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ORIGINAL REPORT

Whole-Genome Profiling of Pediatric Diffuse Intrinsic Pontine Gliomas Highlights Platelet-Derived Growth Factor Receptor α and Poly (ADP-ribose) Polymerase As Potential Therapeutic Targets

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ABSTRACT

Purpose
Diffuse intrinsic pontine glioma (DIPG) is one of the most devastating of pediatric malignancies and one for which no effective therapy exists. A major contributor to the failure of therapeutic trials is the assumption that biologic properties of brainstem tumors in children are identical to cerebral high-grade gliomas of adults. A better understanding of the biology of DIPG itself is needed in order to develop agents targeted more specifically to these children's disease. Herein, we address this lack of knowledge by performing the first high-resolution single nucleotide polymorphism (SNP) –based DNA microarray analysis of a series of DIPGs.

Patients and Methods
Eleven samples (nine postmortem and two pretreatment surgical samples), the largest series thus far examined, were hybridized to SNP arrays (250 k or 6.0). The study was approved by the research ethics board at our institution. All array findings were validated using quantitative polymerase chain reaction, fluorescence in situ hybridization, immunohistochemistry, and/or microsatellite analysis.

Results
Analysis of DIPG copy number alterations showed recurrent changes distinct from those of pediatric supratentorial high-grade astrocytomas. Thirty-six percent of DIPGs had gains in platelet-derived growth factor receptor alpha (*PDGFRA*; 4 to 18 copies) and all showed PDGFR-α expression. Low-level gains in poly (ADP-ribose) polymerase (*PARP) -1* were identified in three cases. Pathway analysis revealed genes with loss of heterozygosity were enriched for DNA repair pathways.

Conclusion
To our knowledge, our data provides the first, comprehensive high-resolution genomic analysis of pediatric DIPG. Our findings of recurrent involvement of the PDGFR pathway as well as defects in DNA repair pathways coupled with gain of *PARP-1* highlight two potential, biologically based, therapeutic targets directed specifically at this devastating disease.

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INTRODUCTION

Brain tumors account for 20% of all neoplasms in children, and are the largest group of solid tumors that develop in childhood.[1] Among the most devastating of brain tumors are gliomas that diffusely involve the brainstem—diffuse intrinsic pontine gliomas (DIPG). These tumors appear almost exclusively during childhood and adolescence and their prognosis is poor. The median survival is shorter than 1 year, and fewer than 10% of patients survive for longer than 2 years.[2,3] Despite collaborative efforts to improve treatments, survival has remained static over the past 20 years, and DIPGs are now the main cause of brain tumor–related death in children.[4]

Substantial advances have been made in the clinical definition of this entity, which is based on a combination of neurological signs, duration of symptoms, and specific magnetic resonance imaging findings. A tissue biopsy sample is not needed for diagnosis, and most children are treated within days of the diagnostic magnetic resonance imaging scan. Radiation offers only symptom control. So far chemotherapy has shown no benefit despite many trials over the past several decades.[4]

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A major contributor to the failure of most of these therapeutic trials is the assumption that biologic properties of brainstem tumors in children are identical to cerebral high-grade gliomas of adults.[5] Thus, current clinical trials employ chemotherapeutic and other biologic agents investigated in trials of adult gliomas which, unfortunately, are not sufficiently similar to DIPGs or even to cerebral high-grade gliomas of children.[6] Generation of biologic studies of DIPG is hampered by the current practice of not obtaining tissue for diagnosis. However, a better understanding of the biology of DIPG itself is needed in order to develop agents targeted more specifically to these children's disease.

To address this lack of knowledge about the biology of pediatric DIPG, and the lack of availability of surgical material to study, we prospectively collected postmortem tumor and matched normal brain samples from a series of these patients. We also retrieved two pretreatment, surgically obtained specimens from our pathology archives, giving us a total of eleven samples. We then performed high-resolution single nucleotide polymorphism (SNP) –based DNA microarray analysis on these samples, the largest series thus examined. Using this approach, we have identified regions of copy number alterations (CNA) found in other studies of adult and pediatric high-grade astrocytomas as well as numerous, novel high frequency gains and losses and regions of loss of heterozygosity (LOH). In particular, we identified frequent gains in platelet-derived growth factor receptor (PDGFR) pathway members raising this as a potential therapeutic target. Further, we identified gains in Poly (ADP-ribose) polymerase (*PARP) -1*, which, coupled with defects in other DNA repair pathways, raises PARP inhibition as another novel therapeutic target for some pediatric DIPGs. The data reported here increases our understanding of the biology of DIPG and will allow the development of more targeted therapies for this devastating disease.

PATIENTS AND METHODS

Patients and Samples

DIPG patients. Between 2003 and 2007, consent was obtained for postmortem examination of nine DIPG patients who presented to the Hospital for Sick Children during this period (DIPG1-9). As soon as possible after death (median post mortem interval 16 hours; range, 9 to 40 hours), the brain was removed and tumor and normal brain (frontal lobe) samples were snap frozen in liquid nitrogen. Two pretreatment, frozen, biopsy samples were also retrieved from our pathology archives (DIPG10 and 11). Sections were cut from all samples to evaluate the amount of tumor present; this ranged from 70 to more than 90% with other tissue comprising infiltrated brain. The study was approved by the research ethics board at our institution.

All samples were examined histologically by two neuropathologists and were diagnosed as either glioblastoma (DIPG3-11) or primitive neuroectodermal tumor (PNET) -like (DIPG1-2). The latter lacked glial differentiation both morphologically and immunohistochemically (glial fibrillary acidic protein-negative). Likewise, they did not mark with more mature neuronal markers (synaptophysin or NeuN), thus the designation as primitive. Table 1 summarizes the clinical information for this patient cohort. The two surgically obtained archival specimens (patients 10 and 11) were from patients who, although presenting with classical symptoms, had atypical imaging characteristics and thus were biopsied before other interventions.

Supratentorial high-grade astrocytoma patients. A cohort of 11 patients with high-grade astrocytoma (HGA; seven WHO grade 4 and four WHO grade 3) and available frozen material was used as a comparison group for the DIPG cohort. The mean age was 7.6 years (range, 0.12 to 16.3 years). Eight patients were dead of their disease (mean survival, 1.3 years; range, 0.07 to 2.5 years) and mean follow-up was 1.3 years in survivors.

Array Hybridization

The Human Mapping 250 SNP Nsp Array (nine samples) and the Genome-Wide Human SNP Array 6.0 (two samples) from Affymetrix (Santa Clara, CA) were used as the platforms for this study. DNA digestion, labeling and hybridization were performed according to the manufacturer's instructions by The Centre for Applied Genomics at our institution. Quality control call rates ranged from 91% to 98% and did not differ significantly between the surgical and postmortem samples, nor did the mean number of SNPs gained/lost, nor the number of regions gained/lost, although the standard deviations were large.

Raw Data Analysis

CEL data were analyzed for CNAs using Partek Genomics Suite version 6.4 (Partek Incorporated, St Louis, MO) and Genotyping Console 2.1 (GC2.1; Affymetrix). The genomic segmentation tool available from Partek Genomics Suite was used with default parameters and cross-referenced with results generated from GC2.1. Only genes with CNAs reported by both algorithms were included in the results.

Table 1. Patient Information

Patient No.	Sex	Age at Dx (years)	OS (months)	Symptoms	Symptom Duration (days)	TMZ		Other Chemotherapy
						During RT	Post RT	
1*	Female	5.1	4.1	LTS, CN	1	No	No	
2*	Female	0	0.39	LTS, CN	4	No	No	
3	Male	5.14	11.51	LTS, ataxia, CN	≤ 180	No	No	Nimotuzumab†
4	Male	10.64	10.16	LTS, ataxia, CN	52	Yes	Yes	
5	Female	8.96	11.64	Ataxia, CN	60	Yes	Yes	Tipifarnib†
6	Male	7.17	6	Ataxia, CN	60	No	Yes	
7	Male	4.98	6.49	LTS, ataxia, CN	2	Yes	No	
8	Female	5.86	4.92	CN	42	Yes	Yes	
9	Male	9.76	10.26	LTS, ataxia, CN	90	Yes	Yes	Nimotuzumab†
10	Male	7.6	2.82	CN	14	No	No	
11	Male	1.66	‡	LTS, ataxia, CN	30	No	No	99703 then focal RT§

Abbreviations: Dx, diagnosis; OS, overall survival; TMZ, temozolomide; RT, radiotherapy; LTS, long-track signs; CN, cranial nerve deficits; 99703, a pilot study of intensive chemotherapy with peripheral stem cell support for infants with malignant brain tumors, Children's Oncology Group.

*Primitive neuroectodermal tumor–like histology, see text for details.

†Treatment at progression.

‡Patient 11 is alive 45 months after diagnosis.

§Treatment at initial diagnosis.

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For loss of heterozygosity (LOH) analysis, raw data was imported to GC2.1 where genotype calling was performed using the Bayesian Robust Linear Modeling using Mahalanobis Distance genotyping algorithm[7] designed for the Human Mapping 500K Array Set. Genotype files were then transferred into Partek 6.4 to obtain LOH calls using default settings.

For both CNA and LOH analyses, where possible, tumors were compared to their respective normal samples while the unpaired samples were normalized to the 270 HapMap samples.

Microsatellite Analysis

Microsatellite markers within and/or flanking *RAD51L1, TCF7L2,* and *MGMT* were used to validate LOH. Details of the microsatellite markers and data analysis are given in Appendix (online only).

Immunohistochemistry

Immunohistochemical staining of paraffin-embedded material was done using standard techniques.[8] Details of the antibody dilutions and sources are given in Appendix (online only).

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) analysis was done on an ABI Step One Plus RT-PCR (Applied Biosystem, Foster City, CA) system using the Qiagen QuantiTect SYBR green PCR mix (Qiagen, Mississauga, Ontario, Canada). Details of primers and controls are given in Appendix (online only).

RESULTS

Genomic Alterations in Pediatric DIPGs Suggest Biologic Differences From Pediatric Supratentorial HGAs

Copy number analysis revealed frequent and multiple CNAs across the DIPG genomes, including the pretreatment surgical specimens (DIPG10 and 11; Fig 1, upper panel). There were numerous recurrent CNAs with multiple overlapping regions identified in more than 30% of cases. As a comparison group, we examined a set of pediatric supratentorial HGAs using the same technique (Fig 1, lower panel). Interestingly, while some overlap is present, the DIPGs show clear differences in the regions of CNA from the HGAs (Fig 2), highlighting their distinct genetic characteristics.

Both the DIPGs and HGAs show large recurrent regions of loss in 13q. However, HGAs show more frequent loss of 9p and 4q while DIPGs show more frequent loss of 11p, 17p, 14q, 18p, and 22q. The region of loss on 17p includes *TP53* which was hemizygously deleted in seven (64%) of 11 DIPGs (Table 2), in contrast to only two HGAs. 17p loss was also reported in a previous study of seven DIPG samples

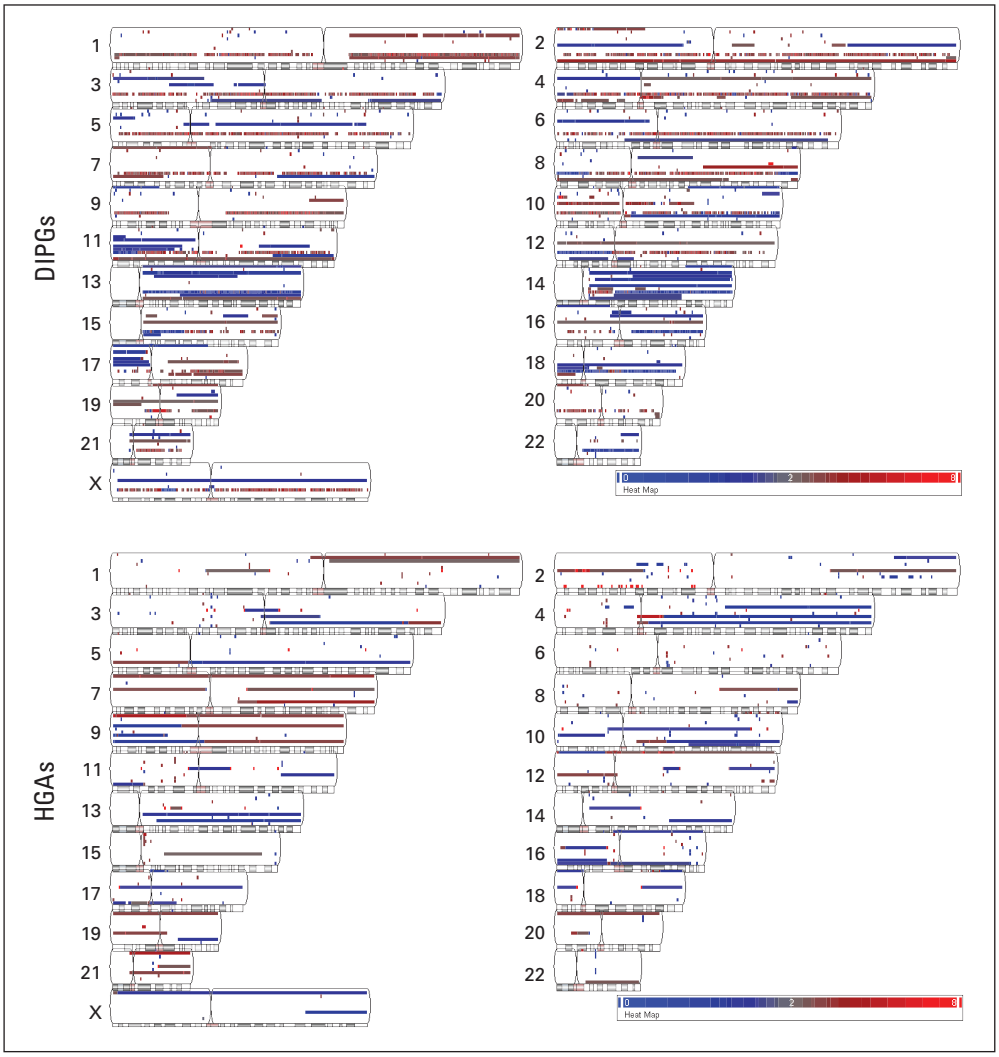

Fig 1. Whole genome view of copy number alterations for pediatric diffuse intrinsic pontine gliomas (DIPGs) versus pediatric supratentorial high-grade astrocytomas (HGAs) highlighting the distinct genetic characteristics of DIPGs. Patients are arranged sequentially with patient 1 at the top and patient 11 at the bottom. Red: copy number gain, blue: copy number loss.

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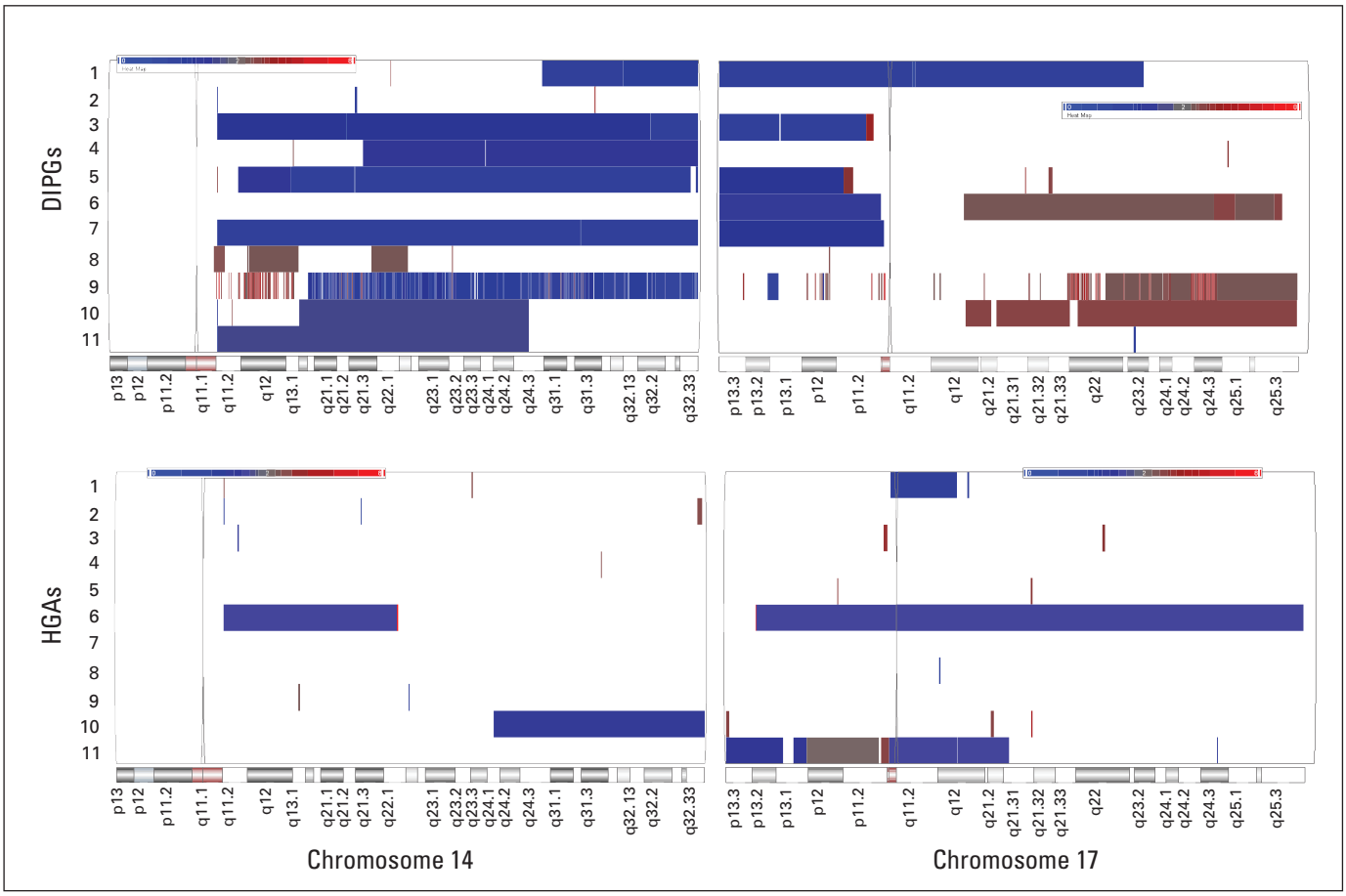

Fig 2. Magnification of chromosomes 14 (left) and 17 (right), highlighting genetic differences between pediatric diffuse intrinsic pontine glioma (DIPGs) and pediatric supratentorial high-grade astrocytomas (HGAs).

which were investigated using a PCR-based technique.[9] *MGMT* was hemizygously deleted in three DIPGs (two postmortem, one pretreatment) but was not expressed in any case (Table 2) suggesting that epigenetic mechanisms may be at play. *PTEN* mutations causing loss of function are found in approximately 20% of adult GBMs[10] with the detection of allelic loss in approximately 50% of patients in one study of pediatric DIPGs.[9,11] *PTEN* was deleted in three (27%) of 11 DIPGs, including one of the surgical samples and in three HGAs as well.

Focal deletions were mostly hemizygous and were largely nonoverlapping. In contrast to a previous report on pediatric malignant

Table 2. Summary of Status of Known GBM Oncogenes and Tumor Suppressor Genes in Pediatric DIPGs

Patient No.	*EGFR* (gain)	EGFR (immuno)	*TP53* (del)	p53 (immuno)	*MGMT* (del)	MGMT (immuno)	*PDGFRA* (gain)	PDGFRA (immuno)	*PDGFA* (gain)	PDGFA/B (immuno)	*PARP1* (gain)	PARP1 (immuno)
1*	Yes	Negative	Yes	Negative	Yes	Negative	No	Strong	Yes	Strong	No	Positive
2*	No	Negative	No	Negative	No	Negative	No	Weak	No	Weak	No	Negative
3	No	Weak	Yes	Negative	No	Negative	No	Weak	No	Weak	Yes	Weak
4	No	Weak	No	Negative	No	Negative	Yes	Weak	No	Weak	No	Weak
5	No	Strong	Yes	50%	No	Negative	No	Weak	No	Weak	No	Negative
6	No	Negative	Yes	Negative	No	Negative	Yes	Strong	No	Weak	No	Negative
7	No	Weak	Yes	20%	No	Negative	No	Strong	No	Weak	No	Positive
8	No	Strong	Yes	10%	No	Negative	No	Strong	No	Strong	No	Negative
9	No	Strong	Yes	30%	No	Negative	Yes	Strong	No	Strong	Yes	Positive
10	No	Negative	No	Negative	Yes	Negative	Yes	Strong	Yes		Yes	Positive
11	No	Weak	No	20%	No	Negative	No	Strong	No		No	

Abbreviation: GBM, glioblastoma multiforme; DIPG, diffuse intrinsic pontine glioma; EGFR, epidermal growth factor receptor; MGMT, O^6-methylguanine methyltransferase; PDGFRA, platelet-derived growth factor receptor alpha; PARP1, poly (ADP-ribose) polymerase; PNET, primitive neuroectodermal tumor.
*PNET-like histology, see text for details.

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gliomas,[12] we did not observe homozygous deletions of the *ELAVL2* (9p21.3) or *HNT* (11q25) loci in our DIPGs or HGAs. *ELAVL2* showed a hemizygous deletion in one DIPG and in three HGAs. The *HNT* region was hemizygously deleted in one and gained in another DIPG and deleted in one HGA. Potential novel DIPG tumor suppressors mapping to focal deletions in at least two tumors are listed in Data Supplement Table 1 (included are regions with three or fewer genes in which at least two tumors showed a copy number of < 1.33).

Large recurrent regions of gain common to both HGAs and DIPGs most frequently involved chromosomes 1q and 9q, while recurrent regions of gain in 17q and 10p were unique to DIPGs. Potential DIPG oncogenes are listed in Data Supplement Table 2 (included are regions with three or fewer genes in which at least two tumors showed a copy number of > 3).

To validate the copy number changes shown by microarray analysis, we tested two genes (*ODZ4* and *MYC* [c-Myc]) reported as highly amplified in DIPG7 and 6, respectively, by microarray analysis using qPCR. Both amplifications were confirmed by qPCR; cases not reported as amplified were run in parallel confirming their normal copy number (Appendix Fig A1, online only).

Similar to previous reports of malignant gliomas of childhood[12,13] Epidermal growth factor receptor (*EGFR*), which is frequently amplified in adult glioblastoma multiforme (GBM),[14,15] was not amplified in any of our DIPG cases but showed a low level copy number gain in one. EGFR showed strong immunopositivity in three cases and weak immunopositivity in a further four (Table 2).

Copy Number Analysis Reveals Potential Role of PDGFR Pathway in Pediatric DIPG

Interestingly, we identified gain of the *PDGFRA* region in four tumors (36%; three postmortem, one surgical; Fig 3A). Gain of this region was validated by FISH (data not shown) and by qPCR (Fig 3B). PDGFR-α immunohistochemistry revealed strong expression (Fig 3C) in seven and weak expression in four of the tumors (Table 2).

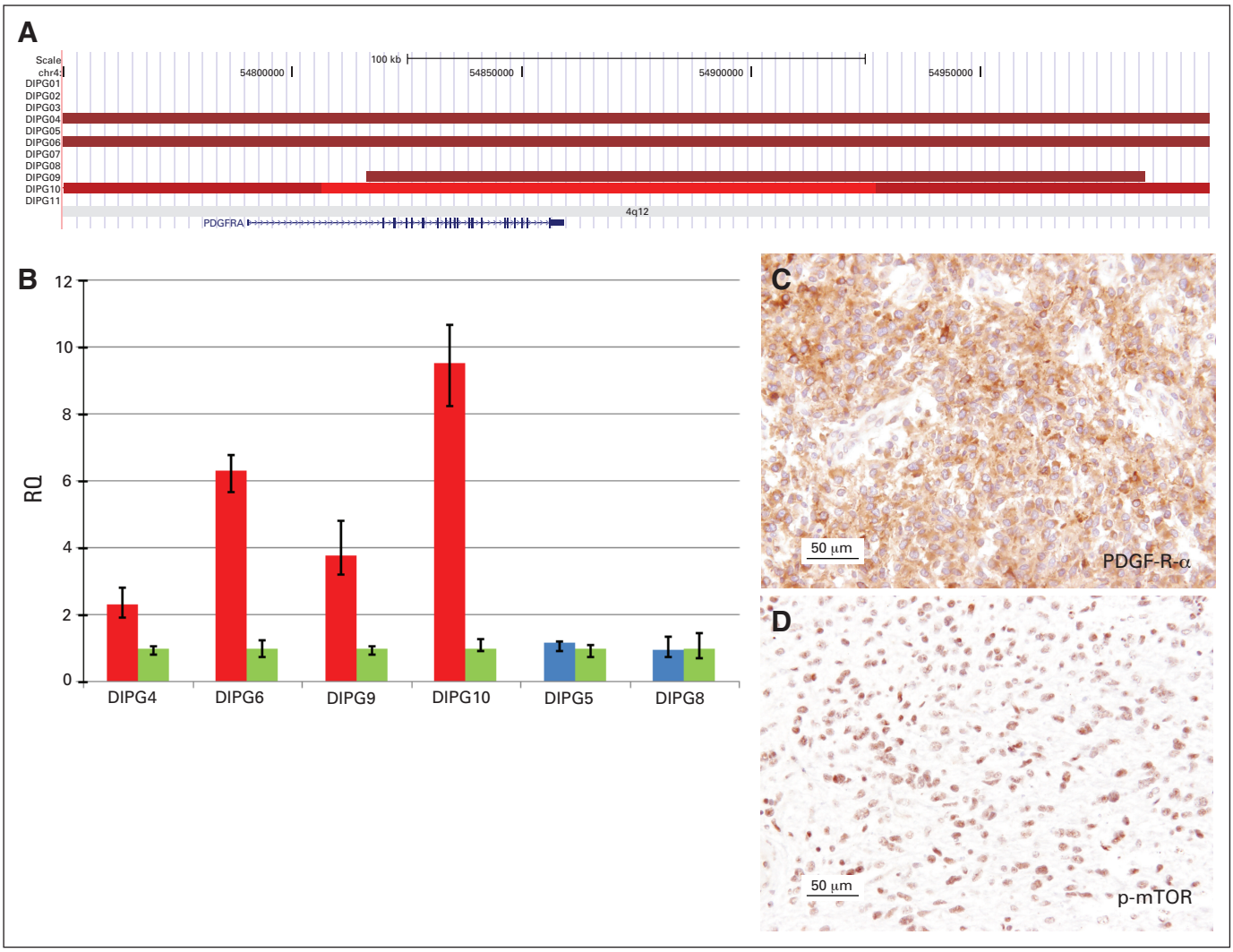

Fig 3. (A) Magnification of the platelet-derived growth factor receptor α (*PDGFRA)* region showing gains in four diffuse intrinsic pontine gliomas (DIPGs). (B) Quantitative polymerase chain reaction validation of copy number gains in the *PDGFRA* region. The *y*-axis represents relative quantity (RQ) values. Red bars represent the tumors where a copy number gain was expected based on the microarray data; blue bars represent tumors where a copy number gain was not expected based on the microarray data. Green bars represent the matched normal brain tissue. Error bars represent the SEM of triplicates. Immunohistochemical detection of (C) PDGFR-α and (D) phospho-mammalian target of rapamycin (p-mTOR) in DIPGs. Both images were taken at original magnification $\times 200$; the scale bars represent 50 μm.

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Remarkably, *PDGFA* was gained in two tumors (one postmortem, one surgical). By immunohistochemistry PDGFA/B showed strong immunopositivity in three DIPGs and weak immunopositivity in a further six (Table 2). In order to test whether expression of the PDGFR correlated with downstream activation of the pathway, we undertook immunohistochemistry for phospho-mammalian target of rapamycin (mTOR). Phospho-mTOR was immunopositive in all 11 cases (strongest in the two surgical samples, moderate in five and weak in four postmortem samples). Thus, 50% of DIPGs show gain of either *PDGFA* or *PDGFRA* and all show expression of PDGFR-α and phospho-mTOR, suggesting that activation of this pathway may be an important mechanism in these tumors.

DIPGs Have Abundant Complex LOH

In the paired analysis, LOH was identified in 2,996 to 27,093 SNP loci. Five hundred forty-three genes showed LOH in at least four samples. Of these, the majority was copy neutral (270 genes). Further, 210 genes showed LOH in association with a deletion and 63 genes had LOH in association with copy number gains.

Pathway Analysis Reveals Recurrent LOH in DNA Repair Pathways

Pathway analysis revealed genes with LOH were enriched for apoptosis, transcriptional regulation, and DNA repair pathways (Appendix Table A1, online only). When pathway analysis was done using three different software applications (Babelomics [http://babelomics.bioinfo.cipf.es/], Ingenuity [Ingenuity Systems, Redwood, CA], and Panther [www.pantherdb.org]), DNA repair pathways were recurrently identified. A list of DNA repair genes and their frequency of involvement in DIPG is presented in Table 3.

Microsatellite markers within and/or flanking *RAD51L1*, *TCF7L2*, and *MGMT* genes involved in DNA repair and/or regulation of transcription, were used to validate our LOH findings. All subjects showed LOH in at least one and at most two of the three polymorphic microsatellite markers. LOH of the microsatellite markers studied was observed in two of six tumors and allelic imbalance of the microsatellite markers consistent with LOH was observed in four of six tumor samples.

Most significantly and of potential therapeutic interest, *PARP-1* was gained in three cases, including one pretreatment case (Table 2). By immunohistochemistry, PARP was expressed in six tumors (strong in four and weak in two). Tumors with compromised ability to repair double-strand DNA breaks by homologous recombination, including those with defects in *BRCA1* and *BRCA2*, are highly sensitive to blockade of the repair of DNA single-strand breaks via PARP inhibition.[16] Pathway analysis of genes showing LOH in DIPG also revealed multiple members of the *BRCA* DNA damage response pathway including deletion or LOH of *BRCA2* in six cases (55%; Table 3). This, coupled with defects in other DNA repair pathways, raises PARP inhibition as a possible therapeutic target for a subset of pediatric DIPGs.

Table 3. DNA Repair Genes Showing LOH and/or Deletions in Pediatric DIPG

Function by Gene	No. of DIPGs		
	Deleted	LOH	Deleted or LOH
Nucleotide excision repair			
RPA1	5	0	5
MNAT1	7*	2	7*
GTF2H3	0	2*	2*
Nonhomologous end-joining			
LIG4	4*	3	5*
XRCC4	1	2*	2*
XRCC5	1	0	1
Homologous recombination			
BRCA1	1	1	1
BRCA2	4	4*	6*
RAD50	1	3*	3*
RAD51L1	7*	4	8*
Modulation of nucleotide pools			
DUT	0	2	2
Base excision repair			
MYH1	5	7*	7*
Mismatch repair genes			
PMS1	1	3*	4*
MLH1	2	1	2
MSH4	1	1	2

Abbreviations: LOH, loss of heterozygosity; DIPG, diffuse intrinsic pontine glioma.
*At least one tumor is a pretreatment specimen.

DISCUSSION

DIPG is one of the most devastating of pediatric malignancies and one for which no effective therapy exists. Here we address the lack of knowledge regarding the biology of these tumors by performing the first high-resolution SNP-based DNA microarray analysis of a series of DIPGs. We have found recurrent regions of CNAs and LOH involving both known genes as well as novel genes not previously described in high grade gliomas.

This represents the first detailed histologic examination of a large series of diffuse brainstem neoplasms. Histologically, nine of these tumors met diagnostic criteria HGA neoplasms. The other two tumors were labeled primitive neuroectodermal as they lacked definitive glial or mature neuronal differentiation. DIPG1 may in fact represent a more undifferentiated glial neoplasm and falls into similar difficulties with categorization as befalls supratentorial pediatric neoplasms which could be called sPNET by some pathologists and GBM by others. Other tumors, DIPG6 for example, had some overlapping histologic features with DIPG1 but maintained glial differentiation, including glial fibrillary acidic protein expression, at least focally. DIPG1 shared overlapping genetic features with the group as a whole.

Previous studies have shown that *EGFR* copy number alteration is the most common genetic alteration in adult glioblastoma but occurs rarely in pediatric glioblastoma even when the protein is overexpressed.[17,18] In our analysis, the *EGFR* gene was not amplified in any of the DIPGs but had a low copy number gain in one. However, EGFR showed immunopositivity in seven cases. EGFR was also found to be expressed in 14 of 16 high-grade gliomas of the brainstem in a study by Gilbertson et al.[19] Together, these findings suggest that EGFR inhibitors may work in a subset of DIPG patients.

PDGFRA and its ligands are expressed in gliomas, particularly in high-grade tumors. Likewise, *PDGFRA* was gained in 36% of our samples. Interestingly, a gain of the *PDGFA* region at 7ptel was found in association with the gain in *PDGFRA* (DIPG10) as well as in an

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additional tumor (DIPG01). PDGF-R-α showed weak or strong immunopositivity in all cases tested. Further, PDGFA/B and phospho-mTOR showed weak or strong immunopositivity in all DIPGs as well. Together these data strongly suggest a role for the PDGF signaling pathway in a large proportion of pediatric DIPGs and suggest that PDGFR inhibitors may be potential therapeutic agents for these tumors.

Temozolomide (TMZ) is a methylating agent currently widely used for treatment of high-grade gliomas. However, trials of TMZ have been unsuccessful in pediatric DIPG. Resistance to alkylating agents via direct DNA repair by O(6)-methylguanine methyltransferase (MGMT) remains a significant barrier to the successful treatment of patients with malignant glioma. However, in our series of DIPGs MGMT was not expressed in any of the tumors, suggesting that this is not the mechanism of TMZ resistance in these patients.

PARP-1 is a nuclear protein involved in sensing and signaling the presence of DNA damage.[20] It then recruits and activates DNA repair proteins.[21-23] PARP-1 binds to single-stranded DNA breaks induced by alkylating agents and to double-stranded breaks formed by ionizing radiation. Enhanced PARP expression and/or activity in tumor cells, compared to normal cells, has been demonstrated in several cancers including malignant gliomas[24] and represents a major mechanism for repairing DNA breaks and escaping apoptosis. PARP-1 activity is particularly important in the base excision repair of N7-methyl guanine damage typically caused by alkylating agents, including TMZ.[25] Thus, PARP-1 over-expression could potentially be associated with both TMZ and radiation resistance.

PARP inhibition should theoretically enhance tumor cells' sensitivity to DNA damaging agents and particularly to radiotherapy. Studies of PARP inhibitors have shown them to enhance the sensitivity of glioma cells to agents that cause DNA damage including both chemotherapy and radiation.[26-29] Pharmacokinetic studies of the PARP-1 and -2 inhibitor ABT-888 in nonhuman primates have demonstrated substantial CSF exposure after oral dosing, with concentrations that are high enough to potentiate antitumor effects of chemotherapy and/or radiation.[30] Phase I and II trials of ABT-888 in combination with radiation and/or TMZ are ongoing.[31]

Interestingly, *PARP-1* was gained in three DIPG cases and expressed in six. Tumors with compromised ability to repair double-strand DNA breaks by homologous recombination, including those with defects in *BRCA1* and *BRCA2*, are highly sensitive to blockade of the repair of DNA single-strand breaks via PARP inhibition.[16] Of note, four DIPGs showed a deletion in *BRCA2* and an additional two showed LOH in this region (Table 3). This, coupled with defects in other DNA repair pathways (Table 3), raises the exciting possibility of using PARP inhibition as a novel therapeutic strategy for some pediatric DIPGs.

To our knowledge, our data provides the first, comprehensive high-resolution genomic analysis of pediatric DIPG. Two potential limitations of the study are the postmortem nature of the samples and the small sample size. To control for the potential therapeutic and postmortem effects on our tissue, all postmortem samples were compared with their own matched normal brain tissue. Further, two of our cases represent archived pretreatment surgical samples. Many of the recurrent changes identified in our postmortem samples, including both the *PDGFRA* and *PARP-1* alterations, were also identified in at least one of the surgical samples as well. Importantly, despite the small sample size, numerous overlapping changes were identified. Our findings of recurrent involvement of the PDGFR pathway as well as defects in DNA repair pathways coupled with gain of PARP-1 highlight two potential, biologically-based, therapeutic targets directed specifically at this devastating disease, relieving the impasse in the development of novel therapeutic strategies for the treatment of pediatric DIPG.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Ute Bartels, Cynthia Hawkins
Financial support: Cynthia Hawkins
Provision of study materials or patients: Ute Bartels, Eric Bouffet, Cynthia Hawkins
Collection and assembly of data: Maryam Zarghooni, Eric Lee, Andrew Morrison, Cynthia Hawkins
Data analysis and interpretation: Maryam Zarghooni, Eric Lee, Pawel Buczkowicz, Andrew Morrison, Annie Huang, Cynthia Hawkins
Manuscript writing: Maryam Zarghooni, Ute Bartels, Eric Lee, Andrew Morrison, Annie Huang, Eric Bouffet, Cynthia Hawkins
Final approval of manuscript: Maryam Zarghooni, Ute Bartels, Eric Lee, Pawel Buczkowicz, Andrew Morrison, Annie Huang, Eric Bouffet, Cynthia Hawkins

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