM patients. These in silico target validation data extend the clinical results on the therapeutic activity of OT101 in adults and young adults and further demonstrate the potential of OT101

as a promising immuno-oncology drug in the treatment of pediatric HGGs, an orphan disease with a low survival rate and no established or effective standard of care. OT101 may offer renewed hope for salvage therapy of DIPG and p-GBM patients who have a rare and fatal disease.

MATERIALS AND METHODS

DIPG dataset

We retrieved an archived transcriptome profiling dataset from the Human Genome U133 Plus 2.0 Array platform measuring gene expression levels in DIPG (n = 29), normal control samples (n = 2), and low-grade glioma (n = 6) samples (https://www.ncbi.nlm.nih.gov/geo/:GSE26576).^[49] Perfect match (PM) signal values for probe sets were extracted utilizing raw CEL files matched with probe identifiers obtained from the Affymetrix provided CDF file (HG-U133 Plus 2.cdf) implemented by Aroma Affymetrix statistical packages ran in RStudio environment (Version 0.99.902, RStudio Inc., running with R 3.3.1). PM signals were quantified using robust multiarray analysis (RMA) in a 3-step process including RMA background correction, quantile normalization, and summarization by median polish of probes in a probe set across 37 samples (RMA method adapted in Aroma Affymetrix). RMA background correction estimates the background by a mixture model, whereby the background signals were assumed to be normally distributed and the true signals are exponentially distributed. Normalization was achieved using a two-pass procedure. First, the empirical target distribution was estimated by averaging the (ordered) signals over all arrays followed by normalization of each array toward this target distribution. Expression values were log, transformed before statistical comparisons between normal, low-grade glioma, and DIPG samples.

p-GBM dataset

GBM dataset was developed from downloading raw Affymetrix CEL files created on the Human Genome U133 Plus 2.0 Array platform by compiling gene chip files from four studies: GSE19578,^[50,51] GSE32374,^[52] GSE34824,^[53] and GSE49822.^[54] PM signals were quantified using RMA in a 3-step process including RMA background correction, quantile normalization, and summarization by median polish of probes in a probe set across 116 samples (n = 53, 21, 27, and 15 for GSE19578, GSE32374, GSE34824, and GSE49822, respectively). From this dataset, we evaluated cases from pediatric/congenital GBM (n = 82), adult GBM (n = 6), and anaplastic oligodendroglioma (AO) cases (n = 5) (for control comparison). Twenty-three samples not belonging to these groups were not considered in the analysis.

Bioinformatic analyses

Protein-protein interaction networks were constructed using STRING10 algorithm (http://string-db.org/) to identify proteins connected to TGF β 2 pathways. In these diagrams,

the nodes depicted protein identifiers. Networks were visualized by first seeding the inputs: TGF^β2, TGF^βR2, TGFBR1, and SMAD2 and then growing the networks to identify connecting hubs between the inputs resulting in identification of 24 nodes: TGFB 3, SMAD7, SMAD2, CREBBP, SMURF2, YAP1, APP, RNF111, SMAD6, SMAD1, SMAD3, SMAD4, CTNNB1, XIAP, TGFβR2, SMURF1, TGF^β2, TGF^βR1, FKBP1A, ACVRL1, CAMK2A, NEDD4L, and ACVR1B. The edges indicated >90% confidence level for the association calculated from various lines of experimental evidence (co-occurrence, coexpression, experiments, and databases). Clustering was performed on the association scores to group protein-protein interaction networks ("K-means with 2 clusters" algorithm provided by the software). Solid lines represented connections within clusters and dotted line connections between clusters. These protein interaction networks were utilized to determine differences in gene expression of probe sets and their error distributions to calculate P-values between normal controls and DIPG patients, and p-GBM versus AO control cases.

We constructed a three-factor mixed model analysis of variance (ANOVA) to compare the expression of TGF β 2 interactome in DIPG patients or TGFB 1/2/3 probe sets in p-GBM comparisons that were extracted from the RMA normalized databases. Two fixed factors were considered in this model: One factor was the expression of probe sets (n = 87 for TGF $\beta 2$ interactome for DIPG comparisons or n = 9 for TGF β 1/2/3 probe sets for p-GBM comparisons), the second factor was comparison of patient subgroups (DIPG vs. normal samples; p-GBM/adult GBM vs. AO control cases) and one interaction term for probe set x patient subgroup. We included a random factor for each sample to correct for multiple measurements taken from each patient and accounting for variation between patients partitioned as a separate variance component. Least squares method was used fit the parameters for these mixed ANOVA models (R packages lme4 v 1.1-12 and lmer test v2.1-32). The root-mean-square error term calculated from the deviation of data points from the model was utilized to determine significant changes in probe set expression in the planned linear contrasts. These contrasts compared the effect sizes (expressed as difference in log, transformed RMA normalized values converted to fold change relative to normal samples) such that the combination of linear parameters to be jointly tested sum to zero for each level of the contrast. Least square mean values were used to construct contrasts between patient subgroups (set to -1) with the comparison group (set to 1) for each of the probe sets by taking the effect sizes and standard error of mean calculated the interaction terms of the model. Two-tailed tests for differences between the least square means with P < 0.05 were deemed statistically significant after controlling for the false discovery rate.

We used a two-way agglomerative hierarchical clustering technique to organize expression of patient samples mean centered to normal samples using the average distance linkage method such that probe sets (rows) having similar expression across patients were grouped together (average distance metric). Dendrograms were drawn to illustrate similar gene expression profiles from joining pairs of closely related gene expression profiles, whereby genes joined by short branch lengths showed the most similarity in expression profile across all samples (R package gplots 3.0.1 were utilized for cluster representations).

RESULTS

Expression of TGF $\beta 2$ and TGF $\beta 2$ interactome in pediatric DIPG

Known protein-protein interaction networks were data mined from the STRING10 database deploying a connecting algorithm that identified 24 nodes with confidence >90% from experimental evidence [Figure 1]. These 24 proteins were represented by 87 probe sets used to interrogate expression levels in DIPG patients compared to normal samples (GSE26576).

Three TGF β 2 probe sets exhibited increased levels of expression in DIPG patients [Figure 2]: Mean fold difference for Probeset 228121_at was 2.48 (linear contrast $P = 3.40 \times 10^{-4}$); Probeset 220407_s_at was 2.00 (linear contrast P = 0.006); and Probeset 209909_s_at was 1.81 (linear contrast P = 0.0185). One of the TGF β 2 probe sets was also exhibited the highest level of mean expression in DIPG patients; TGFB2_228121_at (mean = $9.25 \pm 0.18 \log_2$ RMA) and YAP1_224894_at (mean = $8.55 \pm 0.19 \log_2$ RMA) [Figure 1S].

Expression of TGF β 2 interactome in p-GBM

Similar results were obtained for p-GBM patients [Figure 3]. Three probe sets for TGF β 2 significantly upregulated >2-fold in p-GBM patients (Probesets: 209909_s_at [fold change = 4.03, $P = 1.78 \times 10^{-5}$]; 228121_at [fold change = 3.57, $P = 8.81 \times 10^{-5}$]; and 220407_s_at [fold change = 2.73, P = 0.0019]). The three probe sets with highest expression levels in p-GBM were TGFB2_228121_at (mean = 9.24 ± 0.15), TGFB3_209747_at (mean = 6.99 ± 0.086), and TGFB1_203085_s_at (mean = 6.80 ± 0.16) [Figure 2S].

DISCUSSION

TGF β inhibition is currently being investigated as a treatment option in patients with advanced metastatic cancer.^[46,47,55] The signaling of TGF β can be modulated through several different strategies, including but not limited to using ASO that blocks TGF β an mRNA (e.g., OT101/Trabedersen, using monoclonal antibodies to block TGF β isoforms (lerdelimumab and metelimumab) or using small molecule inhibitors of TGF β R.

Galunisertib (LY2157299 monohydrate; Lilly), the first-inclass selective TGF β RI kinase inhibitor is currently under



Figure 1: Transforming growth factor-beta 2 (TGFβ2) interactome. Protein-protein interaction networks were constructed using STRING10 algorithm (http://string-db.org/) to identify proteins connected to TGFβ2 pathways. In these diagrams, the nodes depicted protein identifiers. Networks were visualized by first seeding the inputs: TGFB2, TGFBR2, TGFBR1, and SMAD2 and then growing the networks to identify connecting hubs between the inputs resulting in identification of 24 nodes depicted in the network diagram



Figure 2: (a and b) Expression of transforming growth factor-beta 2 (TGF β 2) interactome represented probe sets in pediatric diffuse intrinsic pontine glioma (DIPG) patients compared to normal samples. The log₂ transformed fold difference values for each subject (columns [*n* = 29]) and each probe set from the GEO archived dataset GSE26576 are depicted for DIPG patients mean centered to normal samples (*n* = 2). The subjects and probe sets were organized using a two-way clustering algorithm using the average distance metric to determine coregulation of all the probe sets across patients and all patients across probe sets. The heatmap depicts the most significantly up- and down-regulated probe sets ranging from red to blue represent expression greater than normal to lower than normal in DIPG samples, respectively. Fold difference and linear contrast *P*-values are depicted in the table showing three probe sets for TGF β 2 upregulated in DIPG patients

clinical development for a variety of cancers both as a single agent and in combination with immune checkpoint inhibitors nivolumab (NCT02423343) and durvalumab (NCT02734160).^[56] The anti-programmed death-ligand 1 (PD-L1)/TGFB fusion protein M7824 (bintrafusp alfa) (Merck KGaA/Glaxosmithkline) is a first-in-class bifunctional fusion protein comprising a fully human monoclonal antibody against (PD-L1, Avelumab), fused to the extracellular domain of human TGFBR2, which functions as a TGFB "trap."^[57,58] Results from the Phase 1 trial of M7824 (NCT02517398) in metastatic or locally advanced solid tumors provided preliminary evidence of clinical activity. M7824 is being evaluated in the Phase 2 study NCT03631706. SAR439459 (Sanofi) is a neutralizing anti-TGF monoclonal antibody. It blocks the immunosuppressive effects of TGF and inhibits the growth of syngeneic tumors in combination with anti-PD1 and it is being evaluated alone and in combination with anti-PD1 therapy in the early oncology studies CT03192345 and NCT03192345. Anti-TGFB Mab NIS793 (Novartis/ Xoma) is being evaluated in combination with anti-PD1 in NCT02947165. TGFβ-inactivating Mab ARGX-115/ABBV-151 (Argenx/Abbvie) is being evaluated in combination with anti-PD1 ABBV-181 in NCT03821935. TGFB1 inhibitor PF-06952229 (Pfizer) is being evaluated in NCT03685591. TGF_β inhibitor Vactosertib (Medpacto) is being evaluated in combinations with anti-PD1 in NCT03724851 and NCT03732274. AVID200 is a rationally designed and highly potent TGFB 1 and 3 inhibitors. TGFB inhibitor AVID200 (formation biologics) is being evaluated in NCT03834662.

ASOs are short strings of DNA that is designed to downregulate gene expression by interfering with the translation of a specific encoded protein at the mRNA level. Several ASOs have been approved by the FDA, including fomivirsen (indication: Cytomegalovirus retinitis), mipomersen (indication: Familial hypercholesterolemia), eteplirsen (indication: Duchenne muscular dystrophy), and nusinersen (indication: Spinal muscular atrophy) to name a few.^[59] OT101 is a synthetic 18-mer phosphorothioate oligodeoxynucleotide (S-ODN) where all 3'-5' linkages are modified to phosphorothioates. The molecular formula is C177H208N60Na17O94P17S17 and the molecular weight 6143 g/mol. OT101 was designed to be complementary to a specific sequence of human TGFβ2 mRNA following expression of the gene. OT101 is administered through continuous infusion over 7 days, 2 cycles/month \times 6 months, by high-flow microperfusion with an intratumoral catheter using a CED system. Systemic administration of anticancer drugs is often hindered by the blood-brain barrier (BBB), and even drugs that successfully cross the barrier may suffer from unpredictable distributions. CED is a strategy that bypasses BBB entirely and enhances drug distribution by applying hydraulic pressure to deliver agents directly and evenly into a target region. This technique reliably distributes the drug homogeneously through the interstitial space of the target region and achieves high local drug concentrations in the brain.[11,12,19,60,61] Clinical trials involving CED for supratentorial high-grade glial tumors, including Phase II trial of OT101 in adults with R/R HGGs,



p-GBM: Pediatric glioblastoma multiforme

Figure 3: (a and b) Expression of transforming growth factor-beta (TGF β) 1/2/3 probe sets in pediatric glioblastoma multiforme (p-GBM) patients compared to anaplastic oligodendroglioma patients (control group). The log₂ transformed fold difference values for each subject (columns [*n* = 82]) and each of the nine probe sets from four GEO archived datasets (GSE19578 [*n* = 25], GSE32374 [*n* = 15], GSE34824 [*n* = 27], and GSE49822 [*n* = 15]) are depicted for p-GBM patients mean centered to control samples (*n* = 5; from GSE19578). The subjects and probe sets were organized using a two-way clustering algorithm using the average distance metric to determine coregulation of all the probe sets across patients and all patients across probe sets. The heatmap depicts expression changes for each probe set ranging from red to blue representing expression greater than control to lower than control in p-GBM samples, respectively. Fold difference and linear contrast *P*-values are depicted in the table showing three probe sets for TGF β 2 significantly upregulated >2-fold in p-GBM patients (Probesets: 209909_s_at [fold change = 4.03, *P* = 1.78 × 10⁻⁵]; 228121_at [fold change = 3.57, *P* = 8.81 × 10⁻⁵]; and 220407_s_at [fold change = 2.73, *P* = 0.0019])

have demonstrated the feasibility of intratumor catheter placement and intratumor drug administration for the treatment of brain tumors. More recently, CED catheters have also been placed into the brainstem for the treatment of DIPG (e.g., NCT01502917).^[12] CED is an attractive means of delivering therapeutics to DIPG tumors, as it bypasses BBB and allows for the treatment of a relatively large volume of tissue with small amount of infusate.

Our results presented herein demonstrate that TGF β 2 mRNA, the molecular target of OT101, is expressed at very high levels in both pediatric DIPG and p-GBM patients. These *in silico* target validation data extend the clinical results on the therapeutic activity of OT101 in adults and young adults and further demonstrate the potential of OT101 as a promising immuno-oncology drug in the treatment of pediatric HGGs, an orphan disease with a low survival rate and no established

or effective standard of care. OT101 may offer renewed hope for salvage therapy of DIPG and p-GBM patients who have a rare and fatal disease.

CONCLUSIONS

Our findings indicate that TGF β 2 gene product may serve as a target for immunotherapy in pediatric DIPG and GBM. Our findings also significantly expand the current knowledge of TGF β 2 expression in brain tumors and provide new evidence that TGF β 2 gene and its interactome are expressed in pediatric DIPG and GBM at significantly higher levels than in normal tissues or low-grade gliomas. This previously unknown differential expression profile uniquely indicates that TGF β 2 would be an attractive molecular target for the treatment of pediatric DIPG and GBM.

DECLARATIONS, ETHICS APPROVAL, AND CONSENT

Not applicable for this study which utilized *in silico* analyses on archived publicly available datasets.

Competing interest

LH and VT are listed as inventors on patents and patent applications related to OT101. FMU, LH, DN, and VT are employees and SQ is a consultant of Oncotelic/Mateon, a company commercializing a TGF-directed RNA therapeutic. FMU, LH, and VT are shareholders of Mateon. Due to these financial interests in Oncotelic/Mateon, there is a potential conflict of interest in relation to this article. We have made every effort to have a balanced discussion of results with references to other anti-TGF technology platforms to constructively address this potential conflict of interest.

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AUTHORS' CONTRIBUTIONS

FMU and SQ have contributed to the analysis of data. FMU designed the evaluations reported in this paper, directed the data compilation and analysis, and wrote the first draft of the manuscript. All authors reviewed and revised the manuscript, were involved in data interpretation, provided final approval, and made the decision to submit the manuscript for publication. No medical writing support was provided by a professional medical writer.

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SUPPLEMENTAL FIGURES

Figure 1S: Upregulated transforming growth factor-beta 2 (TGF β 2) interactome probe sets in diffuse intrinsic pontine glioma (DIPG) patients. The box and whisker plot depicts the log₂ transformed normalized expression values of the transcripts for TGF β 2 interactome in DIPG (*n* = 29), normal (*n* = 2), and low-grade glioma (*n* = 6). The black line in the box indicates median expression for each probe set, the box indicates the lower and upper quartiles (25% and 75%) of the expression levels, and the whisker depicts 1.5 times the interquartile range above the upper quartile and below the lower quartile. Data points outside the whisker suggest potential outliers in the data. Mean expression level is the joined blue circles. Probe sets are arranged according to the highest mean levels of expression of probe sets for DIPG patients for upregulated transcripts. The highest mean levels of expression for probe sets in DIPG patients were represented by TGFB2_228121_at (mean = 9.25 ± 0.18 log₂ robust multiarray analysis [RMA]); YAP1_224894_at (mean = 8.55 ± 0.19 log₂ RMA); and SMAD4_202527_s_at (mean = 7.54 ± 0.098 log₂ RMA)



Figure 2S: Upregulated transforming growth factor-beta 2 (TGF β 2) 1/2/3 probe sets in glioblastoma multiforme (GBM) patients compared to anaplastic oligodendroglioma control samples. The box and whisker plot depicts the log₂ transformed normalized expression values of the transcripts for TGF β 1/2/3 in control (*n* = 5), pediatric GBM (p-GBM) (*n* = 82), and adult GBM (*n* = 6). The black line in the box indicates median expression for each probe set, the box indicates the lower and upper quartiles (25% and 75%) of the expression levels, and the whisker depicts 1.5 times the interquartile range above the upper quartile and below the lower quartile. Data points outside the whisker suggest potential outliers in the data. Mean expression level is the joined blue circles. Probe sets are arranged according to the highest mean levels of expression of probe sets for TGF β 2 for upregulated transcripts in p-GBM patients. The three highest expressing probe sets in p-GBM were TGFB2_228121_at (mean = 9.24 ± 0.15), TGFB3_209747_at (mean = 6.99 ± 0.086), and TGFB1_203085_s_at (mean = 6.80 ± 0.16)