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RESEARCH ARTICLE

PDGFRA Amplification is Common in Pediatric and Adult High-Grade Astrocytomas and Identifies a Poor Prognostic Group in IDH1 Mutant Glioblastoma

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Abstract

High-grade astrocytomas (HGAs), corresponding to World Health Organization grades III (anaplastic astrocytoma) and IV (glioblastoma; GBM), are biologically aggressive, and their molecular classification is increasingly relevant to clinical management. *PDGFRA* amplification is common in HGAs, although its prognostic significance remains unclear. Using fluorescence *in situ* hybridization (FISH), the most sensitive technique for detecting *PDGFRA* copy number gains, we determined *PDGFRA* amplification status in 123 pediatric and 263 adult HGAs. A range of *PDGFRA* FISH patterns were identified and cases were scored as non-amplified (normal and polysomy) or amplified (low-level and high-level). *PDGFRA* amplification was frequent in pediatric (29.3%) and adult (20.9%) tumors. Amplification was not prognostic in pediatric HGAs. In adult tumors diagnosed initially as GBM, the presence of combined *PDGFRA* amplification and isocitrate dehydrogenase 1 $(IDH1)^{R132H}$ mutation was a significant independent prognostic factor ($P = 0.01$). In HGAs, *PDGFRA* amplification is common and can manifest as high-level and focal or low-level amplifications. Our data indicate that the latter is more prevalent than previously reported with copy number averaging techniques. To our knowledge, this is the largest survey of *PDGFRA* status in adult and pediatric HGAs and suggests *PDGFRA* amplification increases with grade and is associated with a less favorable prognosis in IDH1 mutant de novo GBMs.

INTRODUCTION

High-grade astrocytomas (HGAs), including anaplastic astrocytoma (AA), World Health Organization (WHO) Grade III, and glioblastoma (GBM), WHO Grade IV, occur in both adults and children and are among the deadliest forms of cancer. Current practice stratifies HGAs based upon clinical, histopathologic and limited molecular features. As our understanding of gliomagenesis and tumor response to therapy improves, this stratification will likely undergo multiple revisions with incorporation of additional molecular markers. As such, the continued development and validation of robust methods to assess molecular alterations in HGA is critical.

HGAs are characterized by alterations in receptor tyrosine kinase (RTK) signaling, and abnormal platelet-derived growth factor (PDGF) signaling has been demonstrated in a significant subset of both adult and pediatric tumors. In adult HGAs, PDGF receptor alpha (PDGFRA) is the second most commonly altered RTK receptor after epidermal growth factor receptor (EGFR), with amplification of the *PDGFRA* locus being the most common

mechanism (7, 9, 18, 25, 27, 30). While estimates vary, in a large study using array-based comparative genomic hybridization (CGH), amplification of *PDGFRA* was identified in 11% of patients (27). Increased PDGF pathway activity, however, has been reported in up to 33% of adult GBM (3). Indeed, PDGF signaling pathway alterations are a characteristic feature of many tumors designated as "proneural" based on genomic, transcriptomal and proteomic features (3, 21, 30). However, while tumors with a proneural phenotype may have an improved overall survival (21), other studies have suggested that *PDGFRA* copy number gain/amplification may be associated with worse overall survival in astrocytoma (1, 29).

In children, increased PDGF signaling is also thought to be an important driver of HGAs and *PDGFRA* amplification is similarly considered a common mechanism, with frequencies ranging from 3.4 to 12% (16, 20, 23). In specific clinical subsets of HGA, such as diffuse intrinsic pontine glioma, up to 25% of tumors may have amplification (32), potentially corresponding to its distinctive biologic properties (22). Despite the relatively high frequency of *PDGFRA* amplification in both pediatric and adult HGAs, the prognostic significance of this alteration remains largely unclear.

Mutations in isocitrate dehydrogenase 1 (IDH1) are common in adult AA and in subsets of adult GBM, including GBM that have progressed from a lower-grade astrocytoma (secondary GBM); they are also found in a small subset of tumors diagnosed initially as GBM (de novo GBM) (5, 8, 19, 31). IDH1 mutant tumors, with R132H being the most common mutation, exhibit unique spatial, temporal and biologic characteristics, including enhanced overall survival relative to IDH1 wild-type tumors of a similar grade (15, 17). While infrequent in de novo GBM, IDH1 mutations are enriched in the proneural subtype, which is also characterized by alterations in PDGFRA signaling (21, 30).

Currently, there are no established criteria for the assessment of *PDGFRA* copy number gain/amplification in clinical samples. While many studies have relied on copy number averaging techniques, such as polymerase chain reaction (PCR) and single nucleotide polymorphism arrays, these methods may underestimate the frequency of *PDGFRA* amplifications when only scattered cells are amplified or the degree of amplification is low level. This is particularly true given the tremendous intratumoral heterogeneity of HGAs as recently illustrated for EGFR and PDGFR (24, 25). As such, the simplest and most sensitive technique for detecting copy number gains in routinely processed pathology specimens is fluorescence *in situ* hybridization (FISH). Using this technique, we assess a large series of HGAs, provide practical interpretive guidelines for the clinical assessment of *PDGFRA* copy numbers, and examine the prognostic significance of amplification in both adult and pediatric cohorts.

MATERIALS AND METHODS

Cohort

Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from a total of 123 pediatric HGAs and 307 adult HGAs, including 103 adult AAs, 187 de novo GBMs (i.e. tumors initially diagnosed as GBM), and 17 IDH1 mutant secondary GBMs (i.e. GBM documented progression from a lower-grade astrocytoma) were obtained from 10 institutions: UCSF Brain Tumor Research Center (BTRC) Tissue Bank; Department of Pathology, Newcastle General Hospital; Department of Pathology, Children's Hospital, Los Angeles; Department of Lab Medicine and Pathobiology and Department of Surgery, University of Toronto; Department of Pathology & Laboratory Medicine, University of Kentucky College of Medicine; Department of Pathology and Laboratory Medicine, Emory University; Department of Pathology, Gemelli Hospital, University of Sacred Heart, Rome Italy; Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA; Department of Pathology and Laboratory Medicine, University of Pennsylvania; and Brain Tumor Translational Resource, Department of Pathology and Laboratory Medicine, University of California Los Angeles. These included both whole tissue and tissue microarray (TMA) sections, the latter obtained from ten previously generated HGA TMAs. Clinical characteristics are summarized in Table 1. Pediatric (0.1–20 years of age) and adult (25.7–83 years of age) astrocytoma patients were diagnosed with either AA, WHO grade III, or GBM, WHO grade IV. Clinical and molecular characteristic of the tumor were obtained when available from the respective institutions and included survival from time of initial surgery, age at initial diagnosis, sex, and IDH1 mutation status ($IDH1^{R132H}$) using IDH1(R132H) immunohistochemistry (H09, Dianova GmbH, Hamburg, Germany) or sequencing (12).

Table 1. Clinical and molecular characteristics of 123 pediatric and 263 adult HGAs.

	Pediatric		Adult	
	AA ($n = 66$)	GBM ($n = 57$)	AA ($n = 103$)	GBM ($n = 160$)
Mean age (years ± SD)	9.06 ± 5.16	10.1 ± 5.0	50.6 ± 13.8	55.1 ± 13.1
Sex ratio (M : F)	1.0	1.0	8.1	1.6
Location† (supra- vs. infra-tentorial) (%)	86.0%	72.3%	96.7%	N/A
Location (frontal vs. other)	14.0%	6.38%	47.2%	N/A
IDH1 mutant	N/A	N/A	62.5% ($n = 96$)	7.50% ($n = 160$)
Median survival (days)	490 ($n = 34$)	596 ($n = 29$)	2070 ($n = 69$)	450 ($n = 148$)

AA = anaplastic astrocytoma; GBM = glioblastoma; $IDH1^{R132H}$ = isocitrate dehydrogenase 1; N/A = data are not available; n = number analyzed; SD = standard deviation.
†Data on tumor location was available for 43 pediatric AA, 47 pediatric GBM, and 91 adult AA.

FISH (*PDGFRA*)

Dual-color FISH analysis was performed on 5-μm thick FFPE whole and TMA sections as previously described (12, 14). Briefly, sections were deparaffinized, digested with pepsin, heat denatured and allowed to hybridize with probe sets overnight at 37°C in a humidified oven. A SpectrumOrange (SO)-labeled home brew probe for *PDGFRA* (BAC clone RP11-231C18, CHORI BACPAC Resources Center, Oakland, CA, USA; previously reported in ref (2)) diluted 1:10 in DenHybe (Insitus, Albuquerque, NM) was paired with SpectrumGreen (SG)-labeled centromere enumerating probe (CEP4) 4p11-q11 (reference probe) (Abbott, Downers Grove, IL, USA). Following washes to remove excess unbound probe, the nuclei were counterstained with 10 μL DAPI (Insitus, Albuquerque, NM, USA) and slides were coverslipped. The fluorescent signals were enumerated under an Olympus BX41 fluorescent microscope with appropriate filters (Olympus; Melville, NY, USA). For each hybridization, green and orange signals were enumerated in 100 non-overlapping nuclei. Slides were scanned for regional variability and were considered abnormal regardless of whether the alteration appeared focal or diffuse. Hybridizations were considered non-informative if the FISH signals were either lacking or too weak to interpret.

Statistical analysis

A two-tailed *t*-test was used to compare mean values except where noted. $P < 0.05$ was considered statistically significant. For Kaplan–Meier survival analysis, groups were compared using the log-rank (Mantel–Cox) test. Overall survival was truncated at 750 days for pediatric AAs, as there was a single death after 750 days, and at 4500 days for adult AAs, as there was a single censored patient after 4500 days. Contingency analysis was performed using Fisher's exact test, two-sided. Multivariate Cox proportional hazard regression was used to model survival; while, 10-fold cross-validation and an integrated Brier score (6, 13) were used to compare the predictive error scores. All statistical analyses were carried out using GraphPad software (GraphPad Software Inc., La Jolla, CA, USA) and R (26).

Study approval

All procedures were performed according to protocols approved by the University of California Committee on Research (San Francisco, CA, USA). De-identified FFPE sections of human HGAs and TMAs were obtained from the participating institutions including the UCSF Brain Tumor Research Center Tissue Bank, Department of Pathology, Newcastle General Hospital; Department of Pathology, Children's Hospital, Los Angeles; Department of Lab Medicine and Pathobiology, University of Toronto; Department of Pathology & Laboratory Medicine, University of Kentucky; Department of Pathology and Laboratory Medicine, Emory University; Department of Pathology, Gemelli Hospital, University of Sacred Heart, Rome Italy; The Children's Hospital of Philadelphia; Department of Pathology and Laboratory Medicine, University of Pennsylvania; and Brain Tumor Translational Resource, Department of Pathology and Laboratory Medicine, University of California Los Angeles.

RESULTS

Patient characteristics

The study cohort included 123 pediatric and 263 adult HGAs whose clinical characteristics are summarized in Table 1. For pediatric HGAs, a total of 71 AAs, mean age 9.06 years (range 0.2–20), and 57 GBMs mean age 10.1 years (range 0.1–19) were analyzed. For adult HGAs, 103 AAs, mean age 50.6 years (range 25.7–80) and 160 de novo GBMs, mean age 55.1 years (range 27–79) were analyzed. Based on these data, an additional 27 de novo GBM with mutations in IDH1 and a mean age of 46 years (range 21–83) and 17 IDH1 mutant secondary GBM, with documented progression from a lower-grade astrocytoma, with a mean age of 39.9 years (range 26–55) were analyzed.

Detection of *PDGFRA* copy number gain/amplification by FISH

A wide range of FISH patterns was identified, reflecting both inter- and intra-tumoral heterogeneity. Based on this heterogeneity we devised a scoring system that reflected both the relative number of tumor cells with copy number gain and the magnitude of copy number gain (Figure 1). Cases were scored as: normal (no increase or <10% cells with <6 *PDGFRA* signals); polysomy (>10% cells with 2–6 signals); low-level amplification (<10% of cells with >12 or innumerable signals or >40% cells with 6–12 signals); or high-level amplification (>10% cells with >12 or innumerable signals). Both low-level and high-level amplification were considered as "*PDGFRA* amplified." These definitions were based on similar definitions for amplification of other oncogenes using clinical FISH assays (28). For instance, the definition of low-level amplification with >40% cells containing ≥6 signals is nearly identical to that utilized for EGFR amplification in lung cancer (10). Although biologically, this is probably more accurately described as a "high-level polysomy" rather than true gene amplification, the definition nonetheless correlates strongly with clinical outcome, including therapeutic response to tyrosine kinase inhibitors (10, 28). It was similarly felt that the more common pattern of gene amplification by FISH (>12 or innumerable signals) found in <10% of cells would have a roughly equivalent overall increase in dosage to that of a lower level of gain in larger numbers of cells (i.e., low-level amplification). Often, these two patterns of low-level amplification could be seen together in the same tumor. The decision not to use the *PDGFRA*/CEP4 ratio as part of the definition was based on the finding of several cases in which co-amplification of the centromeric region was found (Figure 1F) wherein a ratio near 1.0 would falsely exclude an interpretation of gene amplification.

Pediatric HGAs

FISH analysis of 123 pediatric HGAs demonstrated that 36 of 123 (29.3%) tumors had *PDGFRA* amplification, including 19 (15.4%) high-level and 17 (13.8%) low-level examples. A high frequency of *PDGFRA* amplification has been reported in a subset of diffuse intrinsic pontine gliomas (DIPGs) (22, 32). In our cohort we did not observe an increase in *PDGFRA* amplification in the brainstem/cerebellum vs. other brain regions ($P = 0.53$); however,

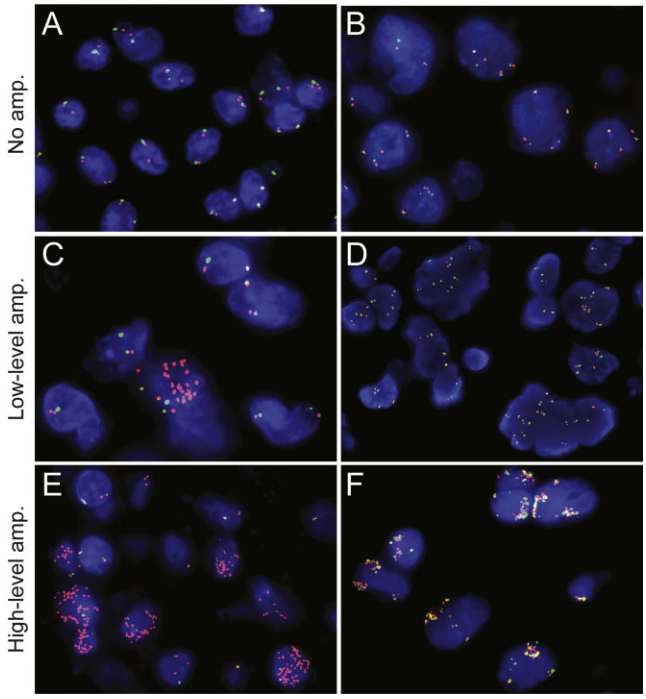

Figure 1. Determination of *PDGFRA* copy number gain/amplification by fluorescence *in situ* hybridization. Fluorescent images illustrating the different patterns of *PDGFRA* amplification in high-grade astrocytomas. **A**. Normal, no increase in *PDGFRA* signals. **B**. Polysomy, >10% of cells with >2, but <6 signals for both *PDGFRA* and centromere enumerating probe (CEP4). **C**. The most frequent pattern of low-level amplification, innumerable *PDGFRA* signals in <10% of cells. **D**. Another pattern of low-level amplification, >40% cells with ≥6 signals (a few signals are beyond the plane of focus). **E**. High-level amplification, > 10% cells with >12 or innumerable *PDGFRA* signals. **F**. Rare tumors demonstrated high-level co-amplification of *PDGFRA* and CEP4. Amp. denotes *PDGFRA* amplification. *PDGFRA* probe (red) and CEP4 (green), magnification ×1000.

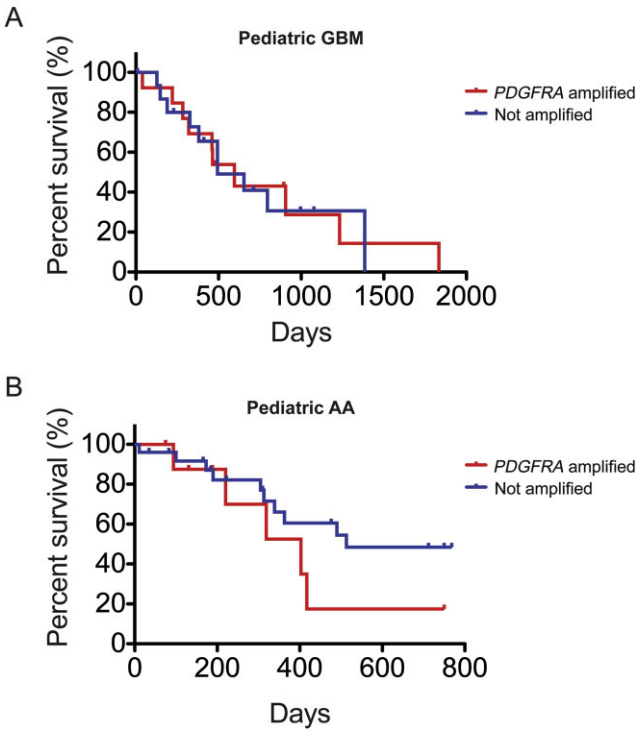

Figure 2. Pediatric high-grade astrocytomas patients have similar overall survival with and without *PDGFRA* amplification. **A**. Pediatric patients with glioblastoma (GBM) with ($n = 13$) and without ($n = 16$) *PDGFRA* amplification have no significant difference in overall survival based on Kaplan–Meier survival analysis, $P = 0.97$. **B**. Kaplan–Meier survival analysis examining overall survival for pediatric patients with anaplastic astrocytoma with ($n = 9$) and without ($n = 25$) amplification of *PDGFRA*, $P = 0.21$. AA = anaplastic astrocytoma.

our cohort included only 12 HGAs that involved the brainstem or cerebellum. To examine potential clinical differences between patients with *PDGFRA*-amplified vs. non-amplified tumors, we analyzed GBM and AA separately.

In pediatric GBM, FISH analysis demonstrated a striking 22 of 57 (38.6%) tumors with amplification of *PDGFRA*, including 11 (19.3%) with low-level and 11 (19.3%) with high-level amplification. Stratification of clinical variables based on *PDGFRA* amplification is shown in Table 2. Patients with *PDGFRA*-amplified tumors tended to be older than patients without amplification; however, this difference was not significant ($P = 0.13$). There was no difference in overall survival between patients with and without *PDGFRA* copy number gain/amplification (Figure 2A).

In pediatric AA, we identified a slightly lower percentage of *PDGFRA*-amplified cases than in GBM (Table 3). A total of 14 of 66 (21.2%) tumors had amplification, including 6 (9.09%) with high-level and 8 (12.1%) with low-level amplification. Although not statistically significant, the age of patients with *PDGFRA*-amplified tumors tended to be older ($P = 0.08$).

Table 2. Clinical characteristics of 57 pediatric patients with glioblastoma.

Patient characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number (%)	35 (61.4%)	22 (38.6%)		
Mean age (years ± SD)	9.23 ± 5.49	11.40 ± 3.82		0.13
Sex ratio (M : F)	1.1	1.0	1.067 (0.3464–3.285)	1.0
Median survival (days)	596	494		0.97
Location: BS/CB vs. other	24.1% ($n = 29$)	11.2% ($n = 18$)	2.545 (0.4656–13.92)	0.45

BS/CB = brainstem/cerebellum; CI = confidence interval; SD = standard deviation.

Table 3. Clinical characteristics of 66 pediatric patients with anaplastic astrocytoma.

Patient characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number (%)	52 (78.8%)	14 (21.2%)		
Mean age (years ± SD)	8.16 ± 5.49	11.2 ± 3.76		0.08
Sex ratio (M : F)	1.38	0.45	3.115 (0.7859–12.35)	0.18
Median survival (days)	513	403		0.21
Location: BS/CB vs. other	6.67% ($n = 30$)	7.69% ($n = 13$)	0.8571 (0.0707–10.38)	1.0

BS/CB = brainstem/cerebellum; CI = confidence interval; SD = standard deviation.

PDGFRA-amplified tumors also tended to be more common in women than men ($P = 0.18$). Similar trends in age and sex in the *PDGFRA*-amplified tumors was noted in the pediatric GBM cohort. There was no significant difference in overall survival between *PDGFRA*-amplified and non-amplified tumors, although there was a trend toward worse survival with amplification (log-rank, $P = 0.21$; Figure 2B).

Adult HGAs

FISH analysis in adult HGAs demonstrated 55 of 263 (20.9%) *PDGFRA*-amplified tumors, including 25 (9.50%) with high-level and 30 (11.4%) with low-level amplification. Similar to pediatric HGAs, *PDGFRA* amplification was more frequent in de novo GBM than AA. In adults, 36 of 160 (22.5%) GBM had *PDGFRA* amplification, including 17 (10.6%) high-level and 19 (11.9%) low-level (Table 4). While the patient's age, sex and overall survival were similar between *PDGFRA*-amplified and non-amplified tumors, there was a significant association between *PDGFRA* amplification and mutations in IDH1 ($P = 0.028$; Table 4 and Figure 3A).

The R132H mutation in IDH1 was present in 12 of 160 (7.5%) adult de novo GBM. Based on the enrichment of *PDGFRA* amplification in IDH1 mutant tumors, we performed a subset analysis focused only on those tumors with mutant IDH1 ($IDH1^{R132H}$). To increase the number of tumors available for analysis, we obtained an additional 27 IDH1 mutant de novo GBM (the clinical characteristics of all 39 tumors are summarized in Table 5). As expected, patients with de novo GBM with IDH1 mutation had significantly better overall survival than patients without IDH1 mutations (median survival 1927 days ($n = 38$) vs. 424 days ($n = 137$), respectively, log-rank, $P < 0.0001$). Stratification of these IDH1 mutant tumors by *PDGFRA* amplification status revealed a striking difference in median overall survival between patients with and without amplification (Figure 3B). Overall median survival was 480 days ($n = 16$) for patients with IDH1 mutant de novo GBM with *PDGFRA* amplification vs. 2179 days ($n = 22$) without *PDGFRA* amplification (log-rank, $P = 0.023$). Other clinical characteristics including age and sex were not significantly different between *PDGFRA*-amplified and non-amplified tumors.

In a multivariate analysis of all de novo GBMs, we examined *PDGFRA* status and IDH1 mutation status. While PDGFRA status alone was not a significant prognostic factor, the interaction of *PDGFRA* amplification and IDH1 mutation status (i.e. the group of tumors which are both *PDGFRA*-amplified and IDH1 mutated)

Table 4. Clinical and molecular characteristics of 160 adult glioblastoma† patients.

Patient and tumor characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number (%)	124 (77.5%)	36 (22.5%)		
Mean age (years ± SD)	55.7 ± 13.3	53.6 ± 12.7		0.56
Sex ratio (M : F)	1.9	1.1	0.5806 (0.2091–1.612	0.31
% IDH1 mutant	5.08% ($n = 124$)	16.7% ($n = 36$)	3.933 (1.184–13.07)	0.028
Median survival (days)	450 ($n = 114$)	455 ($n = 34$)		0.45

BS/CB = brainstem/cerebellum; CI = confidence interval; $IDH1^{R132H}$ = isocitrate dehydrogenase 1; SD = standard deviation.
†All tumors were de novo GBM and diagnosed at initial presentation as GBM.

Table 5. Clinical characteristics of 39 adult patients with IDH1 mutant GBM†.

Patient characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number (%)	22 (56.4%)	17 (43.6%)		
Mean age (years ± SD)	44.7 ± 12.8	46.8 ± 16.8		0.67
Sex ratio (M : F)	1.8	2	0.9167 (0.2121–3.963)	1.0
Median survival (days)	2179 ($n = 22$)	480 ($n = 16$)		0.023

CI = confidence interval; GBM = glioblastoma; $IDH1^{R132H}$ = isocitrate dehydrogenase 1; SD = standard deviation.
†All tumors were de novo GBM and diagnosed at initial presentation as GBM.

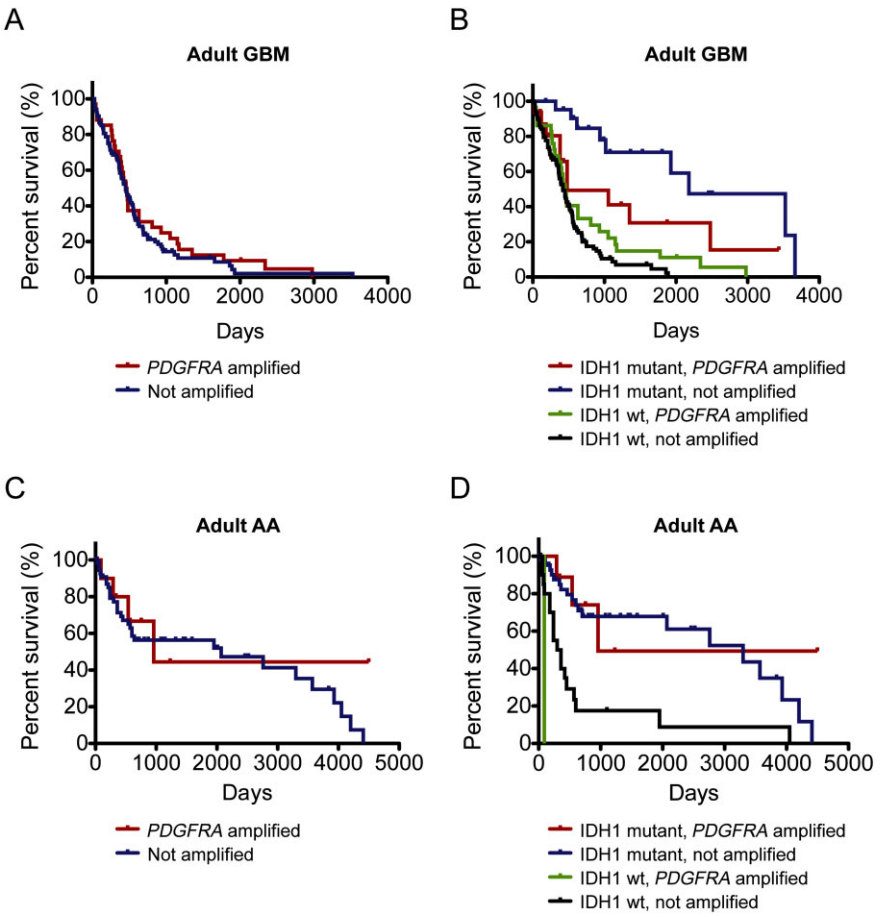

Figure 3. *PDGFRA* amplification is associated with worse overall survival in adult isocitrate dehydrogenase 1 ($IDH1^{R132H}$) mutant glioblastoma (GBM). (**A**) Kaplan–Meier survival analysis of patients with de novo GBM demonstrates no significant difference in overall survival for patients with (red, $n = 34$) and without (blue, $n = 114$) *PDGFRA* amplification, $P = 0.45$. (**B**) Comparison of overall survival in larger cohort of patients stratified for IDH1 mutation ($IDH1^{R132H}$) demonstrating decreased survival in IDH1 mutant GBM with *PDGFRA* amplification (red, $n = 16$) as compared with those without *PDGFRA* amplification (blue, $n = 22$) by Kaplan–Meier survival analysis, $P = 0.023$. Survival curves are also shown for IDH1 non-mutant GBM with (green, $n = 29$) and without (black, $n = 108$) *PDGFRA* amplification. A similar analysis in adult patients with anaplastic astrocytoma (AA) demonstrated overall survival was similar regardless of *PDGFRA* amplification in (**C**) all patients [amplified, red ($n = 11$); not amplified, blue ($n = 58$)] and in (**D**) patients stratified for IDH1 mutation status [IDH1 mutant and amplified, red ($n = 9$); IDH1 mutant and not amplified, blue ($n = 45$); IDH1 non-mutant and amplified, green ($n = 2$); IDH1 non-mutant and not amplified, black ($n = 21$)] by Kaplan–Meier survival analysis, $P = 0.41$ or 0.52, respectively.

was found to be a significant prognostic factor ($P = 0.01$) (Table 6) and remained significant when age was included in the model ($P = 0.049$).

Cross-validation is a technique used for model selection as well as to asses if the model will be useful in an independent data set. Using 10-fold cross-validation and the integrated Brier score, a measure of the strength of the model, we compared the predictive error scores between several models: baseline (no variables in the model); main effect models with IDH1 mutation and/or *PDGFRA* amplification status; and, an interaction model that included the two main effects as well as an interaction term that signifies both IDH1 mutation and PDGFRA amplification. The interaction model had the lowest prediction error and was considered to be the best model, resulting in a marked reduction in error over baseline (16%) and a marginal, but consistent reduction in error over IDH1 mutation alone (2%).

Table 6. Hazard ratios from a multivariate survival analysis in adult patients with de novo GBM†.

	No amplification	*PDGFRA* amplification
IDH1 wt	1.0	0.739 (0.477–1.145) $P = 0.18$
IDH1 mutant	0.140 (0.0639–0.3055) $P = 8.2 \times 10^{-7}$	0.416‡ (0.2131–0.8105) $P = 0.01$

GBM = glioblastoma; $IDH1^{R132H}$ = isocitrate dehydrogenase 1.
†GBM = complete information was available for 136 of the 178 patients, including 38 IDH1 mutant tumors.
‡Both IDH1 is mutated and *PDGFRA* is amplified.

In adult AAs, the frequency of *PDGFRA* amplification was less than in GBMs, with 19 of 103 (18.4%) tumors amplified, including 8 (7.8%) high-level and 11 (10.7%) low-level (Table 7). *PDGFRA*-amplified tumors tended to be located in the frontal lobes, 10 of 15 (66.7%), as compared with the non-amplified tumors, 29 of 69 (42.0%), although this was not statistically significant ($P = 0.15$). *PDGFRA* amplification was not prognostic for overall survival (Figure 3C). Interestingly, *PDGFRA* amplification was not increased in IDH1 mutant AAs, mutations seen in 61.2% of amplified tumors and 62.8% of non-amplified tumors. Next, we analyzed the subset of 60 IDH1 mutant AA to determine whether *PDGFRA* had prognostic benefit in IDH1 mutant adult AA (Table 8). Unlike de novo GBM in which the frequency of *PDGFRA* amplification was highly enriched in IDH1 mutant tumors (17/39; 43.6%) vs. IDH1 non-mutant tumors (30/151; 19.9%), in AA the frequency of *PDGFRA* amplification was similar in IDH1 mutant tumors (11/60; 18.3%) and in IDH1 non-mutant tumors (7/36; 19.5%). Furthermore, in IDH1 mutant AA, there was no statistically significant difference in overall survival between *PDGFRA*-amplified and non-amplified tumors (median

Table 7. Clinical and molecular characteristics of 103 adult anaplastic astrocytoma patients.

Patient and tumor characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number	84 (81.6%)	19 (18.4%)		
Mean age (years ± SD)	50.5 ± 13.2	50.9 ± 16.8		0.91
Sex ratio (M : F)	9.2	5.5	1.673 (0.2857–9.794)	0.62
IDH1 mutant	62.8% ($n = 78$)	61.2% ($n = 18$)	1.075 (0.3750–3.083)	1.00
Median survival (days)	2070 ($n = 58$)	960 ($n = 11$)		0.41
Location: frontal vs. other	42.0% ($n = 69$)	66.7% ($n = 15$)	0.3718 (0.1146–1.206)	0.15

CI = confidence interval; $IDH1^{R132H}$ = isocitrate dehydrogenase 1; SD = standard deviation.

survival 960 days ($n = 9$) vs. 3300 ($n = 45$) days, respectively, $P = 0.52$). While *PDGFRA*-amplified tumors were more common in the frontal lobes with 8 of 9 (88.9%) amplified tumors located in the frontal lobes as compared with 22 of 47 (46.8%) of the non-amplified tumors, this difference was not statistically significant ($P = 0.26$).

IDH1 mutant de novo GBM share many features with secondary GBM, derived from the progression of a lower-grade astrocytoma (15). Similar to IDH1 mutant de novo GBM, *PDGFRA* amplification was frequent in IDH1 mutant secondary GBMs, 7 of 17 (41.2%) tumors. However, unlike in de novo, *PDGFRA* amplification was not associated with shorter overall survival (Table 9).

DISCUSSION

HGAs are a heterogeneous group of tumors and improvements in therapy will likely require stratification of patients based upon clinical, histopathologic and molecular characteristics. Using FISH, we define a set of criteria to evaluate *PDGFRA* copy number alterations and determine the frequency of amplification in a large cohort of pediatric and adult HGAs. Our data suggest that *PDGFRA* amplification is higher than previously estimated in both pediatric (29.3%) and adult (20.7%) HGAs. To our knowledge this represents the largest reported number of pediatric and adult HGAs studied by FISH, and these data support the notion that abnormal *PDGFRA* signaling is important in HGA. In adults, *PDGFRA* amplification was associated with significantly worse overall survival in IDH1 mutant de novo GBM. Indeed, in a multivariate analysis of all adult de novo GBMs, the combination of *PDGFRA* amplification and IDH1 mutation status was identified as a significant prognostic factor. While additional studies in larger patient cohorts are required, our data suggest that IDH1 mutant de novo GBM may be a more heterogeneous group than previously thought.

PDGFR signaling is an important driver of glioma development and progression, and based on whole-genome technologies, *PDGFRA* copy number gain/amplification is the second most common genetic alteration in RTKs in adult GBM, commonly estimated at 11% (27). This reported frequency is nearly identical to that of the high-level amplification pattern that we noted in the current series; however, our techniques also allowed us to uncover a large number of low-level amplifications. Using FISH on routinely processed pathology specimens, we identified a high frequency of *PDGFRA* amplification in adult HGA, including 22.5%

Table 8. Clinical characteristics of 60 adult IDH1 mutant anaplastic astrocytoma.

Patient characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number	49 (81.7%)	11 (18.3%)		
Mean age (years ± SD)	45.1 ± 10.9	41.3 ± 7.30		0.29
Sex ratio (M : F)	10	2.5	4.000 (0.5281–30.30)	0.20
Median survival (days)	3300 ($n = 45$)	960 ($n = 9$)		0.52
Location: frontal vs. other	46.8% ($n = 47$)	88.9% ($n = 9$)	0.5266 (0.1790–1.549)	0.26

CI = confidence interval; $IDH1^{R132H}$ = isocitrate dehydrogenase 1; SD = standard deviation.

Table 9. Clinical characteristics of 17 adult IDH1 mutant secondary GBM†.

Patient characteristics	No amplification	*PDGFRA* amplification	Odds ratio (95% CI)	*P*-value
Number	10 (58.8%)	7 (41.2%)		
Mean age (years ± SD)	41.4 ± 10.2	39.5 ± 7.41		0.89
Sex ratio (M : F)	3	6	0.5000 (0.0415–6.021)	1.0
Median survival (days)	540 ($n = 10$)	317.5 ($n = 7$)		0.15

CI = confidence interval; GBM = glioblastoma; $IDH1^{R132H}$ = isocitrate dehydrogenase 1; SD = standard deviation.
†Secondary GBM = GBM with documented progression from a lower-grade astrocytoma.

in GBM and 18.4% in AA. In contrast to EGFR FISH, where widespread high-level amplification is the rule, focal or low-level *PDGFRA* amplifications were even more common. As such, this alteration may be particularly susceptible to underestimation by dose-averaging techniques, such as PCR and array CGH. To reflect the diversity of *PDGFRA* signals observed by FISH, our scoring system included both low-level and high-level amplification. With the clinical variables available, we did not observe a significant survival difference between tumors with low- and high-level amplification; thus, we considered both "positive" for amplification (data not shown). In future studies, the level of *PDGFRA* amplification may have unique and unexpected prognostic associations as has been seen with EGFR (11).

In adult de novo GBM with IDH1 mutations, the frequency of *PDGFRA* amplification was striking with nearly half of tumors positive for amplification. Interestingly, this was not true in AAs. In IDH1 mutant AA, the frequency of *PDGFRA* amplification was only 18.3%. While the number of IDH1 mutant GBM and AA was relatively small (39 GBM and 60 AA) this difference was significant [43.6 vs. 18.3%, $P = 0.011$ (odds ratio 3.442; confidence interval 1.385–8.554)]. These data suggest that *PDGFRA* amplification may be an important event in the transition from AA to GBM in IDH1 mutant tumors. In support of this idea, analysis of a small number of IDH1 mutant secondary GBMs revealed a high frequency of *PDGFRA* amplification. Furthermore, the percent of cells with PDGFRA amplification tended to be less in IDH1 mutant de novo GBM as compared with IDH1 wild-type GBM, as suggested by the number of cases with low-level amplification (12/17; 71%) vs. (20/36; 56%), respectively. Gene amplification may be particularly important in the progression of IDH1 mutant astrocytoma as Lai *et al* (15) identified EGFR amplification in a smaller percentage of cells in IDH1 mutant vs. IDH1 wild-type tumors. Interestingly, while survival tended to be shorter in *PDGFRA*-amplified, IDH1 mutant AA and secondary GBM this did not reach statistical significance. The difference in survival between IDH1 mutant de novo GBM vs. AA and secondary GBM may reflect an insufficient sample size and high number of censored subjects for the latter or it may suggest potential biologic differences between clinically defined de novo GBM and IDH1 mutant HGAs that progress from a lower-grade tumor.

Reports on the frequency of *PDGFRA* amplification in pediatric HGA vary (range 3.4–12%) with up to 50% reported in irradiation-induced HGAs (16, 20, 23). In a large study of pediatric HGAs, PDGFR amplification as detected by array CGH was identified in 12% of HGAs overall and 17% of GBM (20). In DIPG, *PDGFRA* amplification may be even more common than in other HGAs, with reported estimates of 29% (22, 32). Using FISH we identified *PDGFRA* amplification in 29.3% of HGAs, 38.6% in GBM and 21.2% in AA. While we did not observe a significant association between brainstem location and *PDGFRA* amplification, we did not specifically target this group of tumors for analysis and only 12 HGAs involved the brainstem or cerebellum. *PDGFRA* status was not associated with overall survival in our cohort of pediatric HGAs.

This large multi-institutional study included a broad cross-section of cases from 10 major medical centers; however, it was a retrospective study and has inherent biases including potential case selection bias. In addition, we had access to only limited clinical and molecular data. Indeed, genetic information such as *PDGFRA* mutation status, shown to be common in amplified tumors (18), and co-amplification of EGFR was not assessed (24, 25). Because of several cases with co-amplification of PDGFRA and the centromeric region for which a ratio near 1.0 would falsely exclude PDGFRA amplification, we did not use the PDGFRA/CEP4 ratio to define gene amplification. While this allowed us to identify all cases with PDGFRA amplification we were not able to assess whether PDGFRA was selectively amplified or was co-amplified with other potential oncogenic genes on chromosome 4, particularly KIT and KDR (VEGFR2), given that specific probes for these other genes were not applied. In addition, while our M:F ratio for adult GBM (1.6) was similar to the reported ratio of 1.58, our adult AAs had a very high M:F ratio (8.1) relative to the reported ratio of 1.39 (4). As *PDGFRA* amplification tended to be more common in female patients, we may have underestimated the overall frequency of *PDGFRA* amplification in this population.

In this study we define a set of criteria to assess PDGFR amplification in routine clinical samples and provide an estimate of the frequency of PDGRA amplification in a large set of pediatric and adult HGAs. In our cohort, *PDGFRA* amplification did not have prognostic significance in pediatric HGA. In adults, we identified *PDGFRA* amplification as an independent prognostic factor in IDH1 mutant de novo GBM. These data have important potential implications regarding tumor biology and prognosis and additional studies in a larger number of IDH1 mutant de novo GBM are required.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

REFERENCES

1. Alentorn A, Marie Y, Carpentier C, Boisselier B, Giry M, Labussiere M *et al* (2012) Prevalence, clinico-pathological value, and co-occurrence of PDGFRA abnormalities in diffuse gliomas. *Neuro-Oncol* **14**:1393–1403.

2. Baxter EJ, Hochhaus A, Bolufer P, Reiter A, Fernandez JM, Senent L *et al* (2002) The t(4;22)(q12;q11) in a typical chronic myeloid leukaemia fuses BCR to PDGFRA. *Hum Mol Genet* **11**: 1391–1397.
3. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A, Pedraza A, Holland E (2009) Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PLoS ONE* **4**:e7752.
4. CBTRUS (2011) CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004–2007. Source: Central Brain Tumor Registry of the United States, Hinsdale, IL. Available at: http://www.cbtrus.org (accessed 7 March 2013).
5. Elkhaled A, Jalbert LE, Phillips JJ, Yoshihara HA, Parvataneni R, Srinivasan R *et al* (2012) Magnetic resonance of 2-hydroxyglutarate in IDH1-mutated low-grade gliomas. *Sci Transl Med* **4**:116ra5.
6. Graf E, Schmoor C, Sauerbrei W, Schumacher M (1999) Assessment and comparison of prognostic classification schemes for survival data. *Stat Med* **18**:2529–2545.
7. Guha A, Dashner K, Black PM, Wagner JA, Stiles CD (1995) Expression of PDGF and PDGF receptors in human astrocytoma operation specimens supports the existence of an autocrine loop. *Int J Cancer* **60**:168–173.
8. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A *et al* (2009) Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol* **118**:469–474.
9. Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B, Nister M (1992) Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. *Cancer Res* **52**:3213–3219.
10. Hirsch FR, Herbst RS, Olsen C, Chansky K, Crowley J, Kelly K *et al* (2008) Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. *J Clin Oncol* **26**:3351–3357.
11. Hobbs J, Nikiforova MN, Fardo DW, Bortoluzzi S, Cieply K, Hamilton RL, Horbinski C (2012) Paradoxical relationship between the degree of EGFR amplification and outcome in glioblastomas. *Am J Surg Pathol* **36**:1186–1193.
12. Horbinski C, Miller CR, Perry A (2011) Gone FISHing: clinical lessons learned in brain tumor molecular diagnostics over the last decade. *Brain Pathol* **21**:57–73.
13. Molinaro AM, Lostritto K (2010) Statistical resampling for large screening data analysis such as classical resampling Bootstrapping, Markov chain Monte Carlo, and statistical simulation and validation strategies. In: *Statistical Bioinformatics: A Guide for Life and Biomedical Science Researchers*, JK Lee (ed.), pp. 219–248. John Wiley & Sons, Inc: Hoboken, NJ.
14. Kim YH, Nonoguchi N, Paulus W, Brokinkel B, Keyvani K, Sure U *et al* (2012) Frequent BRAF Gain in Low-Grade Diffuse Gliomas with 1p/19q Loss. *Brain Pathol* **22**:834–840.
15. Lai A, Kharbanda S, Pope WB, Tran A, Solis OE, Peale F *et al* (2011) Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. *J Clin Oncol* **29**:4482–4490.
16. Nicolaides TP, Li H, Solomon DA, Hariono S, Hashizume R, Barkovich K *et al* (2011) Targeted therapy for BRAFV600E malignant astrocytoma. *Clin Cancer Res* **17**:7595–7604.
17. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP *et al* (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* **17**:510–522.
18. Ozawa T, Brennan CW, Wang L, Squatrito M, Sasayama T, Nakada M *et al* (2010) PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. *Genes Dev* **24**:2205–2218.
19. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P *et al* (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**:1807–1812.
20. Paugh BS, Qu C, Jones C, Liu Z, Adamowicz-Brice M, Zhang J *et al* (2010) Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J Clin Oncol* **28**:3061–3068.
21. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD *et al* (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* **9**:157–173.
22. Puget S, Philippe C, Bax DA, Job B, Varlet P, Junier MP *et al* (2012) Mesenchymal transition and PDGFRA amplification/mutation are key distinct oncogenic events in pediatric diffuse intrinsic pontine gliomas. *PLoS ONE* **7**:e30313.
23. Schiffman JD, Hodgson JG, VandenBerg SR, Flaherty P, Polley MY, Yu M *et al* (2010) Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. *Cancer Res* **70**:512–519.
24. Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ *et al* (2011) Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell* **20**:810–817.
25. Szerlip NJ, Pedraza A, Chakravarty D, Azim M, McGuire J, Fang Y *et al* (2012) Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response. *Proc Natl Acad Sci U S A* **109**:3041–3046.
26. Team R Development Core Team (2005) R: a language and environment for statistical computing, reference index version 2.15.0. R Foundation for Statistical Computing. Vienna, Austria.
27. The Cancer Genome Atlas Research Network (TCGA) (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **455**:1061–1068.
28. Tiseo M, Bartolotti M, Gelsomino F, Bordi P (2010) Emerging role of gefitinib in the treatment of non-small-cell lung cancer (NSCLC). *Drug Des Devel Ther* **4**:81–98.
29. Toedt G, Barbus S, Wolter M, Felsberg J, Tews B, Blond F *et al* (2011) Molecular signatures classify astrocytic gliomas by IDH1 mutation status. *Int J Cancer* **128**:1095–1103.
30. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD *et al* (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* **17**:98–110.
31. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W *et al* (2009) IDH1 and IDH2 mutations in gliomas. *N Engl J Med* **360**:765–773.
32. Zarghooni M, Bartels U, Lee E, Buczkowicz P, Morrison A, Huang A *et al* (2010) Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets. *J Clin Oncol* **28**:1337–1344.