PIK3CA Mutations Correlate with Hormone Receptors, Node Metastasis, and ERBB2, and Are Mutually Exclusive with PTEN Loss in Human Breast Carcinoma

Lao H. Saal,[1,4,6] Karolina Holm,[6] Matthew Maurer,[4] Lorenzo Memeo,[2] Tao Su,[5] Xiaomei Wang,[5] Jennifer S. Yu,[2,4] Per-Olof Malmström,[6] Mahesh Mansukhani,[2] Jens Enoksson,[7] Hanina Hibshoosh,[2,5] Åke Borg,[6,8] and Ramon Parsons[2,3,4,5]

[1]Integrated Program in Cellular, Molecular, and Biophysical Studies, Departments of [2]Pathology and [3]Medicine, [4]Institute for Cancer Genetics, [5]Herbert Irving Comprehensive Cancer Center, College of Physicians and Surgeons, Columbia University, New York, New York; Departments of [6]Oncology and [7]Pathology, [8]Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, Lund, Sweden

Abstract

Deregulation of the phosphatidylinositol 3-kinase (PI3K) pathway either through loss of *PTEN* or mutation of the catalytic subunit α of PI3K (*PIK3CA*) occurs frequently in human cancer. We identified *PIK3CA* mutations in 26% of 342 human breast tumor samples and cell lines at about equal frequency in tumor stages I to IV. To investigate the relationship between *PTEN* and *PIK3CA*, we generated a cohort of tumors that had lost PTEN expression and compared it with a matched control set that had retained PTEN. A highly significant association between *PIK3CA* mutations and retention of PTEN protein expression was observed. In addition, *PIK3CA* mutations were associated with expression of estrogen and progesterone receptors (ER/PR), lymph node metastasis, and ERBB2 overexpression. The fact that *PIK3CA* mutations and PTEN loss are nearly mutually exclusive implies that deregulated phosphatidylinositol-3,4,5-triphosphate (PIP_3) is critical for tumorigenesis in a significant fraction of breast cancers and that loss of PIP_3 homeostasis by abrogation of either PIK3CA or PTEN relieves selective pressure for targeting of the other gene. The correlation of *PIK3CA* mutation to ER/PR-positive tumors and PTEN loss to ER/PR-negative tumors argues for disparate branches of tumor evolution. Furthermore, the association between ERBB2 overexpression and *PIK3CA* mutation implies that more than one input activating the PI3K/AKT pathway may be required to overcome intact PTEN. Thus, mutation of *PIK3CA* is frequent, occurs early in carcinoma development, and has prognostic and therapeutic implications. (Cancer Res 2005; 65(7): 2554-9)

Introduction

Phosphatidylinositol-3,4,5-triphosphate (PIP_3) is an important lipid second messenger in tumorigenesis, in particular by activating AKT and other pathways which inhibit apoptosis and promote cellular growth, proliferation, cell motility, angiogenesis, and the accumulation of additional genetic defects. In an elegant "on/off" switch, the phosphatidylinositol 3-kinase (PI3K) holoenzyme, in response to signals transmitted via growth factor receptor tyrosine kinases, phosphorylates phosphatidylinositol-4,5-bisphosphate at the 3′-OH position of the inositol ring to form PIP_3, whereas PTEN catalyzes the precise opposite reaction, thereby reducing the pool of PIP_3, inhibiting growth signals and suppressing tumor formation (1). The importance of this pathway in cancer is highlighted by the following observations: the *PTEN* tumor suppressor is inactivated by mutations in a variety of sporadic human tumors and cancer-predisposing hereditary syndromes, epigenetic silencing of *PTEN* occurs in many forms of cancer, and loss of *PTEN* stimulates tumor development in mice (reviewed in ref. 2). Moreover, one of the catalytic subunits of PI3K, the p110 catalytic subunit α (*PIK3CA*), is a transforming oncogene (3), the 3q26 region where *PIK3CA* is located is amplified in tumors (4, 5), and recently, the *PIK3CA* gene was shown to have activating mutations in nine types of cancer (6–8). Interestingly, Bachman et al. reported a 25% *PIK3CA* mutation rate in breast cancer but did not find any association to clinical markers (8). In the current study, we did a large-scale *PIK3CA* mutational analysis of 292 primary breast tumors and 50 cell lines, and describe for the first time the significant correlation of *PIK3CA* mutations to lymph node metastasis, estrogen receptor α (ER), progesterone receptor (PR), and ERBB2 positivity, and an inverse relationship to functional PTEN.

Materials and Methods

Samples. DNA from 133 primary breast tumor biopsies were obtained along with corresponding normal tissue DNA for a subset of the same patients, from the Herbert Irving Comprehensive Cancer Center Tumor Bank at Columbia University, NY, and 162 surgically dissected sporadic primary breast tumor biopsies or tumor cell pellets as well as normal tissues from a subset were obtained from the South Sweden Breast Cancer Group collected at the Lund University Hospital, Lund. All samples were blinded and anonymized and obtained in accordance with Columbia University's Institutional Review Board and Lund University's ethics committee approval, respectively. The Columbia cohort contains tumors of all stages, a mixture of sporadic cases and those with significant family histories, were collected between 1986 and 2003, and had varied clinical therapies. The Swedish cohort are all sporadic stage II tumors, were collected between 1985 and 1994, received adjuvant tamoxifen for 2 years, and predominantly come from a larger series of patients (9). For the Swedish cohort, a selection was made such that approximately one third were PTEN protein negative ($PTEN^-$) tumors, and the remaining two thirds were PTEN protein positive ($PTEN^+$) and roughly matched to the $PTEN^-$ cases with respect to node and ER status; five tumors with unknown PTEN status were also included.

Note: L.H. Saal and K. Holm contributed equally to this study.
Å. Borg and R. Parsons share senior authorship.
Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org).
Requests for reprints: Ramon Parsons, Institute for Cancer Genetics, 1150 St. Nicholas Avenue, New York, NY 10032. Phone: 212-851-5278; Fax: 212-851-5256; E-mail: rep15@columbia.edu.

A panel of 51 well-established breast cancer cell lines (see Supplementary data) were grown and harvested according to recommended protocols.

Immunohistochemical analysis. The Swedish cohort of tumors was evaluated for PTEN protein status by immunohistochemistry (IHC) on 4-μm formalin-fixed, paraffin-embedded tissue sections essentially as previously described (10, 11). Anti-PTEN Ab-2 (Neomarkers, Fremont, CA) was applied at 1:300 dilution. As ERBB2 analysis was not routine at the time of diagnosis for the majority of Swedish cases, a subset of 97 tumors were evaluated for ERBB2 status using the A0485 antibody (DakoCytomation, Glostrup, Denmark) at 1:1,000. All immunohistochemistry was done using the DakoCytomation TechMate 500 Plus staining system using sodium citrate pH 6.0 antigen retrieval buffer, manufacturer's recommended reagents and standard microwave streptavidin immunoperoxidase protocol, with the exception that anti-PTEN antibody was applied for 2 hours. PTEN staining intensity scores for invasive tumor and nonneoplastic cells were recorded by two independent pathologists (J. Enoksson and H. Hibshoosh) essentially as described in ref. (11) with further details in the Supplementary data. ERBB2 membranous staining was scored (H. Hibshoosh) according to the DAKO system scale: 0, 1+, 2+, 3+, with 0/1+ recorded as ERBB2 negative ($ERBB2^-$), and 2+/3+ recorded ERBB2 positive ($ERBB2^+$).

PCR and sequence analysis. Sequencing of *PIK3CA* exons 1, 2, 4, 5, 7, 9, 12, 13, 18, 20, including intron-exon boundaries was done by PCR amplification and direct sequencing of both strands for all tumors as previously described (6) with some modifications. The Swedish series were also analyzed for mutations in *PTEN* exons 1 to 9 including intron-exon boundaries. Further details are described in the Supplementary data.

Statistical analysis. The Pearson χ^2 test was used for correlation analyses with a $P < 0.05$ used as the cutoff for decisions of statistical significance.

Results and Discussion

To ensure a more complete understanding of the *PIK3CA* mutation spectrum in breast cancer, we did mutational analysis in two large cohorts of breast tumors and a large panel of breast tumor cell lines. All of the 10 exons in which *PIK3CA* mutations have been previously described were analyzed (6). The Swedish cohort was assembled to specifically address genetic associations to PTEN. To investigate the potential correlations to *PIK3CA* mutations in a more diverse sampling of breast tumors, the Columbia cohort was obtained. Patient clinical demographics are summarized in Table 1. In addition, 51 well-established breast cancer cell lines were analyzed (Supplementary Table S1). More than 98% of over 7000 sequence reads were of high quality and informative; three tumors and one cell line were excluded due to consistently poor sequence quality. *PIK3CA* mutations were identified in 77 of 292 (26%) primary breast tumors and 14 of 50 (28%) breast cancer cell lines, similar to the results of Bachman et al., who reported a mutation rate of 22% in a set of 41 breast tumors and 30% among 12 cell lines (8). The *PIK3CA* mutation frequency in the Columbia set (32 of 131) was similar to the Swedish set (45 of 161), notwithstanding the differences in their selection.

Mutations clustered in two previously reported "hotspot" regions in exons 9 and 20, corresponding to the accessory (helical) and catalytic domains of PIK3CA, respectively (Figs. 1 and 2*B*). Expanding upon the observation of Bachman et al., in which three mutations in exon 9 and eight mutations in exon 20 were found among 53 samples (8), we found 31 mutations in exon 9 and 49 mutations in exon 20. This is strong evidence that exon 20 mutations predominate in breast cancer, in contrast to colorectal cancer where exon 9 mutations predominate (6). Whereas mutations in exon 7 (C2 domain) have not been reported in breast cancer owing to small sample size (6) or nonanalysis (8), we found this exon to be a third mutation hotspot with seven mutations. Exons 1, 4, and 13 each had two mutated samples, and we found only one mutation in exon 18. All mutations fall into either the PI3K p85 regulatory subunit binding domain, the C2 domain, the accessory region, or the kinase domain (Fig. 1). The molecular details of how each mutation specifically affects the function and regulation of the kinase requires further study, although the H1047R mutation has been shown to increase its kinase activity (6). Interestingly, two tumors and one cell line contained more than one mutation, each with an exon 20 mutation complemented by a mutation in either exon 7 or 9 (H1047L with E418K, T1025S with E545K, H1047R with P539R, respectively). The significance of these double mutants is unknown, but one may hypothesize that this could indicate a multiclonal tumor, that some mutations are less potent activators of the kinase and that additional mutations to the same allele confer an added growth advantage, or could signify a second hit in the alternate allele. We confirmed the mutational status of all 12 cell lines described by Bachman et al. (8), with the exception of MCF-7 which they report as having E542K, and we found to harbor E545K.

Nine novel nucleic acid substitutions resulting in missense mutations were discovered in our analysis (Fig. 1). For each mutated tumor sample, corresponding normal tissue DNA, when available, was sequenced to verify that the mutation was somatic. Two novel mutations (E453K and N1044K) and an additional 17 mutated tumors with mutations shown somatic in other studies (6–8) were validated in this manner; the remaining seven novel mutations were cross-referenced and not identified in any normal DNAs in another large study (6).[9] Consistent with an oncogenic nature of these mutations, all mutations described in this study were missense, and, except for one mutation in the SUM-185 cell line, seemed heterozygous. Of note, nearly identical sequence to that of *PIK3CA* exons 9, 12, and 13 and their intron-exon boundaries can also be found at 22q11.2, and therefore care must be taken when designing primers and analyzing data (see Supplementary data).

In the Swedish cohort, PTEN protein expression and mutational status were determined. A larger set of 343 stage II cases (9) were evaluated by immunohistochemistry, of which 55 were scored PTEN negative ($PTEN^-$, little to no tumor staining in relation to normal cells on the same tissue section), and in agreement with the literature (12, 13) PTEN protein expression was significantly correlated to ER and PR positivity ($P < 0.0001$ and $P = 0.001$, respectively; data not shown; Fig. 2). From this larger set, 48 of the $PTEN^-$ tumors were selected for this study based on availability of snap-frozen tumor biopsies, and roughly matched to 109 $PTEN^+$ (relatively equal tumor PTEN protein levels when compared with internal control normal cells) tumors, with respect to node and ER status where possible; five cases with undetermined PTEN protein status were also selected and subsequently one $PTEN^-$ case was excluded due to bad sequence (Table 1). Correlation analysis between *PIK3CA* mutations to PTEN IHC status revealed a highly significant association, in that the majority of *PIK3CA* mutations were found in $PTEN^+$ cases ($P = 0.0066$; Table 2). All nine exons of *PTEN* were sequenced in these cases and eight mutations were found (5%). This was not surprising, because in sporadic breast cancer *PTEN* is infrequently altered via mutation (14, 15).

[9] Y. Samuels, personal communication.

Nevertheless, combination of the *PTEN* mutation data with the PTEN expression data improved the inverse correlation between *PIK3CA* mutations and abrogated PTEN (PTEN$^-$ and *PTEN* mutants counted together; $P = 0.0037$; Table 2). Surprisingly, two tumors were double mutants for *PIK3CA* and *PTEN*: *PIK3CA* H701P with a *PTEN* exon 5 frame-shift truncation mutation, and *PIK3CA* C420R with a *PTEN* exon 7 frame-shift truncating mutation. Mutational status of these two cases was rigorously verified (see Supplementary data).

The rare overlap between *PIK3CA* mutations and PTEN inactivation is consistent with the fact that these two proteins catalyze the opposite reaction and may indicate that after activation of PIK3CA or loss of PTEN, either of which results in the net increase of PIP_3 and effective oncogenic activation of the pathway, there is reduced selective advantage for the other gene to be also targeted. This observation also reinforces a recent report by Broderick et al. (7), who analyzed high-grade brain tumors and found no *PIK3CA* mutations in samples harboring *PTEN* mutations (which is the most common way PTEN is inactivated in those tumor types) among 47 tumors analyzed. In this light, the presence of two *PIK3CA*/*PTEN*-double mutants may be an indication that H701P and C420R are less potent activators of the pathway.

We were interested in testing the relationship between *PIK3CA* mutational status and other known clinicopathologic markers (Table 2). We found *PIK3CA* mutations to be highly correlated to lymph node involvement (N^+), ER positivity (ER^+), and PR positivity (PR^+; $P = 0.0375$, $P = 0.0001$, $P = 0.0063$, respectively), three clinical markers associated to patient survival and response to therapy (16, 17). This is in contrast to Bachman et al. who found no correlation to ER/PR in their study (8), which may be due to their small sample size compared with the present study. When only analyzing the stage II tumors from both Swedish and Columbia cohorts together, the correlations to node, ER, and PR status improved ($P = 0.0239$, $P < 0.0001$, and $P = 0.0041$, respectively; Table 2). This is not unexpected given the known associations between tumor stage and ER/PR and node status. Moreover,

Table 1. Clinical demographics of 292 human breast tumors

	Columbia cohort, $n = 131$ (%)	Sweden cohort, $n = 161$ (%)	Combined, $n = 292$ (%)
Stage			
I	31 (24)	0 (0)	31 (11)
II	66 (50)	160 (99)	226 (77)
III	18 (14)	0 (0)	18 (6)
IV	4 (3)	0 (0)	4 (1)
Unknown	12 (9)	1 (1)	13 (4)
PIK3CA mutants by stage			
I	9/31 (29)	0/0 (0)	9/31 (29)
II	14/66 (21)	44/160 (28)	58/226 (26)
III	3/18 (17)	0/0 (0)	3/18 (17)
IV	1/4 (25)	0/0 (0)	1/4 (25)
Unknown	5/12 (42)	1/1 (100)	6/13 (46)
All	32/131 (24)	45/161 (28)	77/292 (26)
Median age (y)	53 (range, 24-89; $n = 122$)	61 (range, 26-77; $n = 160$)	59 (range, 24-89; $n = 282$)
Median tumor size (mm)	25 (range, 5-90; $n = 121$)	27 (range, 2-50; $n = 160$)	26 (range, 2-90; $n = 281$)
Estrogen receptor			
Positive	85 (65)	77 (48)	162 (55)
Negative	35 (27)	79 (49)	114 (39)
Unknown	11 (8)	5 (3)	16 (5)
Progesterone receptor			
Positive	76 (58)	66 (41)	142 (49)
Negative	44 (34)	90 (56)	134 (46)
Unknown	11 (8)	5 (3)	16 (5)
Lymph node			
Positive	58 (44)	106 (66)	164 (56)
Negative	64 (49)	54 (34)	118 (40)
Unknown	9 (7)	1 (1)	10 (3)
ERBB2			
Positive	44 (34)	34 (21)	78 (27)
Negative	60 (46)	76 (47)	136 (47)
Unknown	27 (21)	51 (32)	78 (27)
Recurrence (any)			
Positive	27 (21)	48 (30)	75 (26)
Negative	92 (70)	112 (70)	204 (70)
Unknown	12 (9)	1 (1)	13 (4)
PTEN protein			
Positive	0 (0)	109 (68)	109 (37)
Negative	0 (0)	47 (29)	47 (16)
Unknown	131 (100)	5 (3)	136 (47)
Median follow-up time (y)	2.6 (range, 0-12.8; $n = 121$)	5.2 (range, 0.1-11.4; $n = 160$)	4.4 (range, 0-12.8; $n = 281$)

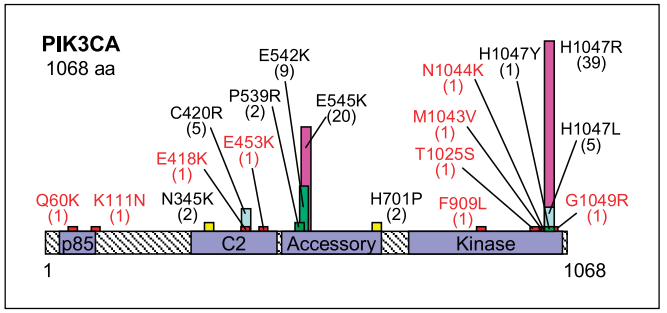

Figure 1. PIK3CA protein and functional domains. The mutations discovered in the present study are graphed with the amino acid substitutions indicated and the frequency of each given within parentheses. Amino acid changes from novel nucleotide mutations (*red*) and previously documented somatic mutations (*black*). Nucleotide substitutions in the *PIK3CA* coding sequence (where 1 is the first base of the start codon) for each mutation is as follows: C178A:Q60K, G333C:K111N, T1035A:N345K, G1252A:E418K, T1258C:C420R, G1357A:E453K, C1616G:P539R, G1624A:E542K, G1633A:E545K, A2102C:H701P, C2727G:F909L, A3073T:T1025S, A3127G:M1043V, T3132A:N1044K, A3140T:H1047L, A3140G:H1047R, C3139T:H1047Y, and G3145C:G1049R. Cell line mutations are presented in Supplementary Table S1. *p85,* PI3K p85 regulatory subunit binding domain; *C2,* C2 calcium/lipid-binding region; *accessory,* PI3K accessory (helical) domain; *kinase,* PI3/4-kinase domain.

PIK3CA mutations were significantly associated with ERBB2 overexpression (P = 0.0415), another predictive marker in breast cancer (18), and the correlation was more significant when testing only stage II cases (P = 0.0371; Table 2). This result is also in contrast to Bachman et al. who found no correlation to ERBB2 staining (8).

The frequency of *PIK3CA* mutations in several clinically relevant groups is worth highlighting. Approximately 33% of tumors positive for either ER, PR, node involvement, or ERBB2 harbored *PIK3CA* mutations (Table 2). Given the strong correlations to individual markers, it is not surprising that the mutation rate increased when we investigated subgroups defined by more than one marker (e.g., to 45% in $ER^+/ERBB2^+$ stage II tumors); in contrast, <7% were mutated among $ER^-/ERBB2^-$ tumors (P = 0.0002, n = 64, data not shown). In both the $ER^+/ERBB2^-$ and $ER^-/ERBB2^+$ subgroups, the mutation frequency was the same as the overall mutation rate (26%) in the complete data set (data not shown). As expected, analyzing further refined subgroups made the differences in mutation rate more extreme: 58% among $ER^+/ERBB2^+/N^+$ stage II cases versus less than 14% in $ER^-/ERBB2^-/N^-$ (P = 0.0062, n = 34, data not shown). It is also worth noting the distribution of clinical markers among *PIK3CA* mutants: 95% were *PTEN* wild type, 86% $PTEN^+$, 78% ER^+, 68% N^+, 65% PR^+, and 48% $ERBB2^+$ (Table 2).

The correlations between *PIK3CA* mutations and PTEN, ER/PR, node, and ERBB2 status present interesting questions. The observation that ER^+/PR^+ tumors more often have *PIK3CA* mutations whereas ER^-/PgR^- tumors more often have loss of PTEN may reflect differences in pathogenesis and disease progression between hormone receptor positive and negative tumors, thereby defining two separate branches of breast tumor development. Furthermore, our finding that *PIK3CA* mutations correlate with node metastasis and a report showing PTEN loss also associated with node metastasis (13) suggest that increased PIP_3 production and activation of the PI3K/AKT pathway may enhance invasion of cancer cells to lymph nodes. This last postulation is corroborated by the findings that PTEN inhibits cell migration (19), and that PIP_3 regulates cell motility (20). Lastly, our result that *PIK3CA* mutations correlate to ERBB2 overexpression argues that multiple positive inputs on the pathway may be needed

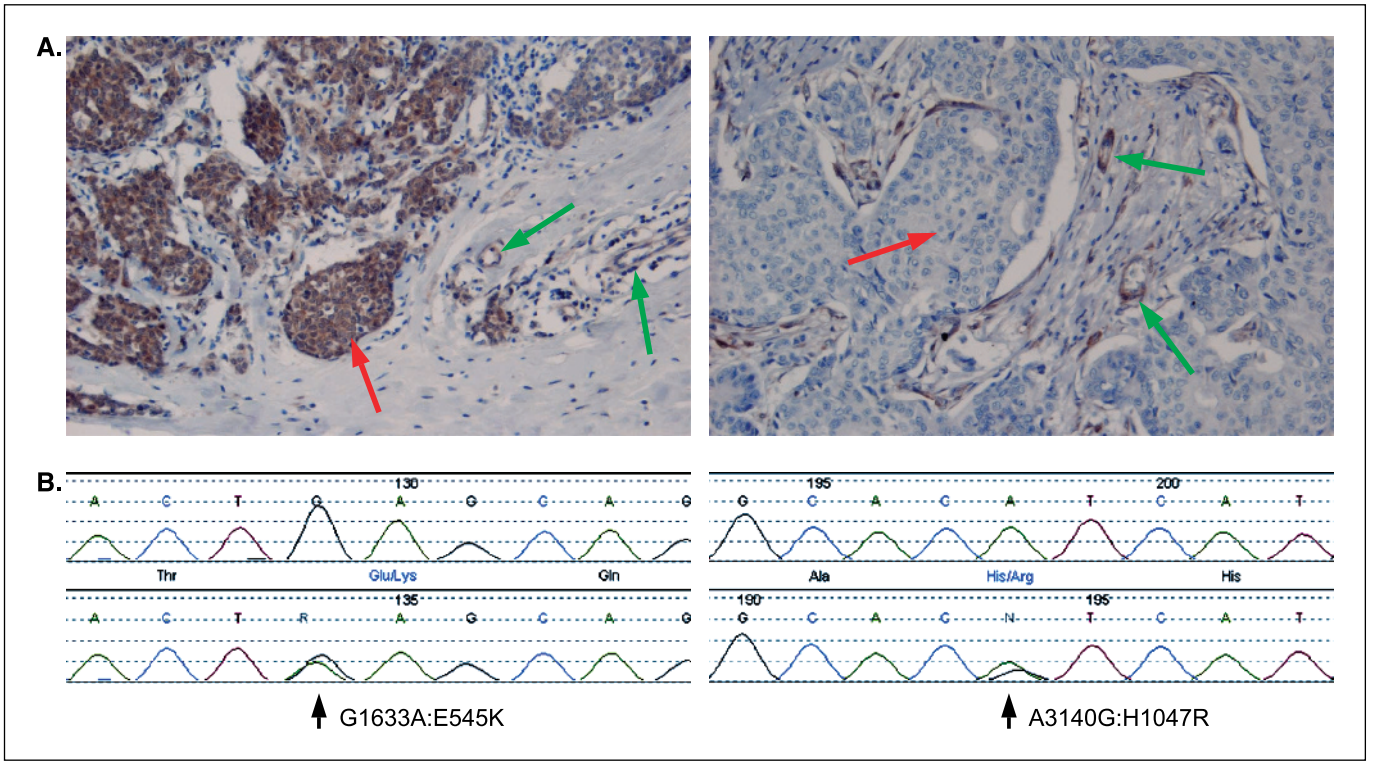

Figure 2. *A,* PTEN immunohistochemical staining. Typical staining results. *Left,* $PTEN^+$ tumor. *Right,* $PTEN^-$ tumor. *Red arrows,* tumor cells. *Green arrows,* $PTEN^+$ normal cells that serve as an internal control. *B, PIK3CA* mutations. Typical chromatogram traces are shown for exons 9 (*left*) and 20 (*right*) with normal wild-type sequence (*top*) and cancer-derived mutant sequence (*bottom*). *Arrows,* position of the missense mutations with nucleotide and amino acid substitutions noted.

Table 2. *PIK3CA* correlation analysis

	PIK3CA mutant (%)	*PIK3CA WT*	*n*	χ^2 *P*
Sweden cohort				
PTEN$^+$	37 (34)	72	156	0.0066
PTEN$^-$	6 (13)	41		
PTEN WT	42 (28)	110	160	NS
PTEN mutant	2 (25)	6		
PTEN "normal"	37 (35)	70	156	0.0037
PTEN abrogated	6 (12)	43		
ER$^+$	33 (43)	44	156	< 0.0001
ER$^-$	11 (14)	68		
PR$^+$	25 (38)	41	156	0.0214
PR$^-$	19 (21)	71		
Lymph node +	36 (34)	70	160	0.0103
Lymph node −	8 (15)	46		
ERBB2$^+$	10 (29)	24	110	NS
ERBB2$^-$	17 (22)	59		
Columbia cohort				
ER$^+$	23 (27)	62	120	NS
ER$^-$	5 (14)	30		
PR$^+$	22 (29)	54	120	0.056
PR$^-$	6 (14)	38		
	14 (24)	44	122	NS
Lymph node +				
Lymph node −	15 (23)	49		
ERBB2$^+$	15 (33)	31	111	0.0323
ERBB2$^-$	10 (15)	55		
All tumors				
ER$^+$	56 (35)	106	276	0.0001
ER$^-$	16 (14)	98		
PR$^+$	47 (33)	95	276	0.0063
PR$^-$	25 (19)	109		
		114	282	0.0375
Lymph node +	50 (30)			
Lymph node −	23 (19)	95		
ERBB2$^+$	25 (31)	55	221	0.0415
ERBB2$^-$	27 (19)	114		
Stage II tumors				
ER$^+$	43 (37)	72	211	<0.0001
ER$^-$	13 (14)	83		
		65	211	0.0041
PR$^+$	36 (36)			
PR$^-$	20 (18)	90		
Lymph node +	43 (31)	96	216	0.0239
Lymph node −	13 (17)	64		
ERBB2$^+$	17 (33)	35	163	0.0371
ERBB2$^-$	20 (18)	91		

NOTE: PTEN$^+$, equivalent PTEN protein expression in tumor and normal cells by immunohistochemistry. PTEN$^-$, loss of PTEN protein expression in tumor cells. PTEN "normal," *PTEN* WT and PTEN+. PTEN abrogated, *PTEN* mutant and/or PTEN$^-$. Estrogen and progesterone receptor status determined by routine clinical assays. ERBB2$^+$, DAKO system immunohistochemistry scores 2+/3+. ERBB2$^-$, 0/1+. Percentages are shown for the *PIK3CA* mutant column in terms of the totals for the cases in the given row. All *P*s < 0.10 are shown, *P*s < 0.05 are significant and underlined.
Abbreviations: WT, wild-type; NS, not significant.

to overcome intact PTEN (notably, both *PIK3CA*/*PTEN*-double mutants are ERBB2$^-$). This is clinically significant as intact PTEN is required for therapeutic response to Herceptin (21). Identification of the tumors that are ER$^+$/ERBB2$^+$ and *PIK3CA* mutation positive, a group likely to have normal PTEN, and treatment with a combination of drugs against the ER, ERBB2, and PIK3CA pathways is a therapeutic approach that should be explored.

Given the significant association of *PIK3CA* mutations to four clinical markers with varied prognostic implications, ER$^+$ and PR$^+$ (good prognosis), and N$^+$ and ERBB2$^+$ (bad prognosis), we did survival analyses. No significant associations were found between *PIK3CA* mutational status and distant disease-free survival, recurrence-free survival, or overall survival within all tumors, or when stratified by cohort, stage, lymph node, or ER status (data not shown). It is possible that the association of *PIK3CA* mutations to good and bad prognostic markers dilutes its association to outcome. Moreover, our selection of tumors was not ideal for survival analysis, as the Swedish set were matched on node and ER status, the Columbia cohort had varied clinical therapies and the duration of follow-up may not have been long enough (Table 1). *PIK3CA* mutations were not associated with tumor size, stage, ploidy, or S-phase fraction (data not shown).

In conclusion, in our study of *PIK3CA* mutations in a large set of breast cancers and breast cancer cell lines, we uncovered a mutation rate of 26%. We found *PIK3CA* mutations in all stages of breast cancer, indicating that mutations are an early event in carcinoma development. To determine the relationship between *PIK3CA* mutational status and PTEN function, we analyzed a subset of our primary tumors for the expression of PTEN protein and for the presence of *PTEN* mutations. We report that *PIK3CA* tumor mutations correlate to intact PTEN, lymph node metastasis, and expression of ER, PR, and ERBB2. We did not find any association with survival, although further study of material better suited for such analysis is warranted and is currently under way. We provide evidence for two separate paths for breast tumor development: hormone receptor positive with *PIK3CA* mutations and hormone receptor negative with PTEN loss. Taken together with the observation that 15% to 35% of breast cancers have loss of PTEN expression, our data would suggest that at least half of all breast cancers have deregulated production of PIP_3. When including aberrations in other upstream and downstream members of the pathway, such as ERBB2 and AKT, it would seem that the majority of breast tumors have activation of the pathway, making this pathway an attractive target for pharmacologic interventions and highlighting the importance of molecular profiling for personalized medicine. In particular, directed small molecule antagonism of PIK3CA proteins may be an effective measure for the treatment of breast cancer, regardless of whether it has a *PIK3CA* mutation or a loss of PTEN protein expression. It is interesting to note that many forms of human cancer have high frequency inactivation of PTEN and activation of PIK3CA. Given that alterations of these two genes tend to be mutually exclusive, it is likely that a very substantial proportion of most common malignancies such as carcinomas of the breast, lung, colon, and prostate have selected for activation of the PI3K pathway.

Note added in proof. Since we submitted this report, Campbell et al. have published a *PIK3CA* mutational study including 70 primary breast tumors but found no association to histologic subtype, grade, ER or node status (22).

Acknowledgments

Received 11/1/2004; revised 12/17/2004; accepted 12/30/2004.

Grant support: NIH, Medical Scientist Training grants 5T32 GM07367-29 (L. Saal and J. Yu) and CA082783 and CA097403 (R. Parsons), Avon Foundation (H. Hibshoosh and R. Parsons), Swedish Cancer Society (Å. Borg), Berta Kamprad Foundation (Å. Borg), Gunnar Nilsson Cancer Foundation (Å. Borg), Lund University Hospital Foundations (Å. Borg), King Gustav V:s Jubilee Foundation (Å. Borg), and Ingabritt and Arne Lundberg Foundation (Å. Borg).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the participating departments of the South Sweden Breast Cancer Group for providing samples; Vladan Miljkovic, Kristina Lövgren, Eva Rambech, and Carina Strand for expert technical assistance; John Jakob for tissue culture work; Ita Horan and Linda Lowenstein for administrative services; Susan Kistler for data retrieval assistance; and Sofia Gruvberger-Saal for helpful discussions.

References

1. Downward J. PI 3-kinase, Akt and cell survival. Semin Cell Dev Biol 2004;15:177–82.
2. Simpson L, Parsons R. PTEN: life as a tumor suppressor. Exp Cell Res 2001;264:29–41.
3. Chang HW, Aoki M, Fruman D, et al. Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3-kinase. Science 1997;276:1848–50.
4. Shayesteh L, Lu Y, Kuo WL, et al. PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet 1999;21:99–102.
5. Ma YY, Wei SJ, Lin YC, et al. PIK3CA as an oncogene in cervical cancer. Oncogene 2000;19:2739–44.
6. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
7. Broderick DK, Di C, Parrett TJ, et al. Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. Cancer Res 2004;64:5048–50.
8. Bachman KE, Argani P, Samuels Y, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol Ther 2004;3.
9. Ferno M, Stal O, Baldetorp B, et al. Results of two or five years of adjuvant tamoxifen correlated to steroid receptor and S-phase levels. South Sweden Breast Cancer Group, and South-East Sweden Breast Cancer Group. Breast Cancer Res Treat 2000;59:69–76.
10. Podsypanina K, Lee RT, Politis C, et al. An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/− mice. Proc Natl Acad Sci U S A 2001;98:10320–5.
11. Bose S, Crane A, Hibshoosh H, Mansukhani M, Sandweis L, Parsons R, Reduced expression of PTEN correlates with breast cancer progression. Hum Pathol 2002;33:405–9.
12. Perren A, Weng LP, Boag AH, et al. Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol 1999;155:1253–60.
13. Depowski PL, Rosenthal SI, Ross JS, Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. Mod Pathol 2001;14:672–6.
14. Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI, Boyd J. Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. Cancer Res 1997;57:3657–9.
15. Ueda K, Nishijima M, Inui H, et al. Infrequent mutations in the PTEN/MMAC1 gene among primary breast cancers. Jpn J Cancer Res 1998;89:17–21.
16. McGuire WL, Clark GM, Dressler LG, Owens MA. Role of steroid hormone receptors as prognostic factors in primary breast cancer. J Natl Cancer Inst Monogr 1986;19–23.
17. Hutter RV Pathological parameters useful in predicting prognosis for patients with breast cancer. Monogr Pathol 1984;175–85.
18. Hayes DF, Thor AD. c-erbB-2 in breast cancer: development of a clinically useful marker. Semin Oncol 2002;29:231–45.
19. Raftopoulou M, Etienne-Manneville S, Self A, Nicholls S, Hall A, Regulation of cell migration by the C2 domain of the tumor suppressor PTEN. Science 2004;303:1179–81.
20. Huang YE, Iijima M, Parent CA, Funamoto S, Firtel RA. Devreotes P, Receptor-mediated regulation of PI3Ks confines PI(3,4,5)P3 to the leading edge of chemotaxing cells. Mol Biol Cell 2003;14:1913–22.
21. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004;6:117–27.
22. Campbell IG, Russell SE, Choong DY, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 2004;64:7678–81.

PIK3CA Mutations Correlate with Hormone Receptors, Node Metastasis, and ERBB2, and Are Mutually Exclusive with PTEN Loss in Human Breast Carcinoma

Lao H. Saal, Karolina Holm, Matthew Maurer, et al.

Cancer Res 2005;65:2554-2559.