Neuro-Oncology 14(11):1393–1403, 2012.
doi:10.1093/neuonc/nos217

Prevalence, clinico-pathological value, and cooccurrence of *PDGFRA* abnormalities in diffuse gliomas

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PDGFRA is a critical gene in glioma biology. Similar to _EGFR_, _PDGFRA_ has been shown to be overexpressed, amplified, mutated, or truncated in gliomas, particularly glioblastomas. In addition, _PDGFRA_ has been recently shown to be rearranged in glioblastoma. However, the frequency, cooccurrence, and clinical value of _PDGFRA_ abnormalities in diffuse gliomas remain unclear. We investigated _PDGFRA_ abnormalities and their clinical impact on 619 primary diffuse gliomas, including 167 grade II, 168 grade III, and 284 grade IV gliomas, with use of BAC-aCGH and validated our findings by quantitative polymerase chain reaction (PCR). We studied PDGFRA expression using reverse-transcription quantitative PCR in 84 gliomas and 12 non-tumor samples. In 138 samples, we also screened PDGFRA point mutations in exons 5, 7, 8, 9, 10, 11, and 23; presence of _KDR-PDGFRA_ fusion gene; and PDGFRA truncation. _PDGFRA_ was amplified and gained in 5.2% and 1.9% of samples, respectively. In addition _PDGFRA_ was point-mutated, rearranged, and truncated in 2.9%, 0%, and 0.7% of cases, respectively. _PDGFRA_ point mutations were observed exclusively in grade IV gliomas and in 12.5% of _PDGFRA_-amplified tumors. High-level _PDGFRA_ amplification was associated with _PDGFRA_ overexpression, high malignancy grade, and older patient age. Of interest, high-level _PDGFRA_ amplification has an independent negative prognostic value for progression-free survival and overall survival among patients with grade III tumors. _PDGFRA_ is altered through various genetic mechanisms in a subset of high-grade gliomas in patients who might be ideal candidates for _PDGFRA_ inhibitor treatment, and _PDGFRA_ gene amplification could be used as a prognostic biomarker in anaplastic gliomas.

Keywords: amplification, glioma, mutation, *PDGFRA*, prognosis.

Gliomas are the most common primary brain tumors in adults.[1] The WHO classifies diffuse gliomas based on the proliferating cell type (ie, astrocytoma, oligodendroglioma, or oligoastrocytoma) and the grade of malignancy (ie, from II to IV).[2]

Although significant progress has been made in the treatment of patients with glioma, this disease remains incurable. Over the past several years, important advancements in the understanding of molecular gliomagenesis have been accomplished, leading to new therapeutic perspectives.[3] Indeed, multiple growth factor receptors with tyrosine kinase activity have been shown to participate in glioma tumorigenesis and are currently targetable by innovative drugs (eg, *EGFR*, *PDGFRA*, or *VEGFR*).[4]

Platelet-derived growth factor receptor A (*PDGFRA*) is the second most frequently mutated tyrosine kinase receptor, following *EGFR*, in glioblastomas (GBMs).[4,5] *PDGFRA* is a transmembrane receptor with 5 immunoglobulin-like repeats in its extracellular domain and a tyrosine kinase (TK) in its intracellular domain. The binding of a ligand to the receptor activates pivotal downstream signal transduction pathways that

Received April 2, 2012; accepted August 2, 2012.

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promote oncogenesis, including MAP kinase, PI3K/AKT, JAK/STAT, and PLC-PKC.[6]

PDGFRA plays an important role in the normal development of the CNS by regulating normal glial cell proliferation and oligodendrocyte differentiation.[7] *PDGFRA* has also been implicated in several cancers, including CNS malignancies.[8] Indeed, several *PDGFRA* abnormalities have been detected in gliomas (eg, amplification, overexpression, in-frame deletion, point mutation, and rearrangement)[4,5,9–15] (Fig. 1A).

The clinical significance, prevalence, and co-occurrence of these *PDGFRA* abnormalities in the various glioma subcategories have not yet been determined. Indeed, these mutations have been studied mainly in GBM.[4,13,14,16] This led us to conduct the present study to assess *PDGFRA* status in diffuse gliomas, because these abnormalities are currently candidate targets for innovative molecular therapies in personalized medicine.

Materials and Methods

Patients

The following inclusion criteria were used for patients and tumors in the present study: age ≥18 years at pathological diagnosis, histological diagnosis of diffuse glioma, primary tumor with no history of brain tumor, detailed clinical information at diagnosis and during follow-up, availability of paired blood and tumor samples, consent form for molecular analysis provided by the patient, and available *PDGFRA* gene copy number status determined via BAC-array based comparative genomic hybridization (BAC-aCGH).

On the basis of the aforementioned inclusion criteria, 619 patients were enrolled in the present study: (1) 167 WHO grade II gliomas (88 oligodendrogliomas, 59 oligoastrocytomas, and 20 astrocytomas); (2) 168 WHO grade III gliomas (27 astrocytomas, 70 oligodendrogliomas, and 71 oligoastrocytomas); and (3) 284 WHO

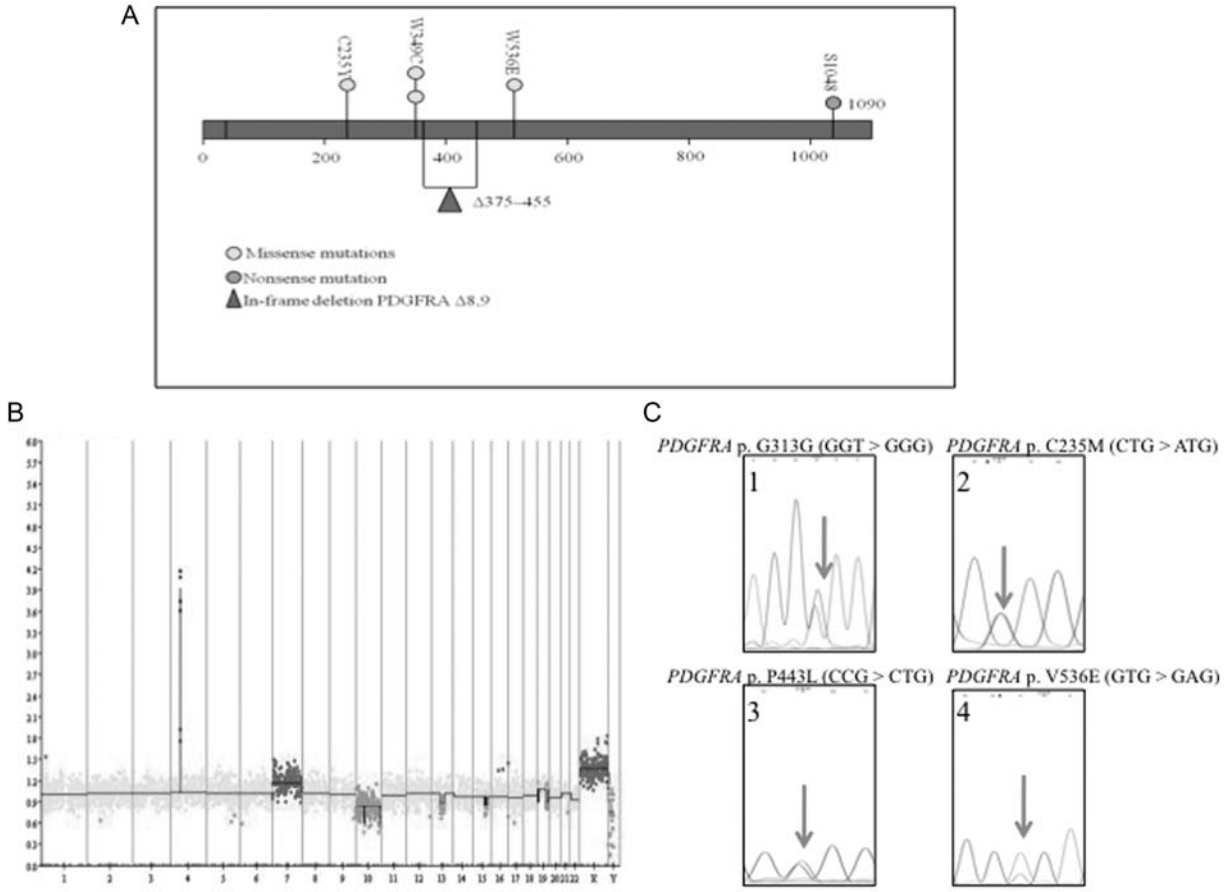

Fig. 1. Previously reported *PDGFRA* mutations in gliomas. (A) Representation of the previously described *PDGFRA* mutations according to the different protein domains. Missense mutations,[5] nonsense mutations,[16] and in-frame deletion.[13–15] (B) BAC-aCGH of a GBM exhibiting *PDGFRA* amplification (green circle). (C) Chromatograms of *PDGFRA* point-mutations reported in glioma.

grade IV gliomas (200 classic GBMs and 84 GBMs with an oligodendroglial component [GBMO]).

DNA Extraction and BAC-aCGH

DNA extraction was performed using the DNeasy Mini kit (Qiagen) according to the manufacturer's recommendations. DNA concentration and quality were determined spectrophotometrically (NanoDrop).

BAC-aCGH experiments were conducted as previously described.[17] Fluorescence levels (cyanine-5–labeled tumor DNA/cyanine-3–labeled control DNA) were measured and processed using GLAD pipeline to determine the genomic status of each BAC (amplification, gain, balanced, hemizygous deletion, or homozygous deletion). Extreme cyanine 5/cyanine 3 ratios of ≥ 3 and ≤ 0.6 were considered to be suggestive of amplifications and homozygous deletions, respectively (Fig. 1B).

Validation of PDGFRA *Copy Number Status Using Quantitative Polymerase Chain Reaction (qPCR)*

PDGFRA-amplified tumors detected on BAC-aCGH were further confirmed using real-time qPCR analysis. The *PDGFRA* primers amplified a genomic fragment overlapping *PDGFRA* intron 15 and exon 15 (TaqMan *PDGFRA* Copy Number Assays [FAM Dye], Assay ID Hs02749151_cn; Applied Biosystems). The reference primers amplified a genomic fragment from RNase P (TaqMan RNase P Detection Reagents [HEX Dye], no. 4316831; Applied Biosystems).

Table 1. Primers used for qPCR testing PDGFRA mRNA expression level

Gene	Forward or Reverse	Sequence	UPL probe
PDGFRA	F	AGGTGGTTGACCTTCAATGG	#80
	R	TTTGATTTCTTCCAGCATTGTG	
PPIA	F	CCTAAAGCATACGGGTCCTG	#47
	R	TTTCACTTTGCCAAACACCA	

Abbreviations: qPCR, quantitative PCR; PDGFRA, platelet-derived growth factor receptor alpha; PPIA, peptidylprolyl isomerase A (cyclophilin A); F, forward; R, reverse; UPL, Universal Probe Library.

RNA Extraction and PDGFRA *Expression Using Reverse Transcription (RT) and qPCR*

Total RNAs were isolated using the RNeasy Lipid Tissue Minikit (Qiagen) according to the manufacturer's recommendations. The RNA concentration was determined using spectrophotometer (NanoDrop). RNA was reverse transcribed using the SuperScript system (Invitrogen) according to the manufacturer's protocol. RNA quality was assessed using Agilent RNA Nano 6000 LabChip kits and an Agilent 2100 Bioanalyzer (minimum RNA integrity number of 7).

RT-qPCR was performed using a Universal Probe Library (Roche Applied Science).[18] The primers are reported in Table 1. cDNA was synthesized from 1 μg of total RNA and used for qPCR. Thermal cycling protocol was 10 min at 95°C, denaturation for 10 s at 95°C, annealing for 30 s at 60°C, and extension for 1 s at 72°C for 45 cycles, followed by 10 s at 40°C of cooling using the LightCycler 480 real-time PCR system. Data were expressed as ΔCt values [ΔCt = Ct of the target gene (*PDGFRA*) – Ct of the control gene (*PPIA*)]. The $2^{-\Delta\Delta Ct}$ equation was used to determine the fold expression changes relative to nontumor tissues.

PDGFRA *mutations*

PDGFRA-amplified tumors were screened for point mutations in exons 5, 7, 8, 9, 10, 11, and 23, as described previously.[19] The primers are reported in Table 2.

Screening for PDGFRAΔ[8,9] *Mutants and for* KDR-PDGFRA *Fusions (*KP *Gene)*

RNA amplified from *PDGFRA* was reverse transcribed to search for truncated genes that lacked exons 8 and 9.

Table 2. Primers used for PDGFRA Sanger sequencing

Exon	F or R	Sequence
5	F	TGTAAAACGACGGCCAGTCCACTGCTGAGGAATGCGGT
	R	CAGGAAACAGCTATGACCTCCCAGAGGTGGGAAGCTAAGG
7	F	TGTAAAACGACGGCCAGTGGCAAAGGCATCACAATGCTG
	R	CAGGAAACAGCTATGACCAGGGATGTGATTTCAAGCATCTCTTA
8	F	TGTAAAACGACGGCCAGTGTCAGGCCGTGGCTGAACTG
	R	CAGGAAACAGCTATGACCAAAGTGCCAGGCTTTCCTTGG
9	F	TGTAAAACGACGGCCAGTTGCTGCTAACCATGTGGGTCTG
	R	CAGGAAACAGCTATGACCGAGCCCTGGACCTTCTTCTCACA
10	F	TGTAAAACGACGGCCAGTGAGAAGGGAGGGCTCCAGGC
	R	CAGGAAACAGCTATGACCTTTGGCGAAAGTCACACGGC
11	F	TGTAAAACGACGGCCAGTAGGCAGCCCTCACACTTCCC
	R	CAGGAAACAGCTATGACCCAGCTGCATCGGGTCCACAT
23	F	TGTAAAACGACGGCCAGTTCCACGTGGCCTACCACAGC
	R	CAGGAAACAGCTATGACCCACACCACTGAGATGCTACTGAGGC

Abbreviations: PDGFRA, platelet-derived growth factor receptor alpha; F, forward; R, reverse.

The same samples were screened for the *KP* fusion gene. All primers and PCR conditions were identical to those described previously.[13,14] In brief, PCR amplification was performed using FastStart Taq DNA polymerase (Roche) in a thermocycling program consisting of 3 min at 94°C, followed by 30 cycles of denaturation for 45 s at 94°C, annealing for 30 s at 55°C, and extension for 90 s at 72°C, followed by a 10 min final extension. To validate these findings, the obtained cDNA was sequenced.

IDH1 *and* IDH2 *Mutational Status*

IDH1 and *IDH2* mutations in the hotspot codons R132 and R172, respectively, were assessed by bidirectional cycle sequencing of PCR-amplified primers, as previously described.[20]

Chromosome Arm 1p/19q Codeletion Was Detected Using BAC-aCGH and Validated Using Microsatellite Analysis

Chromosome arms 1p/19q status was assessed using BAC-aCGH and validated by microsatellites polymorphisms analysis using the following markers, as described previously: D1S450, D1S2667, D1S234, D1S2890, D1S2841, D19S425, D19S219, D19S412, and D19S418.[21]

Statistical analysis

For univariate analysis, χ^2 or Fisher's exact, and Mann-Whitney *U* tests were used to compare categorical and continuous variables, respectively. *PDGFRA* expression level was compared among nontumor tissues, nonamplified tumors, and *PDGFRA*-amplified tumors with use of the nonparametric Kruskal-Wallis test. The Spearman's rank order correlation test was used to analyze the relationship between *PDGFRA* amplification and other alterations (truncation, point mutation, and expression). A Spearman's ρ value of −1 indicated perfect inverse/negative correlation, and a value of 1 indicated perfect/positive correlation. The agreement between BAC-aCGH and microsatellite analysis of 1p19q codeletion detection and between BAC-aCGH and qPCR for *PDGFRA* amplification was assessed using Fleiss κ statistics. A κ value of <0 indicated poor agreement, whereas a κ value of 0.81–1.00 indicated almost perfect agreement.[22]

Overall survival (OS) and progression-free survival (PFS) were evaluated from diagnosis to death and to the first clinical and/or radiological documentation of progression, respectively. All patients without evidence of relapse at the time of the last clinical visit were censored. OS and PFS were estimated using Kaplan-Meier methodology and compared using the log-rank test. A Cox proportional hazards model was used for the multivariate analysis. Two-sided *P* values <.05 were interpreted as statistically significant. Analyses were performed in R, version 2.13.1 (http://www.R-project.org).

Results

Patient and Tumor Characteristics

From 2005 through 2009, 619 diffuse gliomas were characterized using BAC-aCGH (from 345 men and 274 women; sex ratio, 1.3). The median age of the study population was 49.5 years (interquartile range, 37.2–61.2 years). The clinical characteristics of patients with *PDGFRA*-amplified and nonamplified diffuse gliomas are shown in Table 3. All patients provided their written consent for molecular analysis.

PDGFRA *High-Level Amplification and Gain*

PDGFRA high-level amplification and gain were evaluated using BAC-aCGH validated by qPCR ($\kappa = 0.89$; 95% confidence interval [CI], 0.69–1.00); these events were detected in 32 (5.2%) of 619 and 12 (1.9%) of 619 cases, respectively (Fig. 1B).

Correlation with Tumor Pathology.—Both *PDGFRA* high-level amplification and gain were detected in 0 (0%) of 167 WHO grade II, 6 (3.6%) of 168 WHO grade III, and 26 (9.15%) of 284 WHO grade IV gliomas (Table 3). In addition, *PDGFRA* gain was detected in 1 (0.6%) of 167 WHO grade II, 2 (1.1%) of 168 WHO grade III, and 9 (3.1%) of 284 WHO grade IV gliomas.

PDGFRA high-level amplification was not associated with tumor phenotype (18 of 248, 3 of 127, and 11 of 230 of astrocytic, oligodendrocytic, and oligoastrocytic gliomas, respectively; $P = 0.1$) (Table 3), even when GBMO were analyzed as oligodendrocytic tumors.

Table 3. Patients and tumors characteristics

Characteristics	*PDGFRA* Amplified (%)	*PDGFRA* Non-amplified (%)	*P*
No. of tumors (619)	32 (5.2)	587 (94.8)	
Age at diagnosis			
Median (years)	58.8	48.7	0.0002
Interquartile range	51.2–68.6	37–60.9	
Gender			
Female (274)	16 (5.8)	258 (94.2)	NS
Male (345)	16 (4.6)	329 (95.4)	
Tumor WHO grade			
Grade II (167)	0	167 (100)	8.032e-06
Grade III (168)	6 (3.6)	162 (96.4)	
Grade IV (284)	26 (9.1)	258 (90.9)	
Tumor phenotype			
Astrocytoma (248)	18 (7.3)	230 (92.7)	NS
Oligoastrocytoma (130)	3 (2.3)	127 (97.7)	
Oligodendroglioma (including GBMO) (241)	11 (4.6)	230 (95.4)	

Abbreviations: NS, not significant; WHO, World Health Organization; GBMO, glioblastoma with an oligodendroglial component.

PDGFRA high-level amplification was strongly associated with a high grade of malignancy ($P = 8.032e-06$) (Table 3). Likewise, *PDGFRA* gain was also associated with a high grade of malignancy ($P = 6.793e-06$).

Correlation with Clinical Characteristics and Outcome of Patients.—Patients with *PDGFRA*-amplified tumors were significantly older (59.8 years vs 48.7 years; $P = .0002$). No statistically significant association with patient sex was observed (Table 3).

In WHO grade III gliomas, *PDGFRA* amplification had a negative impact on OS and PFS ($P = 5.18e-05$, Fig. 2A; $P = .001$, Fig. 2B) and had an independent prognostic value in multivariate analysis that included chromosome arm 1p/19q status and *IDH* mutation (Tables 4 and 5). Both *IDH* mutation and 1p/19q codeletion were associated with longer PFS and OS in univariate and multivariate analysis (Tables 4 and 5). The agreement between BAC-aCGH and microsatellite analyses was very good ($\kappa = 0.91$; 95% CI, 0.78–1.00). Chromosome arms 1p/19q codeletion was observed in 22% of WHO grade III tumors.

In WHO grade IV tumors, *PDGFRA* amplification does not have any prognostic significance in terms of OS and PFS (Fig. 2C and D).

PDGFRA *Mutations*

Four of the 32 *PDGFRA*-amplified tumors (12.5%) exhibited somatic point mutations in *PDGFRA*. All tumors with point mutations were WHO grade IV. One silent mutation, a G > T transversion at codon G313 (GGT > GGG) (Fig. 1C, Panel 1), was detected. The other 3 were missense mutations: 1 at codon C235M (CTG > ATG) (Fig. 1C, Panel 2), 1 at codon P443L (CCG > CTG) (Fig. 1C, Panel 3), and 1 at codon V536E (GTG > GAG) (Fig. 1C, Panel 4). Each point mutation occurred in different samples, and all have been reported previously.[5] Of interest, among WHO grade IV tumors, *PDGFRA* mutation was observed more frequently in GBMO, although this difference in frequency did not reach statistical significance (75% vs 24%; $P = 0.07$).

Screening for PDGFRA *Truncation and* KDR-PDGFRA *Fusion in* PDGFRA-*Amplified Samples*

PDGFRA truncation (*PDGFRA*Δ[8,9]) was observed in 1 of the 12 *PDGFRA*-amplified tumors (Fig. 3A) and validated by direct sequencing. None of our *PDGFRA*-amplified tumors contained the fusion gene *KDR-PDGFRA*.[14]

PDGFRA mRNA Expression Analysis

The expression level of PDGFRA mRNA in 16 *PDGFRA*-amplified tumors (all WHO grade IV) was compared with that in 68 nonamplified tumors (including 20 WHO grade IV, 26 WHO grade III, and 22 WHO grade II) and 12 nontumor brain tissues (epilepsy

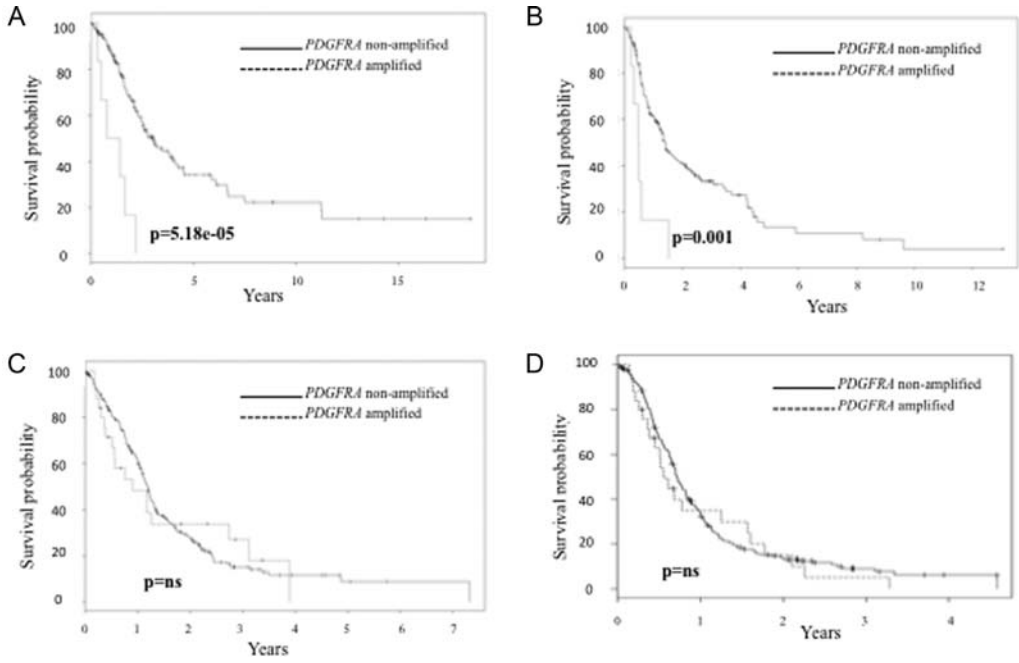

Fig. 2. Prognostic value of PDGFRA abnormalities in gliomas (continuous line indicates *PDGFRA*- non amplified tumors and broken line indicates PDGFRA amplified tumors). (Panel A). OS in PDGFRA amplified vs nonamplified WHO III gliomas. (Panel B). PFS in PDGFRA amplified vs nonamplified tumors in WHO grade III gliomas. (Panel C). OS in PDGFRA amplified vs nonamplified WHO IV gliomas. Panel d. PFS in PDGFRA amplified vs nonamplified tumors in WHO grade IV gliomas.

Table 4. Univariate and multivariate analysis of progression free survival in WHO III tumors

	Variable	*n*	Unvariate analysis		Multivariate analysis	
			median, years (95%CI)	*P* value	HR (95%CI)	*P* value
Sex	Women	77	3.8 (2.4–5.2)	NS		
	Men	91	2.2 (1.5–2.9)			
Age	<45	83	1.7 (0.8–2.6)	.006	1	NS
	≥45	85	3 (2–4.1)		1.6 (0.9–2.6)	
KPS	≥70	9	1.3 (0.2–2.5)	.04	1	NS
	<70	150	0.8 (0.2–1.3)		1.8 (0.8–4)	
Surgery	Biopsy	60	2.1 (1.5–2.7)	4e-04	1	NS
	Resection	100	3.8 (2.7–4.9)		1.4 (0.8–2.3)	
Phenotype	Astrocytoma	27	1.4 (1.1–1.7)	NS		
	Oligodendroglioma	70	1.3 (0.8–1.8)			
	Oligoastrocytoma	71	1.3 (0.9–1.8)			
Treatment	RT alone	40	1.2 (0.4–2.6)	NS		
	All other regimens	128	1.0 (0.5–2.1)			
PDGFRA amplification	No	162	1.4 (1.1–1.7)	.001	1	.004
	Yes	6	1.3 (1.1–1.5)		4.1 (1.5–10.7)	
IDH mutation	No	66	0.7 (0.5–1)	1e-09	1	.001
	Yes	90	3 (1.7–4.4)		0.3 (0.2–0.7)	
1p19q codeletion	No	131	0.9 (0.6–1.2)	3.6e-05	1	.035
	Yes	37	3.5 (2–4.9)		0.5 (0.2–0.9)	

Abbreviations: KPS, Karnofsky Performance Status; PDGFRA, platelet-derived growth factor receptor alpha; IDH 1 and 2, isocitrate dehydrogenase 1 and 2; Oligodendroglial includes oligodendroglioma and oligoastrocytoma.

Table 5. Univariate and multivariate analysis of overall survival in WHO III tumors

	Variable	*n*	Unvariate analysis		Multivariate analysis	
			median, years (95%CI)	*P* value	HR (95%CI)	*P* value
Sex	Women	77	2.3 (1.4–3.1)	NS		
	Men	91	1.5 (0.8–2.9)			
Age	<45	83	2.2 (1.2–3.3)	.0004	1	.004
	≥45	85	1.6 (0.9–2.9)		2.4 (1.3–4.3)	
KPS	≥70	9	3 (2–4.1)	.0064	1	.01
	<70	150	1.5 (0.8–1.7)		2.9 (1.3–7)	
Surgery	Biopsy	60	2 (1.5–2.6)	.0004	1	NS
	Resection	100	3.8 (2.7–4.8)		1.09 (0.6–1.9)	
Phenotype	Astrocytoma	27	2.5 (1.7–3.4)	NS		
	Oligodendroglioma	70	3.1 (1.4–4.8)			
	Oligoastrocytoma	71	2.5 (2.2–2.9)			
Treatment	RT alone	40	2.7 (1.7–4.6)	NS		
	All other regimens	128	1.6 (0.8–2.8)			
PDGFRA amplification	No	162	3.0 (2.2–3.8)	5.18e-05	1	.0003
	Yes	6	0.6 (0.1–1.9)		5.9 (2.2–15.6)	
IDH mutation	No	66	1.5 (1.2–1.8)	1.1e-12	1	.004
	Yes	90	6.6 (2.9–10.5)		0.4 (0.2–0.7)	
1p19q codeletion	No	131	2.2 (1.8–2.5)	2.68e-07	1	.002
	Yes	37	11.2 (2.45–20)		0.2 (0.09–0.59)	

Abbreviations: PDGFRA, platelet-derived growth factor receptor alpha; IDH 1 and 2, isocitrate dehydrogenase 1 and 2; Oligodendroglial includes oligodendroglioma and oligoastrocytoma

samples). Of interest, tumors harboring *PDGFRA* amplification exhibited an increased level of *PDGFRA* mRNA expression compared with their nonamplified counterparts and with nontumor brain tissue ($P = 1.4e\text{-}6$ and $P = 1.5e\text{-}7$, respectively) (Fig. 3B).

PDGFRA *Molecular Abnormalities in* PDGFRA *Nonamplified Gliomas*

We studied a cohort of 106 *PDGFRA* non-amplified glial tumors, including 32 low-grade gliomas (10 astrocytomas,

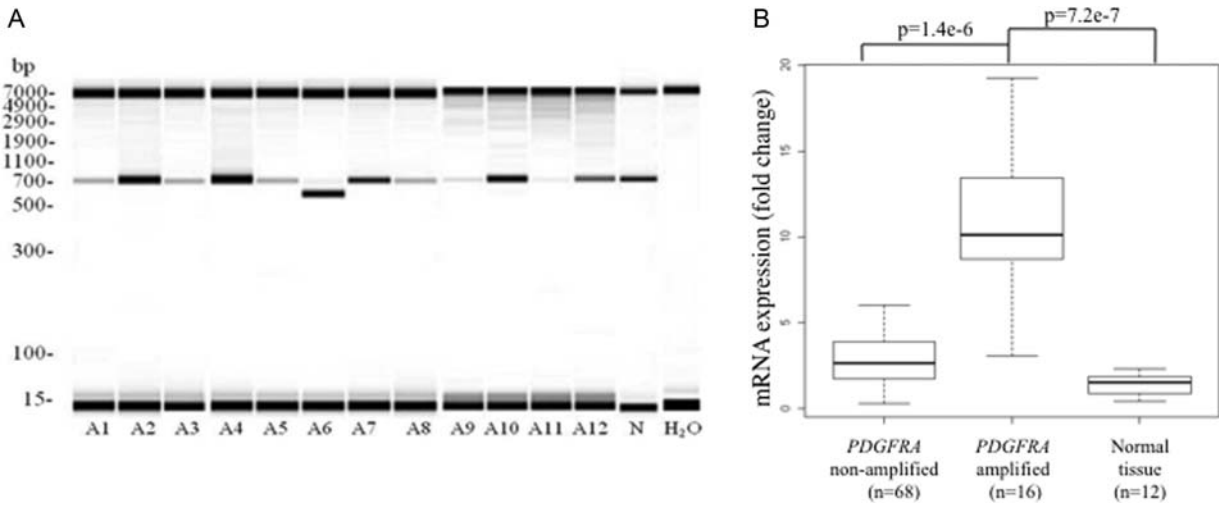

Fig. 3. (A) Reverse-Transcription PCR from total RNA searching for truncated PDGFRA transcript (*PDGFRA*Δ[8,9]). The low weight molecular band (ie, 642 base pairs) and the high weight molecular band (885 base pairs) indicate *PDGFRA* wild-type and truncated PDGFRA transcripts (ie, *PDGFRA*Δ[8,9]) respectively. Each line represents a distinct *PDGFRA*-amplified glioblastoma. PDGFRAΔ[8,9] truncation was detected in 1/12 *PDGFRA*-amplified glioblastoma (line A6). Line "N" indicates a glioblastoma with normal copy number of *PDGFRA*. H_20 indicates water as a negative control of the experiment. (B) mRNA expression of PDGFRA according to *PDGFRA* copy number status (amplified, nonamplified and normal brain tissue).

12 oligodendrogliomas, and 10 oligoastrocytomas); 32 anaplastic gliomas (6 astrocytomas, 18 oligodendrogliomas, and 8 oligoastrocytomas); and 42 GBMs. None of them was *PDGFRA*-point-mutated, *PDGFRA*-truncated, or *KDR-PDGFRA*-rearranged.

Correlation Between PDGFRA *Amplification and* PDGFRA *Mutation, Rearrangement,* IDH *Mutation, and 1p/19q Codeletion*

PDGFRA amplification was strongly associated with the *PDGFRA* expression level (Fig. 3B). In addition, an inverse correlation was observed between mutation and truncation. *PDGFRA* amplification was about twice more frequent in the mutated samples than in the truncated samples.

PDGFRA amplification and *IDH* mutation were inversely correlated; 12.9% of *PDGFRA*-amplified tumors were *IDH* mutated, compared with 2.6% of nonamplified tumors ($P = 0.001$). In addition, tumors featuring *PDGFRA* locus gain were less frequently *IDH* mutated, compared with tumors with normal *PDGFRA* copy number status (16.7% vs 43.2%; $P = .08$). Moreover, the presence of 1p/19q codeletion and *PDGFRA* amplification were mutually exclusive in WHO grade III tumors (0% of 1p/19q codeleted tumors were *PDGFRA*-amplified vs 4.6% of non-codeleted; $P = .03$).

Discussion

PDGFRA is a critical gene in neurogenesis and gliomagenesis. To our knowledge, this study represents the largest cohort of diffuse gliomas and the most comprehensive analysis of the prevalence, the clinicopathological significance, and the co-occurrence of the *PDGFRA* abnormalities most frequently reported in gliomas. Of interest, *PDGFRA* high-level amplification has an independent negative prognostic value in grade III tumors in terms of PFS and OS.

In our study, *PDGFRA* amplification was restricted to high-grade gliomas and was not observed in diffuse low-grade gliomas. A previous study has described *PDGFRA* amplification in low-grade gliomas (this study found amplification in 6 of 20 low-grade gliomas, but 83% of amplified tumors were astrocytomas).[9] Our series included 20 WHO grade II astrocytomas, none of which were *PDGFRA* amplified. These conflicting results require further investigation to specify the frequency of *PDGFRA* amplification in low-grade gliomas (Table 6).

Previous studies have reported that *PDGFRA* amplification occurs in 8.5%–26% of GBMs.[4,5,9,14] This wide range might be attributed to distinct definitions of gene amplification. Taken together, the published data and our results suggest that *PDGFRA* high-level amplification is observed in approximately 10% of GBMs (Table 6).[4,14]

PDGFRA amplification is strongly correlated with deleterious effects in WHO III gliomas in terms of PFS and OS. These results are consistent with our previous study, which focused on anaplastic oligodendrogliomas.[23] This prognostic value of *PDGFRA* amplification is independent of age, KPS, type of surgery, *IDH* mutation, and 1p/19q codeletion. In our series of WHO grade III, tumor phenotype (ie, astrocytoma, oligoastrocytoma, and oligodendroglioma) does not impact significantly patients' survival although a trend is observed. The prognostic value of phenotype in anaplastic

Table 6. Frequency and prognostic significance of PDGFRA alterations in gliomas

***PDGFRA* alteration**	***n***	**Histology and grade**								**Prognostic impact**	**References**
		AI	**AII**	**OII**	**MII**	**AIII**	**OIII**	**MIII**	**GBM**		
Point Mutation	91								1/91		4
	116								4/116	NP	5
	86[†]									NP	9
Rearrangement (truncation)	212								6/212 (6/15*)	NP	14
Rearrangement (fusion *KP*)	212								1/212	NP	
Amplification	206								23/206	NP	4
	170								44/170	NP	5
	57	0/1	5/10	1/10		0/2	2/13	1/2	3/19	No association with survival	9
	87	0/1	0/11**			0/12**			15/63	NP	14
	47								9/47	No association with survival	24
	78		1/35	0/19	0/9	5/15				Poorer prognosis in univariate analysis in the overall population (WHOII and WHOIII gliomas)	25
	390								33/390	No association with survival	26

Abbreviations: PDGFRA. platelet-derived growth factor receptor alpha; [†]histology and grade was not described, 4 silent mutations out of 86 gliomas.
**PDGFRA* amplified samples; **phenotype is not specified; KP. KDR-PDGFRA fusion gene; NP. not performed; A. astrocytoma; O. oligodendroglioma; M. mixed glioma or oligoastrocytoma; GBM. glioblastoma multiforme; I. WHO grade I; II. WHO grade II; III. WHO grade III; IV. WHO grade IV.

oligodendroglial tumors is debated, and some discrepancies might be at least partially explained by some variability in neuropathologic classification.[24] Similarly, intra- and interobserver variability in pathologic diagnosis might induce some variability in the prevalence of 1p/19q codeletion in anaplastic oligodendroglial tumors. Our results are at the lower limit of the range of the prevalence reported in the literature.[25,26]

Techniques used for 1p/19q codeletion testing in anaplastic oligodendroglial tumors might also explain variability in the prevalence of 1p/19q codeletion. Indeed, techniques testing a large number of genomic loci simultaneously (eg, comparative genomic hybridization or multiplex ligation-dependent probe amplification), compared with the techniques investigating a limited number of loci (eg, FISH or microsatellites analysis), allow a better distinction of whole chromosome arms 1p/19q codeletion vs partial 1p/19q codeletion.[27,28] The former alteration corresponding to a reciprocal unbalanced translocation t(1;19)(q10;p10) is actually the favorable prognostic biomarker in anaplastic oligodendroglial tumors.[29,30] Finally, the design of studies (eg, retrospective), because of biases issues, might also explain some variability in biomarkers assessment in oligodendroglial tumors.

No prognostic value for *PDGFRA* amplification was detected in WHO grade IV gliomas. Indeed, the prognostic significance of *PDGFRA* amplification has been debated in the literature (Table 6). Previous studies did not associate *PDGFRA* amplification with worse prognosis in any histological entity.[31] However, Pupitti et al. showed that *PDGFRA* amplification was associated with worse prognosis in a univariate analysis in gliomas.[32] Conversely, Nobusawa et al. studied 390 GBMs and did not find an association between the amplification of *PDGFRA* and survival.[33] As is the case for *EGFR* amplification, *PDGFRA* amplification seems not to have a prognostic value in GBM (Table 6).[34]

The prognostic value of *PDGFRA* mutations in gliomas has not been established previously (Table 6).[9,31,35] Similarly, the *KP*-fusion gene has been described only recently,[14] and there are no studies analyzing the clinical significance of these mutations. Unfortunately, we did not identify any sample with a *KP*-fusion gene; this seems to be a rare phenomenon in glioma.

Our series focused on *PDGFRA*-amplified gliomas because *PDGFRA* truncation and rearrangement has been described as unique to this group.[14] Whether the percentage of *PDGFRA* mutations in nonamplified tumors is similar to that in amplified tumors remains unknown, with the exception of *PDGFRA* deletion, [8,9] which has been detected exclusively in *PDGFRA* amplified gliomas.[14] In a series of 106 *PDGFRA* nonamplified gliomas, we did not find any *PDGFRA* mutation or rearrangement. In our series, the percentage of gliomas with truncations is much lower than that previously reported (8.3% vs 40%).[14]

We found a statistically significant association between *PDGFRA* amplification and the overexpression of *PDGFRA* mRNA ($P = 0.045$). In previous studies, the correlation between *PDGFRA* expression and copy number status was evaluated via immunohistochemistry.[9,36–38] These studies found that *PDGFRA* was overexpressed in approximately 50% of malignant gliomas.[9,36–38] In addition, the prognostic value of PDGFRA expression remains hotly debated.[9,36–38]

PDGFRA is a critical gene in glioma and particularly in GBM. It is amplified in approximately 10% of GBMs, but *PDGFRA* amplification has poor independent prognostic value in anaplastic gliomas. Taken together, our data and those reported in previous studies highlight *PDGFRA* alterations as a potential prognostic biomarker and a therapeutic target. Indeed, multiple *PDGFR* inhibitors are under development in academic and industrial laboratories.[39,40] To date, some have been tested in humans with limited success, but the patients in these trials were not selected on the basis of *PDGFRA* status.[41,42] *PDGFRA* truncation appears as a rare phenomenon in diffuse gliomas. Indeed, it is observed in 8%–40% of *PDGFRA*-amplified GBMs, which means in approximately 0.8%–4% of all GBMs. These results do not support the development of a vaccine therapy against *PDGFRAΔ*,[8,9] similar to that developed for *EGFRvIII* observed in ~20% of all GBM. However, further studies are warranted to specify the exact prevalence of this abnormality in various normal tissues, diffuse gliomas, and other cancer types.[43,44]

Acknowledgments

Institut Hospitalier Universitaire de Neurosciences Translationnelles de Paris.

Conflict of interest statement. None declared.

Funding

This work was supported by Obra Social la Caixa (to A.A.). This work is part of the national program Cartes d'Identité des Tumeurs (http://cit.ligue-cancer.net/), which is funded and developed by the Ligue Nationale Contre le Cancer.

References

1. Central Brain Tumor Registry of the United States. Available at http://www.CBTRUS.org. Accessed 12 December, 2011.

2. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*. 2007;114(2):97–109.

3. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359(5):492–507.
4. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455(7216):1061–1068.
5. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98–110.
6. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature*. 2001;411(6835):355–365.
7. Richardson WD, Pringle N, Mosley MJ, Westermark B, Dubois-Dalcq M. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell*. 1988;53(2):309–319.
8. Shih AH, Holland EC. Platelet-derived growth factor (PDGF) and glial tumorigenesis. *Cancer Lett*. 2006;232(2):139–147.
9. Martinho O, Longatto-Filho A, Lambros MBK, et al. Expression, mutation and copy number analysis of platelet-derived growth factor receptor A (PDGFRA) and its ligand PDGFA in gliomas. *Br J Cancer*. 2009;101(6):973–982.
10. Hermanson M, Funa K, Hartman M, et al. 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*. 1992;52(11):3213–3219.
11. Nistér M, Libermann TA, Betsholtz C, et al. Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor-alpha and their receptors in human malignant glioma cell lines. *Cancer Res*. 1988;48(14):3910–3918.
12. Di Rocco F, Carroll RS, Zhang J, Black PM. Platelet-derived growth factor and its receptor expression in human oligodendrogliomas. *Neurosurgery*. 1998;42(2):341–346.
13. Kumabe T, Sohma Y, Kayama T, Yoshimoto T, Yamamoto T. Amplification of alpha-platelet-derived growth factor receptor gene lacking an exon coding for a portion of the extracellular region in a primary brain tumor of glial origin. *Oncogene*. 1992;7(4):627–633.
14. Ozawa T, Brennan CW, Wang L, et al. PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. *Genes Dev*. 2010;24(19):2205–2218.
15. Clarke ID, Dirks PB. A human brain tumor-derived PDGFR-alpha deletion mutant is transforming. *Oncogene*. 2003;22(5):722–733.
16. Rand V, Huang J, Stockwell T, et al. Sequence survey of receptor tyrosine kinases reveals mutations in glioblastomas. *Proc Natl Acad Sci USA*. 2005;102(40):14344–14349.
17. Idbaih A, Marie Y, Lucchesi C, et al. BAC array CGH distinguishes mutually exclusive alterations that define clinicogenetic subtypes of gliomas. *Int J Cancer*. 2008;122(8):1778–1786.
18. Mouritzen P, Noerholm M, Nielsen PS, et al. ProbeLibrary: A new method for faster design and execution of quantitative real-time PCR. *Nature Methods*. 2005;2:313–316.
19. Sihto H, Sarlomo-Rikala M, Tynninen O, et al. KIT and platelet-derived growth factor receptor alpha tyrosine kinase gene mutations and KIT amplifications in human solid tumors. *J Clin Oncol*. 2005;23(1):49–57.
20. Houillier C, Wang X, Kaloshi G, et al. IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. *Neurology*. 2010;75(17):1560–1566.
21. Hoang-Xuan K, Capelle L, Kujas M, et al. Temozolomide as initial treatment for adults with low-grade oligodendrogliomas or oligoastrocytomas and correlation with chromosome 1p deletions. *J Clin Oncol*. 2004;22(15):3133–3138.
22. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159–174.
23. Idbaih A, Crinière E, Marie Y, et al. Gene amplification is a poor prognostic factor in anaplastic oligodendrogliomas. *Neuro Oncol*. 2008;10(4):540–547.
24. van den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. *Acta Neuropathol*. 2010;120(3):297–304.
25. van den Bent MJ, Carpentier AF, Brandes AA, et al. Adjuvant procarbazine, lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: a randomized European Organisation for Research and Treatment of Cancer phase III trial. *J Clin Oncol*. 2006;24(18):2715–2722.
26. Intergroup Radiation Therapy Oncology Group Trial 9402, Cairncross G, Berkey B, Shaw E, Jenkins R, Scheithauer B, et al. Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. *J Clin Oncol*. 2006;24(18):2707–2714.
27. Idbaih A, Marie Y, Pierron G, et al. Two types of chromosome 1p losses with opposite significance in gliomas. *Ann Neurol*. 2005;58(3):483–487.
28. Jeuken J, Cornelissen S, Boots-Sprenger S, Gijsen S, Wesseling P. Multiplex ligation-dependent probe amplification: a diagnostic tool for simultaneous identification of different genetic markers in glial tumors. *J Mol Diagn*. 2006;8(4):433–443.
29. Griffin CA, Burger P, Morsberger L, et al. Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. *J Neuropathol Exp Neurol*. 2006;65(10):988–994.
30. Jenkins RB, Blair H, Ballman KV, et al. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer Res*. 2006;66(20):9852–9861.
31. Joensuu H, Puputti M, Sihto H, Tynninen O, Nupponen NN. Amplification of genes encoding KIT, PDGFRalpha and VEGFR2 receptor tyrosine kinases is frequent in glioblastoma multiforme. *J Pathol*. 2005;207(2):224–231.
32. Puputti M, Tynninen O, Sihto H, et al. Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. *Mol Cancer Res*. 2006;4(12):927–934.
33. Nobusawa S, Stawski R, Kim Y-H, Nakazato Y, Ohgaki H. Amplification of the PDGFRA, KIT and KDR genes in glioblastoma: a population-based study. *Neuropathology*. 2011;31(6):583–588.
34. Quan AL, Barnett GH, Lee SY, et al. Epidermal growth factor receptor amplification does not have prognostic significance in patients with glioblastoma multiforme. *Int J Radiat Oncol Biol Phys*. 2005;63(3):695–703.
35. Wen PY, Yung WK, Lamborn KR, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99–08. *Clin Cancer Res*. 2006;12(16):4899–4907.
36. Varela M, Ranuncolo SM, Morand A, et al. EGF-R and PDGF-R, but not bcl-2, overexpression predict overall survival in patients with low-grade astrocytomas. *J Surg Oncol*. 2004;86(1):34–40.

37. Ribom D, Andrae J, Frielingsdorf M, Hartman M, Nistér M, Smits A. Prognostic value of platelet derived growth factor alpha receptor expression in grade 2 astrocytomas and oligoastrocytomas. *J Neurol Neurosurg Psychiatr*. 2002;72(6):782–787.
38. Paulsson J, Lindh MB, Jarvius M, et al. Prognostic but not predictive role of platelet-derived growth factor receptors in patients with recurrent glioblastoma. *Int J Cancer*. 2011;128(8):1981–1988.
39. Haberler C, Gelpi E, Marosi C, et al. Immunohistochemical analysis of platelet-derived growth factor receptor-alpha, -beta, c-kit, c-abl, and arg proteins in glioblastoma: possible implications for patient selection for imatinib mesylate therapy. *J Neurooncol*. 2006;76(2):105–109.
40. Bareford MD, Park MA, Yacoub A, et al. Sorafenib enhances pemetrexed cytotoxicity through an autophagy-dependent mechanism in cancer cells. *Cancer Res*. 2011;71(14):4955–4967.
41. Neyns B, Sadones J, Chaskis C, et al. Phase II study of sunitinib malate in patients with recurrent high-grade glioma. *J Neurooncol*. 2011;103(3):491–501.
42. Iwamoto FM, Lamborn KR, Robins HI, et al. Phase II trial of pazopanib (GW786034), an oral multi-targeted angiogenesis inhibitor, for adults with recurrent glioblastoma (North American Brain Tumor Consortium Study 06–02). *Neuro Oncol*. 2010;12(8):855–861.
43. Moscatello DK, Ramirez G, Wong AJ. A naturally occurring mutant human epidermal growth factor receptor as a target for peptide vaccine immunotherapy of tumors. *Cancer Res*. 1997;57(8):1419–1424.
44. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J Clin Oncol*. 2010;28(31):4722–4729.