Breast Cancer Research and Treatment (2006) 96: 91–95
DOI 10.1007/s10549-005-9048-0

Original Report

PIK3CA mutations in breast cancer are associated with poor outcome

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Key words: AKT, breast cancer, *erbB2*, estrogen receptor, mutations, PI3K, prognosis

Summary

The phosphatidylinositol-3-kinase (PI3K)–AKT signaling pathway is considered to play an important role in tumorigenesis. Frequent somatic mutations in the PI3K subunit p110α (*PIK3CA*) occur in a variety of cancer types. We screened 250 primary human breast tumors for mutations in *PIK3CA* in order to determine associations with pathological features and with patient outcome. The frequency of *PIK3CA* mutations in the C2, helical and kinase domains was 35% (88/250). Mutations were associated with larger tumor size ($p = 0.004$) and positive estrogen receptor status ($p = 0.008$). Patients with *PIK3CA* mutations showed significantly worse survival ($p = 0.004$), particularly those with positive estrogen receptor expression or non-amplified *erbB2* (both $p = 0.002$). *PIK3CA* mutation was an independent factor for worse survival in breast cancer patients with non-amplified *erbB2* (RR = 2.6, 95%CI [1.2–5.5], $p = 0.016$).

Introduction

The phosphatidylinositol-3-kinase (PI3K)–AKT signaling pathway regulates various cellular processes including apoptosis, proliferation, growth and cytoskeletal rearrangement [1]. Several components of this signaling pathway are altered in human cancers. These include elevated AKT1 kinase activity [2], amplification of the *AKT2* [3] and *PIK3CA* [4] genes and somatic mutation of the *PTEN* [5] and *PIK3R1* [6] genes. Frequent mutations have also recently been reported in the *PIK3CA* gene coding for the p110α catalytic subunit of PI3K [7–13]. Affected tumor types include colon, brain, gastric, liver, breast and ovarian. The large majority of *PIK3CA* mutations occur in exons 9 and 20, partially encoding the helical and kinase domains, respectively.

The reported frequency of *PIK3CA* mutation in breast cancer ranges from 8 to 40% [7–12]. With the exception of one study [11], all investigations to date have been carried out on relatively small cohorts of less than 100 primary breast tumors, making it difficult to evaluate associations between *PIK3CA* mutation and clinical, pathological and molecular features of this disease. The larger study by Saal et al. [11] on 292 cases reported significant associations with positive steroid receptor status, nodal metastasis and overexpression of *erbB2*. Although these authors found no association between *PIK3CA* mutation and patient survival, they cautioned their selection of tumors was not ideal for this type of analysis. In the current study we screened for *PIK3CA* mutations in a large ($n = 250$) and well characterized series of primary breast cancers with long follow-up. We report for the first time that *PIK3CA* mutation is a marker for worse outcome in this tumor type, particularly for the estrogen receptor (ER) positive and *erbB2* normal patient subgroups.

Materials and methods

Tumor samples

DNA from 250 primary invasive breast tumors diagnosed at the Sir Charles Gairdner Hospital in Western Australia between 1990 and 1994 was obtained for this study. Genomic DNA was extracted from fresh frozen specimens using standard phenol–chloroform extraction procedures. The median age of patients at diagnosis was 59 years (range 18–93 years) and the median follow-up time was 50 months (range 2–78 months). Clinical and pathological features of this tumor series have been described earlier [14]. Disease-specific survival data was obtained from the Death Registry, Health Department of Western Australia. At the end of the study period 45 (18%) patients had died because of disease recurrence. Approximately 92% of node-positive and 23% of node-negative patients received adjuvant systemic therapy comprising either hormone therapy alone or hormone therapy and chemotherapy. Ethics approval for the study was obtained from the University of Western Australia Human Research Ethics Committee.

PIK3CA *mutation screening*

The vast majority (>90%) of *PIK3CA* mutations reported in human cancer occur in exons 7, 9 and 20 [9]. We screened for *PIK3CA* mutations in each of these exons using PCR and fluorescent (F)-SSCP. Details of primer sequences and PCR conditions are shown in Table 1. Primers were designed to cover the entire coding region for each exon. PCR was performed with 10 ng of genomic DNA in a reaction volume of 16 µl comprising HEX-labeled fluorescent primers at a final concentration of 0.4 µM (Geneworks, Australia), 1× reaction buffer (Qiagen, Australia), 0.2 mM deoxynucleotide triphosphates, 1× Q-solution (Qiagen, Australia), the optimal concentration of $MgCl_2$ and 0.2 U of *Taq* Polymerase. After an initial denaturation step of 94 °C for 5 min, 35 cycles of reaction were performed comprising 94 °C for 30 s, the appropriate annealing temperature for 30 s and 72 °C for 45 s. This was followed by a final extension step of 72 °C for 5 min. For F-SSCP, 3 µl of HEX-labeled PCR product was mixed with 9 µl of deionised formamide-loading buffer and denatured at 95 °C for 5 min. A volume of 0.6–1.0 µl of this mix was then loaded onto a non-denaturing polyacrylamide gel and run on the Gel-Scan 2000 instrument (Corbett Research, Australia) as previously described [15]. The sample was pulse loaded for 20 s at 1200 V, the wells rinsed and the gel run for 80–120 min at 1200 V in 0.8× TBE buffer at a constant temperature of 24 °C.

Statistical analysis

The chi-square test was used to evaluate for possible associations between *PIK3CA* mutations and various clinicopathological features. The Student *t*-test was used to compare tumor size between *PIK3CA* normal and mutant groups. Multivariate Cox proportional hazard test and Kaplan–Meier analysis were used to evaluate differences in survival between patient groups. All tests were two-tailed and statistical significance was assumed at $p < 0.05$. The SPSS statistical software package (Chicago, IL, USA) was used throughout.

Results

Examples of *PIK3CA* mutation detection using the F-SSCP technique are shown in Figure 1. Of 250 primary breast cancers screened, 8 (3%) showed mutations in exon 7, 40 (16%) in exon 9 and 47 (19%) in exon 20. Seven tumors contained mutations in two different exons, thus giving an overall *PIK3CA* mutation frequency of 35% (88/250). This is within the range of 8–40% reported in previous studies of breast cancer [7–12].

Associations between *PIK3CA* mutation and clinical, pathological and molecular features of this breast cancer series are shown in Table 2. *PIK3CA* mutations were more frequent in larger tumors and in ER+ and PR+ tumors. The average size of tumors with *PIK3CA* mutation was 25 mm compared to 19 mm for those without mutation ($p = 0.02$, Student *t*-test). The highest frequency of *PIK3CA* mutation (57%) was seen in tumors with well differentiated histology. Trends were also observed for more frequent mutation in node positive tumors and in tumors with ductal histology, but no associations were apparent with patient age, ploidy, *erbB2* amplification or *TP53* mutation.

Kaplan–Meier analysis revealed that *PIK3CA* mutations were associated with significantly worse cancer-specific survival in the overall tumor group (log-rank test $p = 0.004$; Figure 2a). Subgroup analysis revealed that *PIK3CA* mutations showed strong prognostic value within the ER+, PR+ and *erbB2* normal patient groups (Table 3 and Figure 2b). Factors previously shown [14] to be strongly associated with poor prognosis in this tumor series (nodal status, histological grade, tumor size, *TP53* mutation) were included with *PIK3CA* mutation in a multivariate analysis of survival. *PIK3CA* mutation failed to reach significance for independent prognostic value in the overall and ER+ patient groups

Table 1. Primer sequences and PCR conditions used for *PIK3CA* mutation screening

Sequence (5′–3′)	AT (°C)	[$Mg2^+$] (mM)	Size (bp)
Exon 7			
F CCTTTTGGGGAAGAAAAGTG	60	3.5	284
R GAGAGAAGGTTTGACTGCCATAA			
Exon 9			
F_1 TGAAAATGTATTTGCTTTTTCTGT	56	3.5	198
R_1 TCTCCTGCTCAGTGATTTCAGAG			
F_2 GGGAAAATGACAAAGAACAGC	58	2.5	174
R_2 ACATGCTGAGATCAGCCAAA			
Exon 20			
F_1 CATTTGCTCCAAACTGACCA	60	3.5	387
R_1 GGTCTTTGCCTGCTGAGAGT			
F_2 TTGGCTCTGGAATGCCAGAA	60	1.5	167
R_2 TGTGTGGAAGATCCAATCCA			

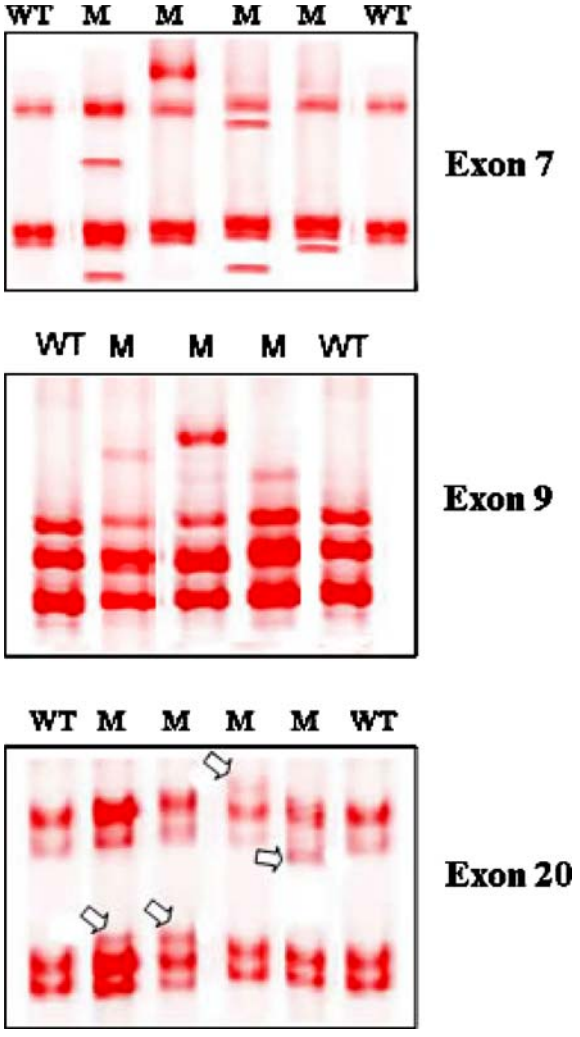

Figure 1. F-SSCP screening for mutations in exons 7, 9 and 20 of *PIK3CA*. Additional bands compared to the wild-type (WT) banding pattern indicate the presence of a mutation (M). These are indicated with arrows for exon 20.

($p = 0.075$ and $p = 0.09$, respectively). However, in patients with normal *erbB2*, *PIK3CA* mutation was an independent factor for worse survival (RR = 2.6, 95%CI [1.2–5.5], $p = 0.016$).

Discussion

The overall frequency of *PIK3CA* mutation in the current SSCP-based study (35%) is slightly more than the 25–27% reported by three other studies on breast cancer [8,10,11] but close to the value of 40% reported in another study that also used SSCP [9]. As suggested earlier [9], SSCP-based mutation analysis is likely to have a superior detection rate compared to direct DNA sequencing because it is much less affected by the high levels of normal DNA contamination found in most solid tumors. Although we did not sequence DNA samples showing aberrant SSCP banding patterns, analysis of 96 germline DNA samples did not reveal the presence of polymorphisms in exons 7, 9 or 20. Therefore we conclude that aberrant banding patterns were due to the presence of somatic mutation. This is supported by another study where the mutations detected in tumor DNA were demonstrated to be somatic by comparison with matching normal DNA [9]. The *PIK3CA* mutation frequency of 35% is more than twice that of *TP53* mutation (16%) and *erbB2* amplification (16%) observed in this tumor series, indicating the likely importance of the PI3K–AKT signaling pathway in breast cancer development.

Exons 9 and 20 encode the helical and kinase domains of *PIK3CA*, respectively. We found a slightly higher frequency of mutation in exon 20 (19%) compared to exon 9 (16%). A predominance of exon 20 mutations in breast cancer has been reported [11] and is believed to contrast with colorectal cancer where exon 9 mutations predominate [7]. Our finding of exon 7 (C2 domain) mutations in 3% of breast cancers is similar to another recent study [11] and suggests this region should be also analyzed in future investigations. Interestingly, 7 tumors in our series contained two mutations. Double mutations in *PIK3CA* have been reported previously in gastric and breast cancers [8,11] and it has been suggested these could indicate multiclonal tumors or a second hit in the alternate allele.

With the exception of Saal et al. [11] who studied 292 primary breast tumors, all other studies on *PIK3CA*

Table 2. Associations between *PIK3CA* mutations and breast cancer phenotype

Feature (n)[a]	*PIK3CA* mutation (%)	p
Total (250)	88 (35)	
Age < 59 years (123)	44 (36)	
Age ≥59 years (127)	44 (35)	NS
Node negative (101)	30 (30)	
Node positive (90)	38 (42)	0.07
Well differentiated (28)	16 (57)	
Moderately differentiated (94)	30 (32)	
Poorly differentiated (75)	27 (36)	0.02[b]
Non-ductal histology (24)	5 (21)	
Ductal histology (203)	80 (39)	0.08
Tumor size ≤ 20 mm (119)	33 (28)	
Tumor size >20 mm (104)	48 (46)	0.004
ER negative (76)	18 (24)	
ER positive (168)	69 (41)	0.008
PR negative (88)	24 (27)	
PR positive (156)	63 (40)	0.04
Diploid (79)	27 (34)	
Aneuploid (73)	23 (32)	NS
ErbB2 normal (189)	65 (34)	
ErbB2 amplified (37)	14 (38)	NS
TP53 normal (211)	76 (36)	
TP53 mutant (39)	12 (31)	NS

[a]Information was unavailable for nodal status on 59 cases, grade on 53 cases, histological type on 23 cases, tumor size on 27 cases, ER and PR on 6 cases, ploidy on 98 cases and *erbB2* amplification on 24 cases.
[b]Well differentiated versus moderate/poorly differentiated.

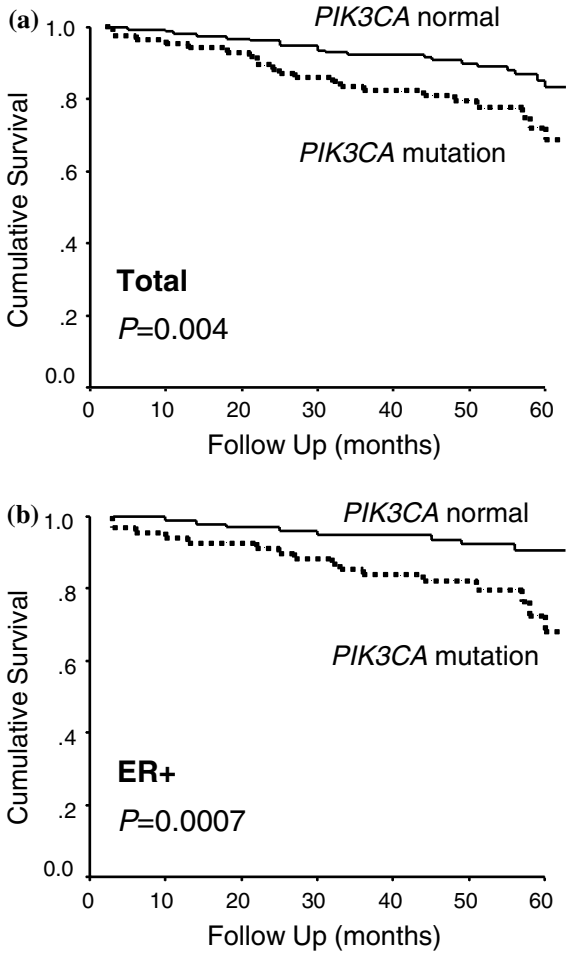

Figure 2. Kaplan–Meier survival analysis for breast cancer patients with and without *PIK3CA* mutation. a, overall group; b, ER+ subgroup of patients.

mutation have been carried out on small series comprising <100 cases. These are generally unsuitable for the investigation of associations with pathological features or with patient outcome because of limited statistical power. In agreement with Saal et al. [11], we found a strong correlation between *PIK3CA* mutation and positive steroid receptor status (Table 2). Also similar to that report and another study on 70 cases [9], we found a trend for association with lymph node involvement. The current study is the first to report an association of *PIK3CA* mutation with tumor size. Almost half the tumors larger than 2 cm were found to carry a mutation.

In view of the associations between *PIK3CA* mutation, nodal involvement and larger tumor size, it is not surprising these mutations were prognostic for worse outcome (Figure 2a). Although *PIK3CA* mutations were also associated with ER+ and PR+ status, these factors had no prognostic value in the current tumor series [14]. In a multivariate model that included the strong prognostic factors of nodal status, histological grade, tumor size and *TP53* mutation, *PIK3CA* mutation failed to reach significance as an independent prognostic factor in both the overall ($p = 0.075$) and ER+ patient subgroups ($p = 0.09$). However, it did reach significance in patients with non-amplified *erbB2* ($p = 0.016$), representing approximately 80% of all breast cancers.

In summary, our results confirm that *PIK3CA* mutation occurs at a high frequency in breast cancer, being more prevalent than both *TP53* mutation and *erbB2* amplification. The association with nodal involvement and large tumor size indicates this mutation plays a major role in determining tumor phenotype. This was particularly evident for ER+, PR+ and *erbB2* normal breast cancers where *PIK3CA* mutation was a strong prognostic factor. Aberrations to the PI3K pathway could provide useful targets for the development of novel therapies.

Table 3. Prognostic significance of *PIK3CA* gene mutations in breast cancer subgroups

Feature (*n*)	Relative risk (95% CI)	*p*
All tumors (250)	2.3 (1.3–4.2)	0.005
ER positive (168)	3.8 (1.6–8.6)	0.002
PR positive (156)	3.4 (1.5–7.9)	0.004
erbB2 normal (189)	3.3 (1.6–7.1)	0.002

Acknowledgement

The authors are grateful to Dr Ian Campbell for information on primer sequences.

References

1. Vivanco I, Sawyers CL: The phosphatidylinositol-3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2: 489–501, 2002
2. Sun M, Wang G, Paciga JE, Feldman RI, Yuan ZQ, Ma XL, Shelley SA, Jove R, Tsichlis PN, Nicosia SV, Cheng JQ: AKT1/PKBalpha kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. Am J Pathol 159: 431–437, 2001
3. Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V, et al.: Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int J Cancer 64: 280–285, 1995
4. Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, Collins C, Pinkel D, Powell B, Mills GB, Gray JW: PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet 21: 99–102, 1999
5. Saito M, Okamoto A, Kohno T, Takakura S, Shinozaki H, Isonishi S, Yasuhara T, Yoshimura T, Ohtake Y, Ochiai K, Yokota J, Tanaka T: Allelic imbalance and mutations of the PTEN gene in ovarian cancer. Int J Cancer 85: 160–165, 2000
6. Philp AJ, Campbell IG, Leet C, Vincan E, Rockman SP, Whitehead RH, Thomas RJ, Phillips WA: The phosphatidylinositol-3′-kinase p85alpha gene is an oncogene in human ovarian and colon tumors. Cancer Res 61: 7426–7429, 2001
7. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE: High frequency of mutations of the PIK3CA gene in human cancers. Science 304: 554, 2004
8. Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH: PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 24: 1477–1480, 2005

9. Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE, Pearson RB, Phillips WA: Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 64: 7678–7681, 2004
10. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, Konishi H, Karakas B, Blair BG, Lin C, Peters BA, Velculescu VE, Park BH: The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol Ther 3: 772–775, 2004
11. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R: PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 65: 2554–2559, 2005
12. Levine DA, Bogomolniy F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J: Frequent mutation of the PIK3CA gene in ovarian and breast cancers. Clin Cancer Res 11: 2875–2878, 2005
13. Wang Y, Helland A, Holm R, Kristensen GB, Borresen-Dale AL: PIK3CA mutations in advanced ovarian carcinomas. Hum Mutat 25: 322, 2005
14. Grieu F, Li WQ, Iacopetta B: Genetic polymorphisms in the MMP-2 and MMP-9 genes and breast cancer phenotype. Breast Cancer Res Treat 88: 197–204, 2004
15. Iacopetta B, Elsaleh H, Grieu F, Joseph D, Sterrett G, Robbins P: Routine analysis of p53 mutation in clinical breast tumor specimens using fluorescence-based polymerase chain reaction and single strand conformation polymorphism. Diagn Mol Pathol 9: 20–25, 2000

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