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 factor-beta 2 (TGFβ2) gene expression in pediatric diffuse intrinsic pontine glioma (DIPG) and glioblastoma multiforme (GBM). Materials and Methods: We performed a meta-analysis of TGFβ2 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 TGFβ2 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 TGFβ2 probe sets was also exhibited the highest level of mean expression in DIPG patients; TGFB2_228121_at (mean = 9.25 ± 0.18 log2 robust multiarray analysis [RMA]) and YAP1_224894_at (mean = 8.55 ± 0.19 log2 RMA). Similar results were obtained for p-GBM patients (n = 82). Three probe sets for TGFβ2 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 TGFβ2 gene product may serve as a target for immunotherapy in pediatric DIPG and GBM. Our findings also significantly expand the current knowledge of TGFβ2 expression in brain tumors and provide new evidence that TGFβ2 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 TGFβ2 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

Central 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]
DIPG is a group of very aggressive brain tumors affecting the brainstem and representing the second most common malignant brain tumor of childhood. DIPGs are inoperable due to their anatomic location and almost invariably fatal. Radiation therapy (RT) provides only temporary symptom relief with no overall survival (OS) benefits. Conventional chemotherapy drugs, including temozolomide, are not effective and do not have an established role in the management of DIPG. 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%. Five-year survival is <3% and long-term survivors have evidence of substantial cognitive impairment, likely as a consequence of RT. While GBM is the most common and lethal primary brain tumor in adults, it represents only a small minority of primary brain tumors in children. Like DIPG, pediatric GBM (p-GBM) is a devastating disease in children with substantial morbidity and mortality and no effective standard therapy. 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%. 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. 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 neuro-oncology clinical trials.

The transforming growth factor (TGF)-beta superfamily comprises three isoforms, TGF-beta 1 (TGFβ1), TGFβ2, and TGFβ3. TGFβ binds to the serine/threonine kinase TGFβ receptor 2 (TGFβR2), which leads to the formation of a heterotetrameric complex between the serine/threonine kinases TGFβR1 and TGFβR2. The constitutive active TGFβR2 phospholyses TGFβR1, which, in turn, recruits phospholyses and activates Smad2 and Smad3 activating a Smad-dependent signal transduction pathway which results in activation of a signature transcriptome. TGFβ can also stimulate Smad-independent signaling through activation of the phosphatidylinositol 3-kinase-Akt pathway and other serine kinases. TGFβ 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, chemotraction, migration, and invasiveness. TGFβ 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. TGFβ expression in solid tumors has been associated with poor prognosis as well as resistance to RT, targeted therapeutics, and chemotherapy. In addition, TGFβ controls the tumor microenvironment (TME) to restrain antitumor immunity by restricting T-cell infiltration and suppressing both the innate and adaptive immune system elements. TGFβ-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. TGFβ also plays a role in tumor tolerance by recruiting Treg cells toward the primary tumor site as a means of immune evasion. Inhibition of TGFβ2 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, TGFβ induces the self-renewal capacity of GICs. Notably, GBM is characterized by a T-cell exhaustion signature and pronounced T-cell hyporesponsiveness of the TME and TGFβ2 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, TGFβ has emerged as an attractive target for the therapeutic intervention of glioblastoma. OT101, a TGFβ2-specific phosphorothioate antisense oligodeoxyxynucleotide (ASO), is a first-in-class RNA therapeutic designed to abrogate the immunosuppressive actions of TGFβ2. OT101 is intended to reduce the level of TGFβ2 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 TGFβ2 production/secretion, (ii) inhibit proliferation as well as invasive migration, and (iii) enhance sensitivity to lymphokine-activated killer cell-mediated cytotoxicity of TGFβ2-expressing human HGG cells. 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. 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. 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 and anaplastic astrocytomas. Here, we provide unprecedented evidence that TGFβ2 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](https://www.ncbi.nlm.nih.gov/geo/: GSE26576)). 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 $\log_2$ 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 (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 $\log_2$ transformed before statistical comparisons between normal, low-grade glioma, and DIPG samples.

**Bioinformatic analyses**

Protein-protein interaction networks were constructed using STRING10 algorithm ([http://string-db.org/](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: TGFβ2, TGFβR2, TGFβR1, and SMAD2 and then growing the networks to identify connecting hubs between the inputs resulting in identification of 24 nodes: TGFβ 3, SMAD7, SMAD2, CREBBP, SMURF2, YAP1, APP, RNF111, SMAD6, SMAD1, SMAD3, SMAD4, CTNNB1, XIAP, TGFβR2, SMURF1, TGFβ2, TGFβR1, FKBP1A, ACVR1L, 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 TGFβ2 interactome in DIPG patients or TGFβ 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 TGFβ2 interactome for DIPG comparisons or $n = 9$ for TGFβ 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 $\log_2$ 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 TGFβ2 and TGFβ2 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 TGFβ2 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 TGFβ2 probe sets was also exhibited the highest level of mean expression in DIPG patients; TGFB2_228121 at (mean = 9.25 ± 0.18 log2 RMA) and YAP1_224894 at (mean = 8.55 ± 0.19 log2 RMA) [Figure 1S].

Expression of TGFβ2 interactome in p-GBM

Similar results were obtained for p-GBM patients [Figure 3]. Three probe sets for TGFβ2 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^{-3} \]); 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

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

Galunisertib (LY2157299 monohydrate; Lilly), the first-in-class selective TGFβRI 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: TGFβ2, TGFβRI, TGFβR1, 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.](image)
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)/TGFβ 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 TGFβR2, which functions as a TGFβ “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-TGFβ 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. TGFβ1 inhibitor PF-06952229 (Pfizer) is being evaluated in NCT03685591. TGFβ inhibitor Vactosertib (Medpacto) is being evaluated in combinations with anti-PD1 in NCT03724851 and NCT03732274. AVID200 is a rationally designed and highly potent TGFβ 1 and 3 inhibitors. TGFβ 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 was designed to be complementary to a specific sequence of human TGFβ2 mRNA following expression of the gene. OT101 was administered through continuous infusion over 7 days, 2 cycles/month × 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,
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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 TGFβ2 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 TGFβ2 gene product may serve as a target for immunotherapy in pediatric DIPG and GBM. Our findings also significantly expand the current knowledge of TGFβ2 expression in brain tumors and provide new evidence that TGFβ2 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 TGFβ2 would be an attractive molecular target for the treatment of pediatric DIPG and GBM.

**Figure 3:** (a and b) Expression of transforming growth factor-beta (TGFβ) 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 TGFβ2 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 220406_s_at [fold change = 2.73, \( P = 0.0019 \)]).

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p-GBM: Pediatric glioblastoma multiforme
**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 with references to other anti-TGF technology platforms to constructively address this potential conflict of interest.

**FUNDING INFORMATION**

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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.

**REFERENCES**


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SUPPLEMENTAL FIGURES

Figure 1S: Upregulated transforming growth factor-beta 2 (TGFβ2) 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 TGFβ2 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 TGF82_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 (TGFβ2) 1/2/3 probe sets in glioblastoma multiforme (GBM) patients compared to anaplastic oligodendroglioma control samples. The box and whisker plot depicts the log_2 transformed normalized expression values of the transcripts for TGFβ1/2/3 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 TGFβ2 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)