PIK3CA Gene Mutations in Pediatric and Adult Glioblastoma Multiforme

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Abstract

The phosphatidylinositol 3-kinases (PI3K) are a family of enzymes that relay important cellular growth control signals. Recently, a large-scale mutational analysis of eight PI3K and eight PI3K-like genes revealed somatic mutations in *PIK3CA*, which encodes the p110α catalytic subunit of class IA PI3K, in several types of cancer, including glioblastoma multiforme. In that report, 4 of 15 (27%) glioblastomas contained potentially oncogenic *PIK3CA* mutations. Subsequent studies, however, showed a significantly lower mutation rate ranging from 0% to 7%. Given this disparity and to address the relation of patient age to mutation frequency, we examined 10 exons of *PIK3CA* in 73 glioblastoma samples by PCR amplification followed by direct DNA sequencing. Overall, *PIK3CA* mutations were found in 11 (15%) samples, including several novel mutations. *PIK3CA* mutations were distributed in all sample types, with 18%, 9%, and 13% of primary tumors, xenografts, and cell lines containing mutations, respectively. Of the primary tumors, *PIK3CA* mutations were identified in 21% and 17% of pediatric and adult samples, respectively. No evidence of *PIK3CA* gene amplification was detected by quantitative real-time PCR in any of the samples. This study confirms that *PIK3CA* mutations occur in a significant number of human glioblastomas, further indicating that therapeutic targeting of this pathway in glioblastomas is of value. Moreover, this is the first study showing *PIK3CA* mutations in pediatric glioblastomas, thus providing a molecular target in this important pediatric malignancy. (Mol Cancer Res 2006;4(10):709–14)

Introduction

Phosphatidylinositol 3-kinases (PI3K) are a family of lipid kinases involved in diverse cellular signaling pathways, including proliferation, differentiation, migration, trafficking, and glucose homeostasis (1). Recently, Samuels et al. (2) did a large-scale mutational analysis of PI3K and PI3K-like genes in several human cancers and found somatic point mutations in 25% to 32% of colorectal cancers, glioblastomas, and gastric cancers and 3% to 8% of breast, lung, and colorectal adenomas. All of the mutations were identified in *PIK3CA*, which codes for a catalytic subunit of the class IA PI3K multiprotein enzyme. Mutations were found in 10 of the 20 exons of this gene, with the helical (exon 9) and kinase (exon 20) domains harboring the most mutations. Since this initial discovery, *PIK3CA* mutations have been described in ovarian, hepatocellular, thyroid and endometrial cancers, acute leukemia, as well as in other central nervous system malignancies (3-9).

With respect to glioblastomas, Samuels et al. (2) first reported *PIK3CA* mutations in 4 of 15 (27%) samples. Since this initial description, several groups have reported a markedly lower *PIK3CA* mutation rate ranging from 0% to 7% (3, 10-12). In addition, there have been no reports of the mutation frequency in pediatric glioblastomas, which may have a different molecular etiology from that of glioblastomas in adult patients. In the present study, we have aimed to determine the frequency of *PIK3CA* mutations and copy number in a cohort of 73 glioblastoma samples derived from adult and pediatric primary tumors, xenografts, and cell lines.

Results

PIK3CA mutational analysis was done on 10 of 20 exons of *PIK3CA* by PCR amplification of genomic DNA followed by direct DNA sequencing. Exons 1, 2, 4, 5, 7, 9, 12, 13, 18, and 20 were specifically examined because previous work showed that *PIK3CA* mutations, although clustered in exons 9 and 20, were all observed within these 10 exons (2). Analysis of these

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Table 1. *PIK3CA* Mutations Found in 73 Glioblastomas

No.	Sample	Age (y)	Source	*PIK3CA* Mutations*			Previously reported	
				Exon	Nucleotide change	Amino acid change	Glioblastoma	Other malignancies
1	As3-104	16	P	1	G263A	R88Q	+	+
2	G-141T †	7	P	4	**C892A**	**P298T**	–	–
3	G-8T	52	P	4	**C928T**	**R310C**	–	–
4	SK-MG 17	NA	CL	4	**T1031G**	**V344G**	–	–
5	SK-MG 9	NA	CL	7	G1357A	E453K	–	+
6	X #5	57	X	9	G1624A	E542K	+	+
7	G-174T	62	P	9	G1633A	E545K	+	+
8	G-141T †	7	P	20	A3062G	Y1021C	–	+
9	G-41T	19	P	20	G3129T	M1043I	–	+
10	As3-9	78	P	20	**A3131G**	**N1044S**	–	–
11	SK-MG 26	NA	CL	20	C3139T	H1047Y	–	+
12	G-83T	77	P	20	G3145A	G1049S	+	+

Abbreviations: P, primary tumor; NA, not available; CL, cell line; X, xenograft.
*Nucleotide position refers to the position within the coding sequence, where position 1 corresponds to the first position of the start codon. Bold mutations have not been previously reported.
† Same sample.

exons in 73 glioblastoma samples identified 12 missense mutations in 11 (15%) samples (Table 1). *PIK3CA* mutations were found in 7 of 38 (18%), 1 of 11 (9%), and 3 of 24 (13%) primary tumors, xenografts, and cell lines, respectively (Fig. 1A). Corresponding normal tissue was available for each primary glioblastoma sample, and in all cases, the mutations were somatic. Mutations were identified in exons 1, 4, 7, 9, and 20. One mutation was found in exon 1 (G263A, R88Q), three in exon 4 (C892A, P298T; C928T, R310C; T1031G, V344G), one in exon 7 (G1357A, E453K), two in exon 9 (G1624A, E542K; G1633A, E545K), and five in exon 20 (A3062G, Y1021C; G3129T, M1043I; A3131G, N1044S; C3139T, H1047Y; G3145A, G1049S). One sample harbored two mutations, C892A, P298T and A3062G, A1021C. Representative sequence chromatograms are shown in Fig. 2.

The 38 primary glioblastoma samples examined in this study were derived from both pediatric (ages ≤21 years) and adult patients. Overall, somatic missense *PIK3CA* mutations were present in 7 of 38 (18%) of the total primary tumor samples examined. Fourteen samples were derived from pediatric patients, and 24 samples were obtained from adult patients. *PIK3CA* mutations were found in 3 of 14 (21%) and 4 of 24 (17%) pediatric and adult glioblastoma samples, respectively (Fig. 1B).

To determine if *PIK3CA* gene amplification was present in the samples examined, quantitative real-time PCR was done. No evidence of significant gene amplification (>4-fold) was found in any glioblastoma sample. Figure 3 illustrates the number of *PIK3CA* gene copies for each primary adult and pediatric glioblastoma sample, xenograft, and cell line. Samples of squamous cell carcinoma of the lung were included as a positive control with *PIK3CA* amplification (>4-fold) detected in 3 of 14 samples.

Discussion

In this study, 10 exons of *PIK3CA* were evaluated in 73 glioblastoma samples for mutations. Twelve missense mutations in the *PIK3CA* gene were identified in 11 samples with an overall mutation rate of 15% (Table 1). *PIK3CA* mutations were found in 18% of primary tumor samples, 9% of xenografts, and 13% of cell lines examined; mutations were found in 17% and 21% of the adult and pediatric primary tumor samples, respectively. Four of the 12 mutations, C892A, C928T, T1031G, and A3131G, are novel. Four additional mutations, G1357A, A3062G, G3129T, and C3139T, although described in other malignancies (2, 6), have not yet been reported in

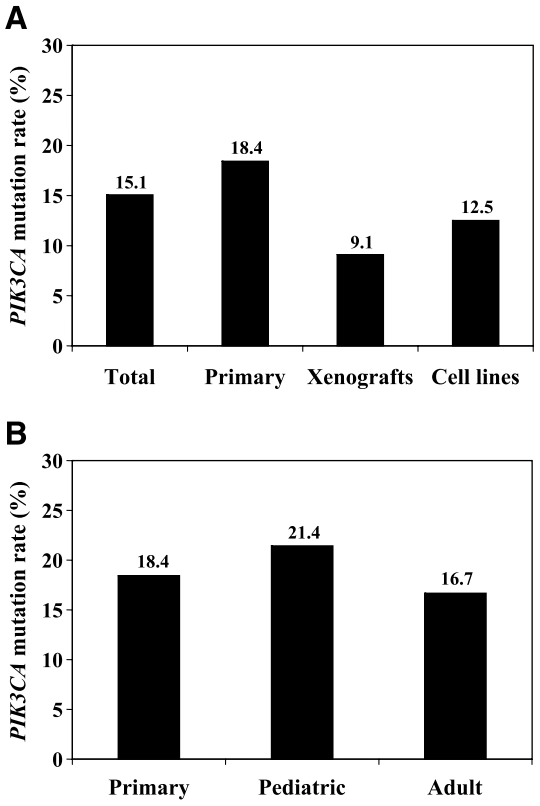

FIGURE 1. A. *PIK3CA* mutation rate in glioblastoma samples and primary tumor, xenograft, and cell line subgroups. **B.** *PIK3CA* mutation rate in primary glioblastoma samples and pediatric and adult subgroups.

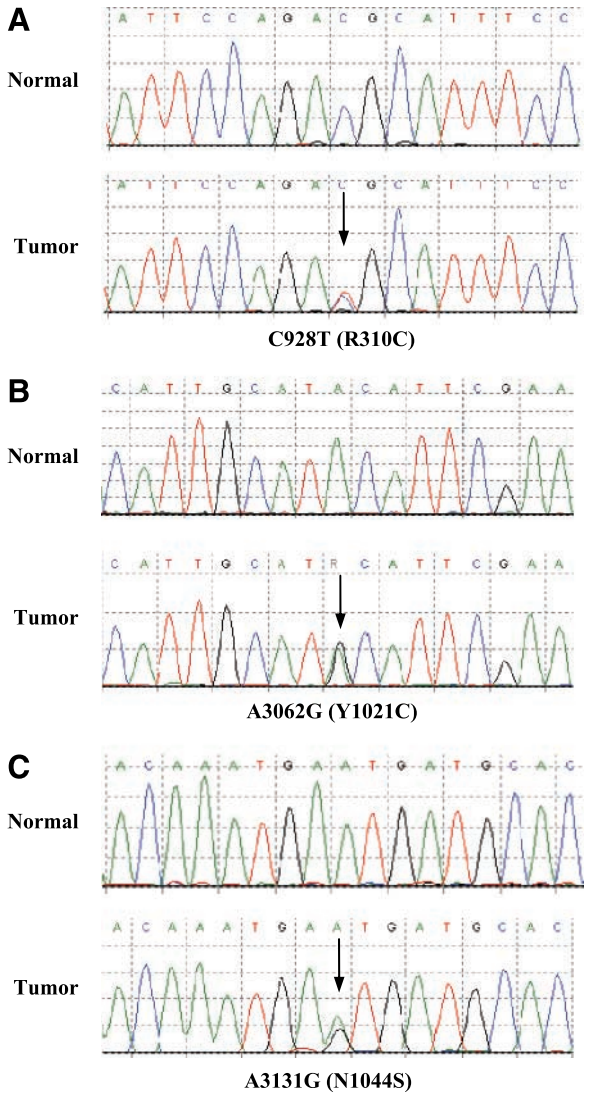

FIGURE 2. *PIK3CA* mutations in glioblastoma. Representative examples of somatic missense mutations detected in exons 4 (**A**) and 20 (**B** and **C**) in a series of 73 glioblastoma samples. Bottom sequence chromatogram, obtained from the tumor tissue; top sequence chromatogram, obtained from the corresponding normal control tissue. Bottom of each chromatogram, nucleotide and amino acid alterations depicting the mutation.

glioblastomas. The remaining mutations in exons 1, 9, and 20 have been described previously in glioblastomas (2, 3, 10).

For each mutated sample, corresponding matched normal DNA, when available, was sequenced to verify that the mutation was somatic. Eight *PIK3CA* mutations were identified in seven of the primary tumor samples examined. Corresponding normal matched DNA was available for each of these primary samples; in each case, the mutations, including three of the novel mutations (P298T, R310C, and N1044S), were somatic. With respect to the novel mutation at amino acid position 1,044, a mutation has been reported (T3132A), which changes the amino acid at that position from an N to K (13). In our study, we found a somatic missense mutation at nucleotide position 3,131, which resulted in an N to S change at amino acid position 1,044. Four missense mutations (V344G, E453K, E542K, and H1047Y) were identified in xenografts and cell lines, samples for which no corresponding normal DNA was available. Three of these four mutations have been previously identified in other malignances (E453K, E542K, and H1047Y; refs. 2, 4, 13) and/or glioblastoma (E542K; ref. 10). In the previous reports, these mutations were found to be somatic mutations and thus it is likely in our current study that these mutations represent acquired changes. One novel mutation (V344G) observed in a sample for which there was no matched normal DNA was not identified in any DNA sample from our study or the initial large *PIK3CA* mutation report (2) and may either be a mutation or a rare polymorphism.

In the initial large-scale mutational analysis of *PIK3CA*, Samuels et al. (2) investigated all 20 exons of *PIK3CA* in several tumor types by PCR amplification and direct sequencing. Although >75% of alterations occurred in exons 9 and 20, mutations were observed in 10 of the 20 exons. In that study, the authors reported *PIK3CA* mutations in 4 of 15 (27%) glioblastomas. Since that initial report, several additional studies have also investigated glioblastomas for *PIK3CA* mutations (3, 10-12) and reported a significantly lower *PIK3CA* mutation rate (≤7%; Table 2). Broderick et al. (3) analyzed exons 9 and 20 in 105 glioblastomas cell lines, xenografts, and primary tumors by PCR amplification and DNA sequencing and found mutations in 5 (5%) samples. Mueller et al. (11) examined all 20 exons of *PIK3CA* by single-strand conformational polymorphism (SSCP) analysis and direct sequencing of exons 9 and 20; no somatic mutation was found in 30 glioblastomas. Two additional studies examining 20 (10) and 10 (12) exons of *PIK3CA* by SSCP found mutations in 5 of 70 (7%) and 5 of 97 (5%) samples, respectively. In the current study, we investigated 10 exons of *PIK3CA*, previously shown to harbor mutations, by PCR amplification and direct DNA sequencing and found mutations in 11 of 73 (15%) samples. Several factors, which are discussed below, can account for the disparity between the current study and initial report of *PIK3CA* mutations in glioblastoma with mutation rates of 15% and 27%, respectively, and numerous other studies (3, 10-12) reporting a *PIK3CA* mutation rate of <7%.

Although >75% of mutations reported in the initial study by Samuels et al. (2) were in exons 9 and 20, only one of the four (25%) mutations reported in glioblastoma samples was in those two exons. In our study, exon 20 was the most frequently affected exon with 45% of samples with mutations containing mutations within this exon. Sixty-four percent of samples with mutations possessed mutations in exons 9 and 20. Analysis of the current study in combination with others examining at least the 10 exons in which *PIK3CA* mutations were originally described (2, 10-12) reveals that 56% (14 of 25) of glioblastoma samples with *PIK3CA* mutations contain a mutation in either exon 9 or 20. Thus, studies examining only exons 9 and 20 are likely to underestimate the mutation rate. It is also possible that our mutation rate of 15% may be an underestimate if any of the tumor samples in our study contained mutations within the 10 exons not examined. Indeed, *PIK3CA* mutations have been described in non–central nervous system malignancies in exons that were not investigated in this study, such as exon 6 and possibly exon 14 (4).

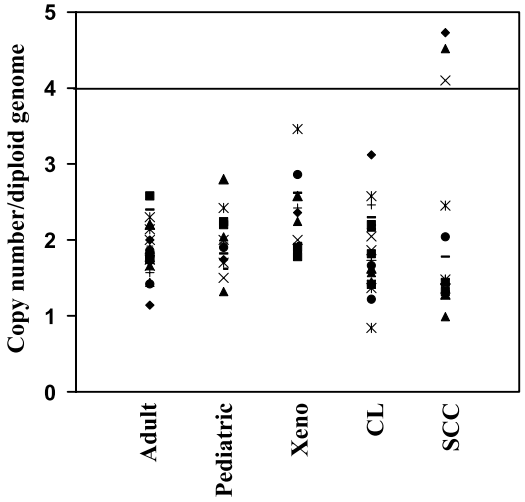

FIGURE 3. *PIK3CA* gene amplification in glioblastoma and squamous cell carcinoma (*SCC*) of the lung. Quantitative real-time PCR was done on adult and pediatric primary glioblastoma samples, xenografts (*Xeno*), and cell lines (*CL*). Squamous cell carcinoma samples were included as a positive control. Copy number per diploid genome is shown for each sample.

There is a significant amount of variability reported in the *PIK3CA* mutation rate in glioblastomas even in the studies examining at least the 10 exons in which *PIK3CA* mutations were originally described (2), ranging from 0% to 27% (2, 10-12). One possible explanation for this range in the mutation rate is the technique used to identify the mutations. The three studies using SSCP to evaluate *PIK3CA* exons (10-12) have found lower mutation rates (0-7%) than the initial study (2) in which all 20 exons were PCR amplified and directly sequenced. This is not surprising as SSCP screening is a less sensitive technique for mutation detection and underestimates the true mutation rate (14, 15). Thus, the studies using SSCP may underestimate the *PIK3CA* mutation rate in glioblastomas (10-12). Combining the initial report by Samuels et al. (2) and the current study (which examined nonoverlapping samples), our best estimate for the *PIK3CA* mutation rate in glioblastoma patients of all ages is 17% (15 of 88).

Several of the mutations found in our study (E542K, E545K, and M1043I) are oncogenic or increase kinase activity, albeit in nongliomatous systems (16-20). Another mutation that has been studied functionally (H1047R; refs. 16-20) was not found in our study; however, we found a mutation that changed the amino acid at the same position (H1047Y). Thus, for many mutations in the current study, there are data supporting a functional role in oncogenesis.

Interestingly, one novel mutation (P298T) was found in a pediatric sample, which contained two mutations, one in exon 4 and one in exon 20. Although the significance of this double mutant is unknown, *PIK3CA* double mutants have previously been described (2, 6, 13, 21). In the study by Samuels et al. (2), each of seven tumors contained two somatic alterations; several other groups have also reported double *PIK3CA* mutations (6, 13, 21). Although not specified in Samuels et al. (2), each of the double mutants described by these other groups (6, 13, 21), like the current case, contained one mutation in exon 20. One possible explanation for double mutations is that only one mutation is functional. This is supported in part by the observation that the only double mutation sample from our study was found to exhibit low-level microsatellite instability (22). The mismatch repair instability within these tumors increases not only the chances of point mutations that activate oncogenes or inactivate tumor suppressors but also the background mutation rate in these genomes.

In addition to gene mutations, another mechanism of oncogenic activation involves gene amplification. Many studies, using various techniques, have investigated *PIK3CA* gene amplification in glioblastomas with somewhat differing results. Hui et al. (23) investigated 14 glioblastomas via an array-based comparative genomic hybridization and reported

Table 2. Comparison of Studies Investigating *PIK3CA* Mutations in Glioblastomas

	Samuels et al., 2004	Broderick et al., 2004	Mueller et al., 2005	Hartmann et al., 2005	Knobbe et al., 2005	Current study
No. samples	15	105	30	70	97	73
Source	Primary tumors Xenografts Cell lines	Primary tumors Xenografts Cell lines	Primary tumors	Primary tumors	Primary tumors	Primary tumors Xenografts Cell lines
Age range (y)	NR	NR	NR	NR	10-83	0.4-78
Exons screened	20	2 (9, 20)	20	20	10 (1, 2, 4, 5, 7, 9, 12, 13, 18, 20)	10 (1, 2, 4, 5, 7, 9, 12, 13, 18, 20)
Method	PCR/sequencing	PCR/ sequencing	SSCP (20), sequencing (Ex 9, 20)	SSCP	SSCP	PCR/sequencing
Samples with mutations, *n* (%)	4 (27)	5 (5)	0 (0)	5 (7)	5 (5)	11 (15)*
Mutation location	Ex 4 Ex 5 Ex 13 Ex 20	Ex 9 (2) Ex 20 (3)		Ex 1 Ex 9 (3) Ex 20	Ex 1 (del) Ex 2 Ex 5 Ex 20 (2)	Ex 1 Ex 4 (3) Ex 7 Ex 9 (2) Ex 20 (5)
Amplification	NR	0/50	NR	NR	0/97	0/73

Abbreviations: NR, not reported; Ex, exon; Del, deletion.
*Twelve mutations identified in 11 samples.

PIK3CA gene copy number gains in 9 (64.3%). Mizoguchi et al. (24) investigated 10 glioblastoma samples with fluorescence *in situ* hybridization and reported one case with an extra copy of *PIK3CA*. No amplification of *PIK3CA*, however, was detected in glioblastoma samples using a duplex PCR assay (12, 25). In our study and the study reported by Broderick et al. (3), no evidence of significant *PIK3CA* gene amplification was observed in 73 and 50 glioblastoma samples by quantitative PCR analysis, respectively.

In summary, our results confirm previous findings of *PIK3CA* mutations in human glioblastomas. In this study, we report that 15% of glioblastomas possess *PIK3CA* mutations. Moreover, we show that *PIK3CA* mutations are prevalent in glioblastomas from both pediatric and adult patients. As such, therapeutics targeting this pathway may be beneficial to both adult as well as pediatric patients with the diagnosis of glioblastoma.

Materials and Methods

Tumor Samples

DNA was extracted from glioblastoma primary tumor samples (n = 38), xenografts (n = 11), and cell lines (n = 24) obtained from Johns Hopkins Division of Neuropathology (Baltimore, MD), Duke University Medical Center Tissue Bank (Durham, NC), Mayo Clinic (Rochester, MN), University of São Paulo School of Medicine (Ribierão Preto, São Paulo, Brazil), Memorial Sloan-Kettering Cancer Center (New York, NY), and the American Type Culture Collection (Manassas, VA). Primary samples were derived from patients whose ages ranged from 0.4 to 78 years, with 14 samples obtained from pediatric patients (ages ≤21 years) and 24 samples derived from adult patients (ages >21 years). All brain tumors were histologically classified according to the WHO classification (26), and the acquisition and use of the specimens were approved by the institutional review board.

PCR, Sequencing, and Mutational Analysis

Exons 1, 2, 4, 5, 7, 9, 12, 13, 18, and 20 were individually amplified from genomic DNA using PCR primers and conditions described previously (2). The amplified products were sequenced by Agencourt Bioscience Corp. (Beverly, MA) using a custom sequencing primer that was complementary to an internal region of the PCR amplicon. Sequence traces were analyzed to identify potential genomic alterations using the Mutation Surveyor software package (SoftGenetics, State College, PA). All cases with mutations were verified by repeat PCR amplification and sequencing. Genomic DNA from corresponding normal brain tissue or peripheral blood was isolated and sequenced to confirm the somatic nature of the mutations in primary tumor samples.

Quantitative Real-time PCR

Copy number was determined by quantitative real-time PCR on an iCycler apparatus (Bio-Rad, Hercules, CA). Tumor DNA content was normalized to that of Line-1, a repetitive element whose copy number per haploid genome is similar among normal and neoplastic tissues. All PCR amplifications were carried out in triplicate, and the threshold cycle numbers were averaged. The following primers were used: *PIK3CA*, 5′-TCAGATTTACGGCAAGATATGC-3′ (forward) and 5′-TGCCTTACTGGTTACCTACCG-3′ (reverse); Line-1, 5′-AAAGCCGCTCAACTACATGG-3′ (forward) and 5′-TGCTTTGAATGCGTCCCAGAG-3′ (reverse). Real-time PCR conditions and copy number calculations were done as described previously (27).

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