

Research Paper

The *PIK3CA* Gene is Mutated with High Frequency in Human Breast Cancers

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KEY WORDS

PI3 kinase, *PIK3CA*, oncogene, mutation, breast cancer

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ABSTRACT

The phosphatidylinositol 3-kinases (PI3Ks) are known regulators of cellular growth and proliferation. It has recently been reported that somatic mutations within the PI3K subunit p110 α (*PIK3CA*) are present in human colorectal and other cancers. Here we show that thirteen of fifty-three breast cancers (25%) contain somatic mutations in *PIK3CA*, with the majority of mutations located in the kinase domain. These results demonstrate that *PIK3CA* is the most mutated oncogene in breast cancer and support a role for *PIK3CA* in epithelial carcinogenesis.

INTRODUCTION

PI3Ks are lipid kinases that regulate diverse cellular signaling pathways and are often altered in human malignancies.¹ Studies suggest important roles for PI3K signaling in the regulation of cell growth, cell migration, epithelial to mesenchymal transition, alternative splicing events, and maintenance of tissue morphologies.²⁻⁶ In breast malignancies, changes in PI3K activity have also been associated with an increased resistance to chemotherapeutic and radiation therapies.⁷⁻¹⁰

Although deregulation of PI3Ks has been previously documented in tumorigenesis,¹¹ the presence of somatic point mutations within this family of genes in human cancers has only recently been demonstrated.¹² In this landmark study, a high frequency of *PIK3CA* mutations was found in colorectal and other human cancers. However, of the twelve human breast cancers analyzed in this study only one (8%) contained a mutation, suggesting that mutational activation of this gene in breast cancers is an uncommon event. Due to the small sample size of breast cancers used in this study, we expanded this analysis using forty-one primary invasive breast cancers and twelve human breast cancer cell lines. In addition, we examined the relationship of *PIK3CA* mutational status with known determinants of breast cancer prognosis and therapy.

MATERIALS AND METHODS

Cell Lines and Primary Samples. Cell lines and cell line genomic DNA were purchased from American Type Culture Collection (ATCC, Manassas, VA). Primary samples were obtained as paraffin embedded slides from archival materials and were coded and kept anonymous according to procedures approved by the Johns Hopkins Institutional Review Board.

Genomic DNA Preparation and Sequencing. Primary human invasive breast cancers and adjacent normal tissue were micro-dissected and genomic DNA prepared as previously described.¹³ DNA from tumor and normal tissue was polymerase chain reaction (PCR) amplified, and directly sequenced as previously described.¹² Genomic sequencing was performed using primers described by Samuels et al.¹² In some cases, only a small amount of DNA could be recovered from microdissected samples. Therefore, a nested or hemi-nested PCR approach was used with the following primers: Exon 1 template PCR; forward 5' CTCCACGACCATCATCAGG 3' and reverse 5' GAT-TACGAAGGTATTGGTTTAGACAG 3'. Exon 1 hemi-nested PCR; forward 5' ACTTGATGCC-CCCAAGAATC 3' and reverse from template PCR. Exon 1 sequencing primers were 5' CTGCTT-CTTGAGTAACACTTACG 3' and 5' CCCCCTCCATCAACTTCTTC 3'. Exon 9 template PCR; forward 5' GATTGGTTCTTTCCCTGTCTCTG 3' and reverse 5' CCACAAATATCAATTTACAA-CCATTG 3'. Exon 9 nested PCR; forward 5' TTGCTTTTTCTGTAAATCATCTGTG 3' and reverse 5' TATGGTAAAAACATGCTGAG 3'. Exon 9 sequencing primer was 5' TAAATCATCT-GTGAATCCAGAG 3'. Exon 20 was sequenced in two parts, exon 20-1 and 20-2. Primers for exon 20-1 template PCR were forward 5' TGACATTTGAGCAAAGACCTG 3' and reverse 5' GGATTGTGCAATTCCTATGC 3'. Exon 20-1 hemi-nested PCR; forward 5' TTAATTATAG-GTTTCAGGAG 3' and exon 20-1 template reverse primer. Exon 20-1 sequencing primer was 5' CCTATGCAATCGGTCTTTGC 3'. Primers for exon 20-2 template PCR were forward 5' TTTTCTCAATGATGCTTGGC 3' and reverse 5' GGGGATTTTTGTTTTGTTTTGG 3'; exon 20-2 hemi-nested PCR; forward 5' TTGCATACATTCGAAAGACC 3' and exon 20-2 template

Table 1 SUMMARY OF PIK3CA SOMATIC MUTATIONS FOUND IN PRIMARY BREAST CANCERS

Tumor #	Exon1	Exon9	Exon20	Her2/neu	ER	PR	Stage	Type
1				3+	+	+	T1CN2	IDC
2			A3140G:H1047R	1+	+	+	T1CN0	ILC
3				1+	+	-	T2N2	ILC
4				0	-	-	T3N2	IDC
5				1-2+	+	+	T2N1	IDC
6			A3140G:H1047R	3+	+	+	T1N2	IDC
7				0	+	+	T2N0	IDC
8				3+	+	+	T2N0	IDC
9	G263A:R88Q			0	+	+	T2N1	ILC
10				0	-	-	T2N0	IDC
11				2-3+	+	+	T3N2	IDC
12				1+	-	+	T2N2	IDC
13				1+	-	-	T1CN0	IDC
14				3+	+	-	T3N1	IDC
15			A3140G:H1047R	0-1+	-	-	T2N2	IDC
16			A3140G:H1047R	1+	+	+	T2N2	ILC
17				1-2+	+	-	T3N3	ILC
18		G1624A:E542K		2+	+	+	T2N0	IDC
19				N/A	-	-	T3N3	IDC
20			A3140G:H1047R	3+	+	+	T1CN0	IDC
21				3+	+	+	T1CN0	IDC
22				1+	+	+	T3NX	IDC
23				0-1+	+	-	T2N1	IDC
24				N/A	N/A	N/A	T1BN2	IDC
25				3+	+	+	T3N2	IDC
26				3+	+	+	T1CN0	IDC
27				3+	+	+	T1CN1	IDC
28			C3137T:A1046V	3+	-	-	T1CNX	IDC
29				0-1+	+	+	T1CN0	IDC
30				2+	+	+	T1CN3	IDC
31				3+	+	-	T1CNX	IDC
32				3+	-	-	T1CN1	IDC
33				2+(FISH -)	+	+	T1CN0	IDC
34				2+(FISH -)	+	+	T1CN0	IDC
35	G328A:E110K			0	-	-	T1CN0	IDC
36				1+	-	-	T2N0	IDC
37				0-1+	+	+	T1CN0	IDC
38				0	-	-	T1CN0	IDC
39				1+	+	-	T1CN0	IDC
40				3+	-	-	T2NX	IDC
41				0-1+	+	+	T2N0	ILC

Mutations are shown as nucleotide change followed by amino acid change. Mutations in bold are unique to this sample set. Abbreviations are: ER, estrogen receptor; PR, progesterone receptor; FISH, fluorescence in situ hybridization, IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; N/A, information not available.

reverse primer. Exon 20-2 sequencing primer was 5' TGGACTTAAGGCA-TAACATG 3'. All mutations were verified at least twice, as were the normal controls.

Immunohistochemistry Analysis. Immunohistochemistry for estrogen and progesterone receptors was done using a standardized laboratory protocol for formalin-fixed, paraffin-embedded tissue sections on the Ventana Benchmark XT (Ventana, Tuscon, Arizona). For Estrogen receptor, the 6F11 monoclonal antibody (Novacastra, Newcastle upon Tyne, UK) was

used at a dilution of 1:50. For Progesterone receptor, the PGR636 monoclonal antibody (Dako, Carpinteria, CA) was used at a dilution of 1:60. Only nuclear labeling was scored as positive. Normal breast tissue surrounding the tumors served as internal controls. For Her-2/neu status, we used the Dako Herceptest Kit (Dako, Carpinteria, CA) as per the manufacturers instructions for formalin-fixed, paraffin-embedded tissue sections. Tumors were scored as negative (0 or 1+), weak positive (2+), or strong positive (3+) based upon the intensity and extent of membranous labeling.

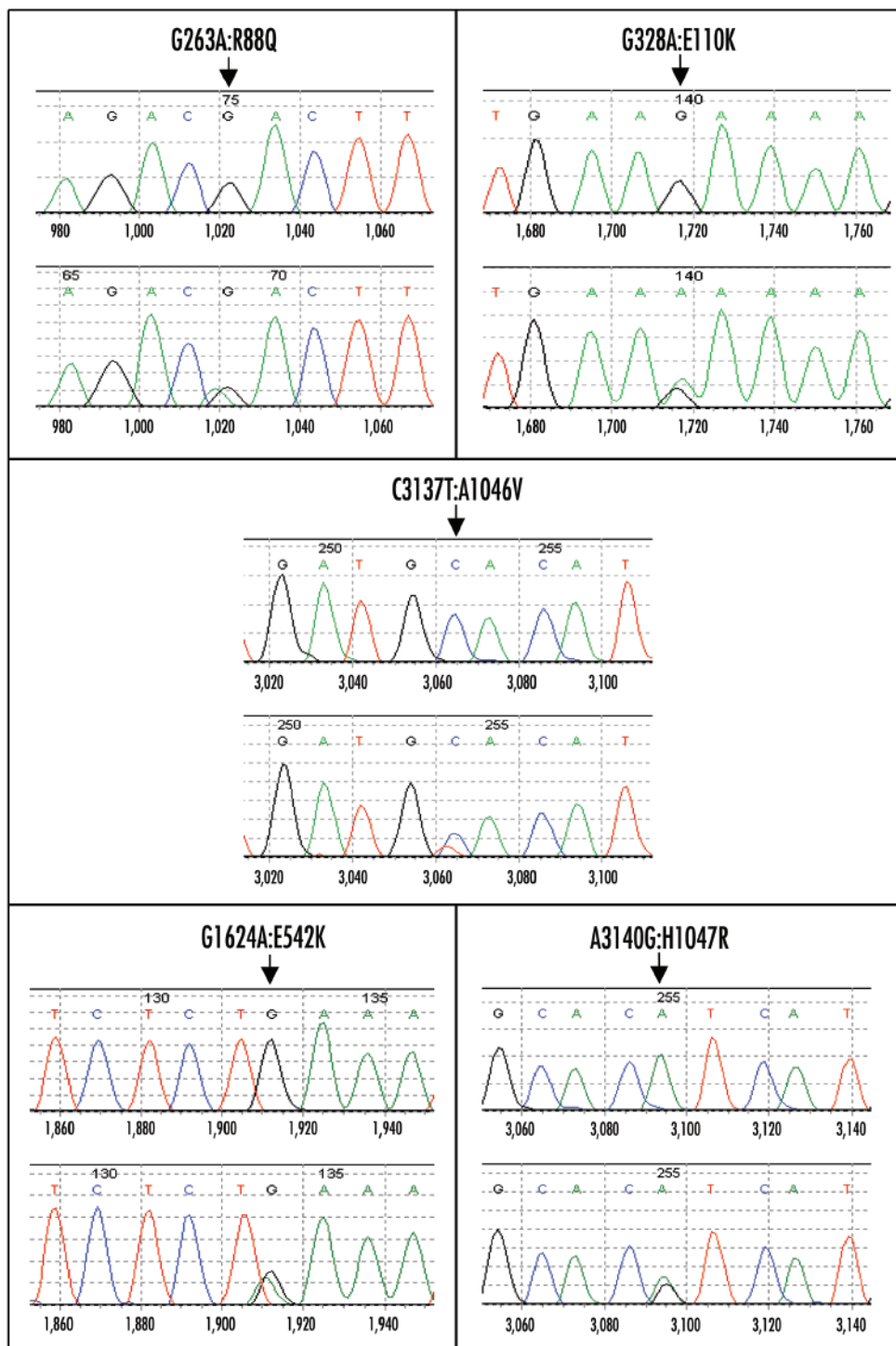


Figure 1. Somatic mutations found in the *PIK3CA* gene in primary breast cancers. Examples of somatic mutations found in the p85 binding domain, helical and kinase domain of *PIK3CA*. Top sequence in each chromatogram is derived from normal tissue and bottom sequence from tumor. Arrows indicate the position of the missense mutations. The nucleotide and amino acid changes are given above the arrows.

RESULTS

Due to the scarce amount of DNA derived from these samples, we chose to examine only exons 1, 9 and 20 as these exons have been shown to harbor >91% of the somatic mutations found in colorectal and other cancers.¹² Exon 1 of the *PIK3CA* gene encodes for the p85 binding domain, while exons 9 and 20 encode for the helical and kinase domains respectively. The frequency of observed mutations is shown in Table 1. Of the forty-one

primary breast cancers used in our study, nine contained somatic missense mutations. Two of these mutations were found in exon 1, one mutation was present in exon 9, and the remaining mutations were located within exon 20. In all cases where mutations were demonstrated, DNA from adjacent normal mammary or lymphoid tissue revealed the presence of wild-type sequence only, confirming that the mutations were indeed somatic. In addition to our primary samples, we also sequenced genomic DNA from twelve well established human breast cancer cell lines (Table 2). This analysis demonstrated that four out of twelve cell lines contained *PIK3CA* mutations (30%), half of which were in exon 9 and half in exon 20. Taken together, our combined analyses show that *PIK3CA* mutations are present in 25% of human breast cancers.

The status of estrogen and progesterone receptors (ER/PR), as well as the presence or absence of Her-2/neu amplification, are independent determinants of breast cancer prognosis, as well as predictors of response to targeted therapy.^{14,15} To determine the relationship between *PIK3CA* mutational status with these known determinants, we analyzed our primary samples for receptor status using immunohistochemical methods and fluorescence in situ hybridization for Her-2/neu when indicated. As seen in Table 1, there was no correlation between *PIK3CA* mutations and the presence or absence of ER/PR labeling. In a similar manner, Her-2/neu expression status did not correlate with the presence or absence of *PIK3CA* mutations. Finally, the nodal status and histologic type of breast cancers (ductal versus lobular) used in our study suggest that *PIK3CA* mutations are not exclusive to any specific subgroup of breast cancer. These data suggest that *PIK3CA* mutations affect signaling pathways independent from those of ER/PR and Her-2/neu.

DISCUSSION

The spectrum of mutations found in our study is similar to the analysis by Samuels et al., but there are some important differences. In their study, forty-three exon 9 and twenty-seven exon 20 mutations were found suggesting exon 9 mutations are the most common *PIK3CA* mutations in colorectal and other cancers. In contrast, we found

only three mutations within exon 9, but uncovered eight exon 20 mutations within our fifty-three samples, suggesting that exon 20 mutations predominate in human breast cancers. In addition, three novel somatic mutations were discovered including two in exon 1 and one in exon 20 (Table 1 and Fig. 1). It should be noted that no truncating mutations were found and all of the mutations were heterozygous consistent with previous reports describing the oncogenic nature of these mutations.¹²

Table 2 SUMMARY OF PIK3CA MUTATIONS FOUND IN BREAST CANCER CELL LINES

Cell Line	Exon1	Exon9	Exon20
BT-483		G1624A:E542K	
MDA-MB-175-VII			
MDA-MB-231			
MDA-MB-415			
MDA-MB-435S			
SK-BR-3			
T-47D			A3140G:H1047R
UACC-812			
UACC-893			A3140G:H1047R
ZR-75-1			
ZR-75-30			
MCF-7		G1624A:E542K	

Mutations are shown as nucleotide change followed by amino acid change.

Our data expand the work of Samuels et al. by providing a more comprehensive analysis of *PIK3CA* mutations in human breast cancers. The higher percentage of mutations found in our study likely stems from the larger sample size that we used. To our knowledge, these data place the *PIK3CA* gene as the most mutated oncogene in human breast cancer. In addition, it appears that *PIK3CA* mutational status does not segregate with ER/PR, Her-2/neu or nodal status in primary invasive breast cancers. It will therefore be of interest to determine in larger randomized trials whether *PIK3CA* mutations can be used as independent markers for risk and prognosis. More importantly, this work allows for the development of therapies specifically targeted towards the substantial number of women with breast cancers harboring these mutations.

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