

PI3K Pathway Mutations and PTEN Levels in Primary and Metastatic Breast Cancer

Ana M. Gonzalez-Angulo^{1,2}, Jaime Ferrer-Lozano⁵, Katherine Stemke-Hale², Aysegül Sahin³, Shuying Liu¹, Juan A. Barrera⁶, Octavio Burgues⁷, Ana M. Lluch⁶, Huiqin Chen¹, Gabriel N. Hortobagyi¹, Gordon B. Mills², and Funda Meric-Bernstam⁴

Abstract

The purpose of this work was to determine whether there are differences in PIK3CA mutation status and PTEN protein expression between primary and matched metastatic breast tumors as this could influence patient management. Paraffin sections of 50 μm were used for DNA extraction and slides of 3 μm for immunohistochemistry (IHC) and FISH. Estrogen receptor, progesterone receptor, and HER2 IHC were repeated in a central laboratory for both primary tumors and metastases. PTEN levels were assessed by IHC and phosphoinositide 3-kinase (PI3K) pathway mutations were detected by a mass spectroscopy-based approach. Median age was 48 years (range: 30–83 years). Tumor subtype included 72% hormone receptor positive/HER2 negative, 20% HER2-positive, and less than 7.8% triple receptor negative. Tissues were available for PTEN IHC in 46 primary tumors and 52 metastases. PTEN was lost in 14 (30%) primary tumors and 13 (25%) metastases. There were five cases of PTEN loss and eight cases of PTEN gain from primary tumors to metastases (26% discordance). Adequate DNA was obtained from 46 primary tumors and from 50 metastases for PIK3CA analysis. PIK3CA mutations were detected in 19 (40%) of primary tumors and 21 (42%) of metastases. There were five cases of PIK3CA mutation loss and four cases of mutation gain (18% discordance). There was an increase of the level of PIK3CA mutations in four cases and decrease in one case from primary tumors to metastases. There is a high level of discordance in PTEN level, PIK3CA mutations, and receptor status between primary tumors and metastases that may influence patient selection and response to PI3K-targeted therapies. *Mol Cancer Ther*; 10(6); 1093–101. ©2011 AACR.

Introduction

Given the ability of hormone receptors and HER2 levels to predict response to therapy, it is currently recommended that they should be determined on every primary invasive breast cancer. However, changes in receptor status over disease progression and treatment have been described that could alter response to therapy. Concordance rates between primary tumors and recurrence site of 71% and 56% have been reported for estrogen receptor and progesterone receptor expression, respectively, and discrepancy rates for HER2 expression between primary tumors and matched metastases may be as high as 20% (1, 2). Furthermore, in a series of

patients with HER2-positive breast cancer treated preoperatively with trastuzumab, we reported that the HER2 status assessed by gene amplification changed to negative in over 30% of the cases who had extensive residual disease at the time of surgery (3). We recently reported that discordance of receptor status between primary tumors and metastases correlates with survival in patients with breast cancer (4). This suggests that optimal patient outcomes and responses in clinical trials could potentially be achieved by obtaining and evaluating tissue from metastatic sites.

The phosphoinositide 3-kinase (PI3K)/Akt/ mTOR pathway mediates multiple cellular functions critical to tumor initiation, progression, and outcomes including growth and proliferation, metabolism, motility, migration, invasion, angiogenesis, survival, and autophagy (5). Tight regulation of this pathway is paramount to ensure that multiple cellular inputs are integrated for appropriate cellular outcomes. Frequent deregulation and aberrations of this pathway have been implicated not only in breast cancer development and progression (6) but also in breast cancer therapy resistance (7–10). Thus, multiple drugs targeting the PI3K/Akt/mTOR pathway are in development and in early clinical trials as monotherapy and combination therapy in breast cancer (6). Our group

Authors' Affiliations: Departments of ¹Breast Medical Oncology, ²Systems Biology, ³Pathology, and ⁴Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas; and ⁵Fundación para la investigación, and Departments of ⁶Hematology-Oncology and ⁷Pathology, Hospital Clínico Universitario de Valencia, Valencia, Spain

Corresponding Author: Ana M. Gonzalez-Angulo, Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Unit 1354, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-2817; Fax: 713-794-4385; E-mail: agonzalez@mdanderson.org

doi: 10.1158/1535-7163.MCT-10-1089

©2011 American Association for Cancer Research.

has also shown significant changes from primary tumors to metastases on phosphorylation events that serve as biomarkers for response to PI3K/Akt/mTOR signaling-targeted therapies (48% discordance on p-Akt and p-4E-BP1; ref. 11).

A discordance between the level of potential biomarkers in primary tumor and metastasis could occur due to alterations in gene expression, possibly due to different microenvironmental stimuli, from stochastic events during metastasis, from clonal selection during metastasis, or from clonal evolution either at the primary or at the metastatic site. However, as new targeted therapies enter clinical trials in the metastatic setting using signatures from the primary tumor for patient selection may have profound outcome implications if biomarkers are discordant between the primary and metastatic site(s). *PIK3CA* mutation and loss of PTEN expression are being pursued as potential predictors of response to novel PI3K pathway inhibitors. Thus, to optimize patient selection for PI3K-targeted therapy, it is critical to determine whether mutation status of PI3K pathway components and PTEN loss in the primary tumor is concordant with the status of these markers in the metastases.

The objective of this study was to determine whether there are differences in mutation status of components of the PI3K pathway and in PTEN protein expression between primary tumors and metastases.

Materials and Methods

Under a research collaboration between MD Anderson Cancer Center and the Hospital Clinico Universitario de Valencia, Spain, we located paraffin blocks and corresponding clinical information of 50 matched pairs of primary breast cancer and biopsies of their corresponding asynchronous metastasis (distant nodes, skin, liver, lung, and bone). Paraffin blocks were sectioned. Two 50- μ m thick cuts were used for DNA extraction and 3- μ m slides were used for immunohistochemistry (IHC) and FISH. All cases were reviewed by dedicated breast pathologists. Estrogen receptor, progesterone receptor, and HER2 staining were repeated in a central laboratory in both primary tumors and metastases. FISH analysis was done in all cases that had 2+ staining by IHC or if there was a discordant result between the primary tumor and the metastasis. The Institutional Review Board of both institutions approved the laboratory studies and chart reviews.

Immunohistochemical analysis to determine estrogen receptor (clone SP1) and progesterone receptor (clone 1E2) status was conducted on 3- μ m sections of formalin-fixed, paraffin-embedded tissues using a Benchmark XT instrument (Ventana). Both Allred score (12) and percentage of nuclear staining were determined. Tumors with moderate to intense nuclear staining of at least 1% or more (13) or an Allred score equal or greater than 3 or 8 (12) were considered estrogen receptor or progesterone receptor positive.

Immunohistochemical analysis for HER2 was conducted under similar conditions, using the Pathway

anti-HER-2/neu (4B5) monoclonal antibody (Ventana). FISH analysis was conducted using HER2 FISH Pharm Dx (Dako) according to the manufacturer's instructions. HER2 positive was defined as 3+ receptor overexpression on Immunohistochemical staining (strong membranous staining in at least 30% of cells) and/or gene amplification was found on FISH. A gene copy/CEP-17 ratio greater than 2.2 was considered amplified (14).

PTEN IHC was conducted using monoclonal mouse Anti-Human PTEN antibody clone 6H2.1 from Dako at 1:100 dilution. Negative control slides without primary antibody were included for each staining. Both cytoplasmic and nuclear PTEN staining in the tumor and non-neoplastic ductal epithelium and stroma were quantified. PTEN staining in the nonneoplastic normal epithelium, intratumoral, and extratumoral stromal cells served as the internal positive control. Cases in which stromal staining was not observed were considered inevaluable. PTEN expression level was scored semiquantitatively on the basis of staining intensity and distribution using the immunoreactive score (IRS) as follows: IRS = SI (staining intensity) \times PP (percentage of positive cells). SI was determined as 0, negative; 1, weak; 2, moderate; and 3, strong. PP was defined as 0, <1%; 1, 1%–10%; 2, 11%–50%; 3, 51%–80%; and 4, >80% positive cells. Ten visual fields from different areas of each tumor were used for the IRS evaluation. Tumors with IRS of 0 were considered to have PTEN loss.

At least 70% tumor nuclear cellularity was confirmed in the samples used for DNA extraction. DNA was extracted using the QiaAMP Microkit (QIAGEN) according to manufacturer's instructions. A mass spectroscopy-based approach evaluating single nucleotide polymorphisms was used to detect known mutations in *PIK3CA* (*PIK3CA*_A1046V, *PIK3CA*_C420R, *PIK3CA*_E110K, *PIK3CA*_E418K, *PIK3CA*_E453K, *PIK3CA*_E542K, *PIK3CA*_E545K, *PIK3CA*_F909L, *PIK3CA*_G1049R, *PIK3CA*_G451L456_V, *PIK3CA*_H1047L, *PIK3CA*_H1047R, *PIK3CA*_H1047Y, *PIK3CA*_H701P, *PIK3CA*_K111N, *PIK3CA*_M1043V, *PIK3CA*_N345K, *PIK3CA*_P539R, *PIK3CA*_Q060K, *PIK3CA*_Q546E, *PIK3CA*_R088Q, *PIK3CA*_S405F, and *PIK3CA*_T1025S), *AKT1* (*AKT1*_E17K_G49A, *AKT1*_G173R_G517C, and *AKT1*_G173R_G517C), *AKT2* (*AKT2*_E17K_G49A and *AKT2*_G175R_G523C), and *AKT3* (*AKT3*_E17K_G49K and *AKT3*_G171R_G511A). PCR and extension primers for each gene were designed using Sequenom, Inc. Assay Design. PCR-amplified DNA was cleaned using EXO-SAP (Sequenom, Inc.) primer extended by IPLEX chemistry, desalted using Clean Resin (Sequenom, Inc.), and spotted onto Spectrochip matrix chips using a nanodispenser (Samsung). Chips were run in duplicate on a Sequenom MassArray MALDI-TOF MassArray system. Sequenom Typer Software and visual inspection were used to interpret mass spectra. Reactions where 8% or more of the resultant mass run in the mutant site in both directions were scored as positive. The MassArray approach allows quantification of the percentage of the DNA present that shows the *PIK3CA* mutation, which

Table 1. Patients and primary breast cancer characteristics

Characteristic	Total		PIK3CA mutations		PTEN loss	
	N	%	N	%	N	%
	51	100	19/47	40.4	14/46	30.4
Age at diagnosis, y						
Median	48	(30–83)	50	(30–83)	45	(31–70)
Menopausal status						
Premenopausal	30	58.8	8/26	30.8	9/29	31
Postmenopausal	21	41.2	11/21	52.4	5/17	29.4
Histology						
Ductal	40	78.4	12/36	33.3	12/37	32.4
Lobular	8	15.7	4/8	50	1/6	16.7
Other	3	5.9	3/3	100	1/3	33.3
Nuclear grade						
1	2	3.9	1/2	50	0/0	0
2	21	41.2	8/20	40	6/20	30
3	28	54.9	10/25	40	8/26	37.8
Lymphovascular invasion						
Positive	45	88.2	17/41	41.5	11/41	26.8
Negative	6	11.8	2/6	33.3	3/5	60
Breast cancer subtype						
Hormone receptor positive	37	72.5	15/34	44.1	10/35	28.6
HER2 positive	10	19.6	3/9	33.3	2/9	22.2
Triple receptor negative	4	7.8	1/4	25	2/3	66.7
Systemic chemotherapy						
Anthracycline based	23	45.1	9/18	50	7/21	30
Anthracycline and taxane based	27	52.9	9/27	33.3	6/25	24
No	2	3.9	1/2	50	1/2	50
Systemic endocrine therapy						
Tamoxifen	35	68.6	13/32	40.6	8/33	24.2
Aromatase inhibitor	2	3.9	2/2	100	1/2	50

reflects the fraction of cells with the mutations. Reactions in which there was more than a 50% increase or decrease in the value of DNA present that shows the *PIK3CA* mutation, the mutation score were also reported. In preliminary work, we directly compared standard Sanger sequencing and the Sequenom MassARRAY detection on 100 tumor samples. Sanger sequencing showed 11 of the 100 samples to have *PIK3CA* mutations. Using the Sequenom method, we detected all of the *PIK3CA* somatic mutations expected from Sanger sequencing analysis plus an additional 11 putative mutations. The utility and accuracy of the probes as well as the ability to quantify aberrations were confirmed with analysis of breast cancer cell lines with known *PIK3CA* mutations before analyzing patient samples. As shown by mixing experiments using cells with known mutations, the Sequenom approach can detect a mutation even if it is present in only 5% of the cell population and can give quantitative information on each mutation.

Patients were categorized according to their *PIK3CA* mutation and/or *PTEN* status. Patient and tumor characteristics were tabulated and compared between the χ^2 and the Fisher test as appropriate. Time to recurrence was

measured from the date of diagnosis to the date of first documented local or distant recurrence (metastasis biopsy). The Kaplan–Meier product limit method was used to estimate the time to recurrence of all patients by concordant and discordant marker (*PIK3CA* and/or *PTEN*) status and groups were compared with the log-rank statistic. Values of $P < 0.05$ were considered statistically significant; all tests were 2 sided. Statistical analyses were carried out using SAS 9.2 (SAS Institute Inc.) and S-Plus 7.0 (Insightful Corporation).

Results

Patients were categorized according to breast cancer subtype and their tumor characteristics are given in Table 1. Median age was 48 years (range: 30–83 years), and 58.8% of the patients were postmenopausal. Seventy-eight percent of the tumors had a ductal histology and more than 50% had a high nuclear grade and lymphovascular space invasion. Distribution by tumor subtype included 72.5% hormone receptor-positive and HER2-negative (HR), 19.6% HER2-positive (HER2), and less than

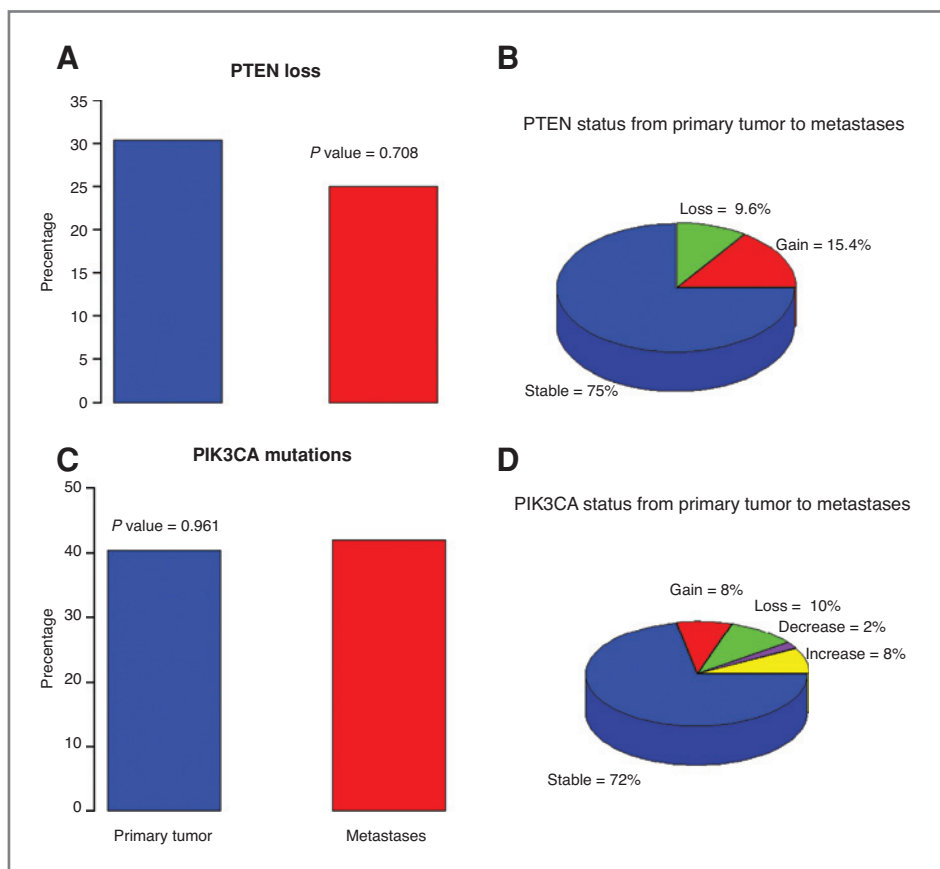


Figure 1. A and C, proportion of primary tumor and metastases with PTEN loss and PIK3CA mutations. B and D, distribution of the changes in PTEN levels and PIK3CA mutation status from primary tumor to metastases.

10% triple receptor–negative breast cancer (TNBC). Ninety-eight percent of the patients received systemic chemotherapy with an anthracycline or an anthracycline/taxane-based regimen (53%). All patients with hormone receptor tumors received adjuvant endocrine therapy.

Distant disease free survival was 90 months (range: 5–210 months). Initial distribution of the metastatic sites included bone only in 6 (11.8%) patients, visceral only in 6 (11.8%) patients, and bone and visceral in 39 (76.4%) patients. Metastasis tissues were obtained from at least 1 metastatic site in all cases, 2 metastatic sites in 7 cases, and 3 metastatic sites in 1 case. The time between obtaining samples of primary tumor and metastases was 46.5 months (range: 10–229 months). Metastatic samples were obtained from soft tissue 17 (28.8%); lymph nodes 9 (15.2%); lung 8 (11.8%); bone 6 (10.2%); liver 3 (5.1%); brain 2 (3.4%); pleura 2 (3.4%); ovary 2 (3.4%), peritoneum 1 (1.7%); and bowel 1 (1.7%).

Tissues were available for PTEN IHC in 46 primary tumors and 52 metastases. PTEN loss was found in 14 (30.4%) of the primary tumors and 13 (25%) of the metastases. There were no significant differences in the proportion of tumors with PTEN loss between primary tumors and metastasis ($P = 0.708$; Fig. 1). Adequate DNA was obtained from all 46 primary tumors and from 50 of the 59 metastases. Mutations in *AKT1*, *AKT2*, or *AKT3* were not detected. Mutations in *PIK3CA_H1047_A3140*

and *PIK3CA_E545_G1633* were found in this sample set. All others tested were not detected. A mutation in *PIK3CA* was found in 19 (40.4%) of the primary tumors and 21 (42%) of the metastases. Mutations in *PIK3CA_E545_G1633* occurred in only 1 primary tumor and in 1 of its corresponding metastasis. All other *PIK3CA* mutations occurred at *PIK3CA_H1047_A3140*. There were no significant differences in the proportion of tumors with *PIK3CA* mutations between primary tumors and metastasis ($P = 0.961$; Fig. 1).

The results of the frequency of PTEN loss and *PIK3CA* mutations by tumor subtype are summarized in Table 2.

When looking at standard-of-care breast cancer markers (estrogen receptor, progesterone receptor, and HER2), there were 51 pairs of primary tumors and metastases, samples for 2 metastatic sites in 7 cases, and 3 metastatic sites in 1 case for a total of 59 comparisons. There was a 25.4% discordance rate from primary tumors to metastasis: 4 tumors lost estrogen receptors, 7 tumors lost progesterone receptors, and 1 tumor lost HER2. Two tumors became progesterone receptor positive and 1 tumor became HER2 positive in their metastatic sites.

For PTEN analysis, there were 51 pairs of primary tumors and metastases, samples for 2 metastatic sites in 7 cases, and 3 metastatic sites in 1 case for a total of 59 comparisons. Ten slides (9 comparisons) were considered invaluable due to lack of adequate tissue. There

Table 2. PTEN loss and PIK3CA mutations by breast cancer subtype

Primary tumor	HR positive (n = 37)	HER2 positive (n = 10)	Triple negative (n = 4)	Total (n = 51)
PTEN loss	10/32	2/10	2/4	14/46 (30.4%)
PIK3CA mutations	15/34	3/9	1/4	19/47 (40.4%)
Both	4/32	1/10	1/4	6/46 (13%)
Either or both	19/34	4/9	2/4	25/47 (53.2%)
Metastasis	HR positive (n = 38)	HER2 positive (n = 12)	Triple negative (n = 6)	Total (n = 56)
PTEN loss	10/34	2/12	1/6	13/52 (25%)
PIK3CA mutations	15/34	4/11	2/5	21/50 (42%)
Both	2/34	1/12	1/6	4/50 (8%)
Either or both	17/34	5/12	3/6	25/50 (50%)

Abbreviation: HR, hormone receptor.

were 5 cases of PTEN loss and 8 cases of PTEN gain from primary tumors to metastasis for a 26% discordance rate. When looking at PTEN loss, 3 of the 5 primary tumors were hormone receptor positive and stayed hormone receptor positive in their metastatic sites. One tumor was HER2 positive and had a *PIK3CA* mutation detected in both primary tumors and metastases. Interestingly, 1 tumor was a primary TNBC at diagnosis, was found to be HR-positive at the metastatic site, and also gained a *PIK3CA* mutation. All these patients received chemotherapy (anthracycline- and taxane-based), and all but the TNBC patient received adjuvant tamoxifen. When looking at the cases that became PTEN positive, 7 of the 8 tumors were hormone receptor positive in both primary tumors and metastases and 3 of them had a *PIK3CA* mutation in both primary tumors and metastases. However, 1 tumor showed a significant increase in the number of cells with *PIK3CA* mutations in the metastatic site. One tumor classified as HER2 positive in the primary site became a TNBC at the metastatic site. All these patients received chemotherapy (anthracycline- and taxane-based) and adjuvant tamoxifen. The patient with the HER2-positive primary tumor that changed to TNBC in the metastasis did not receive trastuzumab.

In 2 cases in which there were more than 1 metastases, there were discordant findings between the metastases. One primary tumor in which PTEN was lost, PTEN continued to be lost in the bone biopsy; however, there was PTEN gain (score of 8) in the mediastinal node metastasis. In a second primary tumor in which PTEN was positive, PTEN continued to be positive in the liver biopsy; however, there was PTEN loss in the ovary biopsy. Both tumors had *PIK3CA* mutations in the primary and all metastatic sites. Figure 2 shows representative matched cases of primary tumors and metastases, including stable positive and negative staining, and shows changes in PTEN levels from absent to gain and from present to loss.

For *PIK3CA* mutation analysis, there were 51 pairs of primary tumors and metastases, samples for 2 metastatic sites in 7 cases, and 3 metastatic sites in 1 case for a total of 59 comparisons. There was insufficient DNA in 13

samples (11 comparisons). There were 5 cases where *PIK3CA* mutations were detected in the primary tumor but not in the metastasis and 4 cases where *PIK3CA* mutation was not detected in the primary tumors but

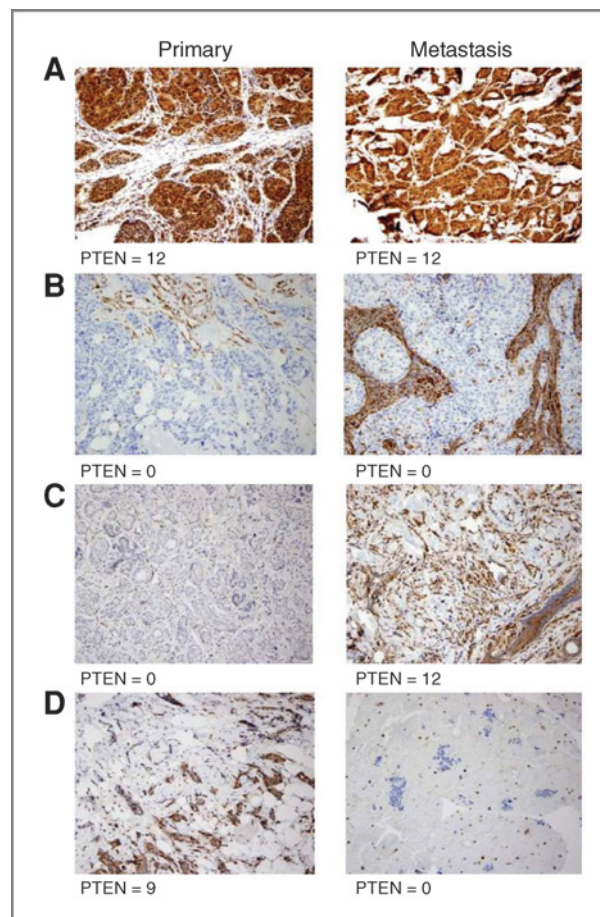


Figure 2. Immunohistochemical staining for PTEN in different cases of primary and metastatic disease. A and B, stable positive and negative staining, respectively. C and D, changes in PTEN levels from absent to gain and from present to loss, respectively.

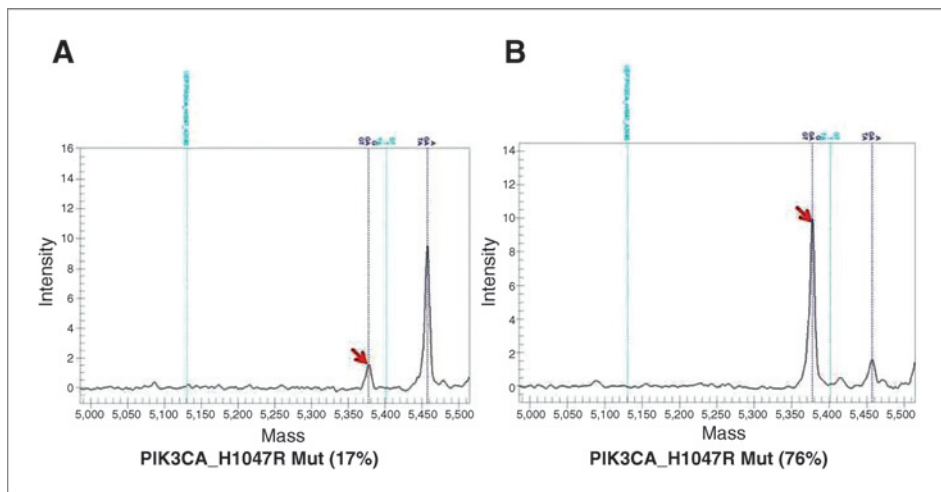


Figure 3. A representative case of marked increase levels of *PIK3CA* mutation at *PIK3CA_H1047R* from primary tumor (A; 17%) to metastasis (B; 76%). Mutation peak noted by red arrow.

was detected in the metastasis for an 18% discordance rate. Figure 3 shows a representative case of a marked increase levels of *PIK3CA* mutation. There was increase in the fraction of DNA with the *PIK3CA* mutation by more than 50% in 4 cases between the primary tumors and the metastasis and a decrease in the percentage of cells with the *PIK3CA* mutation by more than 50% in 1 case from primary tumors to metastasis.

When looking at cases where *PIK3CA* mutation was detected in the primary tumors and not the metastasis, 4 tumors were hormone receptor positive and 1 was a TNBC. Interestingly from the 4 hormone receptor-positive tumors, 1 tumor was found to be HER2 positive at the metastatic site and 1 tumor was found to be a TNBC in the metastatic site. There were no changes in PTEN IHC. Four of these patients received chemotherapy (anthracycline- and taxane-based) and adjuvant tamoxifen, and 1 patient did not receive chemotherapy but adjuvant letrozole. There was 1 case in which the primary tumor had a *PIK3CA* mutation (69%), the mutation was detected in a mediastinal metastasis, but with a significant decrease (24%), and where the mutation was not detected in a liver metastasis. PTEN was lost in the primary tumor and metastatic samples. This case was an hormone receptor-positive tumor that became TNBC in both metastatic sites and received chemotherapy (anthracycline- and taxane-based) and adjuvant tamoxifen.

When looking at cases where *PIK3CA* mutations were detected in the metastasis but not the primary tumors, 2 of the 4 tumors were hormone receptor positive, 1 was HER2 positive, and 1 was a TNBC. One of the hormone receptor-positive tumors became PTEN positive in the metastasis. The TNBC became hormone receptor positive in the metastatic site and lost PTEN. Patients with hormone receptor-positive tumors received chemotherapy (anthracycline- and taxane-based) and adjuvant tamoxifen. The patient with the HER2-positive tumor received adjuvant tamoxifen (tumor was estrogen receptor positive), and the patient with TNBC did not receive adjuvant systemic chemotherapy.

When looking at cases with more than 50% changes in the fraction of DNA with the *PIK3CA* mutation, all 4 tumors with increased fractions were hormone receptor positive. One case gained PTEN levels from a score of 0 to 4. All four received chemotherapy (3 anthracycline-based and 1 anthracycline- and taxane-based) and adjuvant tamoxifen.

Table 3 and Fig. 1 summarize the changes in markers between primary and metastatic breast cancer. Table 4 shows the significant changes in the percentage of the DNA fraction with *PIK3CA* mutations in the primary tumors and metastases.

There were no differences in median time to recurrence in between patients who had discordant tumors compared with patients who had concordant tumors by PTEN levels and *PIK3CA* mutation status (37 months vs. 40 months, $P = 0.922$).

The therapy for breast cancer patients is currently different on the basis of hormone receptor and HER2 expression. The breast cancer subtype was different between the primary tumors and the metastasis in 4 patients, which would result in a change in therapy based on current guidelines. For 2 hormone receptor-positive primary tumors, 1 was a TNBC and lost PTEN in both metastases while maintaining a *PIK3CA* mutation in the primary tumors in 1 metastatic site but with the *PIK3CA* mutation not being detected in a second metastatic site. One HER2-positive cancer became TNBC and gained PTEN, and 1 TNBC became HER2 positive, which was associated with the detection of a *PIK3CA* mutation in the metastatic but not the primary site.

Discussion

The PI3K/Akt/mTOR signaling pathway is an emerging therapeutic target for cancer therapy. As a result, there is an urgent need to identify robust markers that can determine pathway activity and likelihood of benefit from pathway targeting therapy. Mutations at the *PIK3CA* gene and the levels of PTEN are being studied

Table 3. Changes in marker status from primary to metastatic breast cancer

Marker	Changes from primary tumor to metastases				
PTEN and PIK3CA mutations status					
PTEN	≥1 to Loss	0 to Gain	Stable		
Primary tumors (N = 46)	5 (9.6%)	8 (15.4%)	39 (75%)		
Metastases (N = 52)					
PIK3CA mutation	Detected to absent	Absent to mutation	Increase in PIK3CA	Decrease in PIK3CA	Stable
Primary tumors (N = 47)	5 (10%)	4 (8%)	4 (8%)	1 (2%)	36 (72%)
Metastases (N = 50)					
Standard of care breast cancer markers					
Estrogen receptor	Positive to negative	Negative to positive	Stable		
Primary tumors (N = 51)	4 (6.8%)	0 (0%)	55 (93.2%)		
Metastases (N = 59)					
Progesterone receptor	Positive to negative	Negative to positive	Stable		
Primary tumors (N = 51)	7 (11.9%)	2 (3.4%)	50 (84.7%)		
Metastases (N = 59)					
HER2	Positive to negative	Negative to positive	Stable		
Primary tumors (N = 51)	1 (1.7%)	1 (1.7%)	57 (96.6%)		
Metastases (N = 59)					

as possible predictive markers for PI3K pathway inhibitors. However, it is unknown whether the assessment of these markers in the primary tumor accurately reflects the status of these markers in metastases arising from the same tumor. This is particularly important in breast cancer, where the primary tumor is removed surgically and the target for therapy is usually occult micrometastases. We report the results of a systematic evaluation of concordance in PTEN levels and PI3K pathway mutations in matched primary tumor and metastases. We found that, overall, PI3K pathway aberrations are common and that half of primary tumors as well as metastases have either *PIK3CA* mutations, PTEN loss, or both. The overall rates of *PIK3CA* mutation and PTEN loss were similar in the primary tumors and matched metastases. Furthermore, *PIK3CA* and PTEN loss were found together with approximately the expected frequency indicating that there was neither coselection nor mutual exclusion for these events. However, we found marked

discordances in PTEN levels (26%), *PIK3CA* mutations (18%), and receptor status (25%) between the primary tumor and metastases. Strikingly, there was both a gain and a loss of ability to detect PTEN and *PIK3CA* mutations between the primary tumors and metastases suggesting that at least in terms of *PIK3CA* and PTEN, metastasis is likely a stochastic event with metastatic competence not being dependent on aberrations in the PI3K/Akt/mTOR pathway.

Several studies have addressed the issue of discordance in expression of individual breast cancer markers (estrogen receptor, progesterone receptor, and HER2) between primary tumor and recurrence/metastasis, but discordance rates varied substantially from study to study (1, 2, 11, 15–17). Others have studied different markers with similar conflicting results. Lacroix and colleagues looked at the membrane protein levels of HER2 and epidermal growth factor receptor as well as gene aberrations affecting these oncogenes in human primary tumors and metastatic lesions. Among 57 patients, expression level and gene copy numbers of HER2 or epidermal growth factor receptor were similarly altered in the primary tumor and metastatic lesions derived from the same patient (18). Using IHC or sandwich enzyme immunoassay, investigators determine expression levels of HER2 and p53 proteins in 42 breast cancer samples from 21 patients who underwent surgery for primary tumors and surgical resection of asynchronous metastases. Estrogen receptors and progesterone receptors were also measured by enzyme immunoassay in each case. Although, there was no difference in the positivity rate of HER2 and p53 expression between the primary tumors and the metastases, there was 50% discordance in estrogen receptor and progesterone receptor

Table 4. Significant changes in the levels of *PIK3CA* mutations in the primary tumors and metastases

Direction	PIK3CA mutation level, %	
	Primary tumor	Metastases
Increase	12	30
Increase	17	27
Increase	17	76
Increase	9	47
Decrease	69	24

expression (19). Using IHC, Cardoso and colleagues reported the percentage of discordant biomarker status in the primary tumor and its metastatic lymph nodes to be 2% for HER2, 6% for *p53*, 15% for *bcl-2*, 19% for topoisomerase II- α , 24% for HSP27, and 30% for HSP70. For the subgroup of patients with positive biomarkers in the primary tumor, the percentage of discordance was 6% for HER2, 7% for *p53*, 14% for *bcl-2*, 19% for HSP70, 21% for topoisomerase II- α , and 36% for HSP27. For the subgroup of patients with positive biomarkers in the lymph nodes, the percentage of discordance was 9% for *bcl-2*, 15% for HER2 and *p53*, 21% for topoisomerase II- α , 22% for HSP27, and 25% for HSP70 (20).

Previous reports have not compared PI3K pathway mutational status and PTEN levels in primary tumors and their corresponding metastases. However, investigators recently reported the frequency of *PIK3CA* mutations in *in situ* and invasive breast cancer. They sequenced exons 9 and 20 of *PIK3CA* in pure ductal carcinoma *in situ* (DCIS), DCIS adjacent to invasive carcinoma, and invasive ductal breast carcinomas. In a subset of cases, both *in situ* and invasive areas were analyzed from the same tumor. They found that the frequency of *PIK3CA* mutations was approximately 30% in all 3 histologic groups, consistent with previous reports for invasive cancer (21). Interestingly, in third case, *in situ* and invasive areas of the same tumor were discordant for *PIK3CA* status, and in 2 cases in which multiple invasive and adjacent *in situ* areas within the same tumor were analyzed independently, they detected intratumor heterogeneity for *PIK3CA* mutations (22). Thus, there seem to be multiple tumor subclones with different mutation status present in primary tumors. Using IHC, we previously looked at the discordance in the expression of p-Akt (Ser473) and p-4E-BP1 (Ser65) between primary breast cancer and matched surgically resected metastases. We found that most primary breast tumors and metastases expressed p-Akt (76% of each). However of the 23 matched evaluable pairs, 11 (47.8%) had discordant immunohistochemical results. Similarly, although most of the primary tumors and metastases were positive for p-4E-BP1 (75% and 74%), of the 23 matched evaluable pairs, 8 (47.8%) were discordant (11).

Our study suggests that *PIK3CA* mutation status and PTEN levels may differ between primary tumors and metastases. We had anticipated that if there were discordances, these would be consistent with higher PI3K/Akt/mTOR signaling in metastases because of molecular evolution, therapy exposure/selection, and increased metastatic potential of active pathway clones. Unexpectedly, we found that almost the same proportion of patients had activating *PIK3CA* mutations in their primary tumor and not in the metastasis, as had activating mutations in their metastasis but not in the primary tumors. We found similar results when looking at PTEN levels. The gain and loss frequencies between primary and metastatic sites indicate that the aberrations in the PI3K pathway assayed are not obligatory for the metastatic process, did not drive the

metastatic process, and are not selected during the metastatic process. There were also some cases in which we detected marked differences in the percent of cells with *PIK3CA* mutations between primary tumors and metastases indicative of dominance of different clones in the primary tumors and metastases. The underlying mechanism and clinical significance of these changes should be formally studied. However, it is clear that this should be considered in the design and implementation of trials of PI3K pathway-targeted therapy.

Changes in receptor expression may either account for a true biological phenomenon or may result from inconsistent measurement. It has been argued that changes in receptor expression may occur at different time points of disease (molecular evolution) and possible reasons include the following: change during tumor progression as a consequence of genetic instability or clonal selection (23), intratumoral heterogeneity (24, 25), selection by therapy or metastatic process, and mutations induced by cellular exposure to systemic agents such as chemotherapy or hormonal therapy or targeted therapy. However, evidence derived from transcriptional profiling suggests that the hard-wired characteristics of individual tumors do not change by chance throughout the course of the disease. Weigelt and colleagues (26) showed that gene expression profiles of metastases clustered closely to their corresponding primary tumors, which may indicate that the metastatic capability may be an intrinsic feature of the primary tumor. As an alternative hypothesis for this observation, the particular genomic aberrations driving the tumor may only allow the tumor to explore a limited transcriptional space and that processes regulating metastasis are not able to dominate the underlying transcriptional pattern.

Further studies are needed to confirm our findings and whether they represent a true biological discordance as discussed before or the limitations of marker development such as the poor reproducibility of IHC, or pre-analytic variables including variation in processing and fixation of tumors, tumor hypoxia times, and tumor cellularity. However, it is important to emphasize that the MassArray approach applied to assess DNA mutations is highly robust and unlikely to show a significant false-positive or -negative rate and also to be able to detect relatively rare events in the tumor mass. As new targeted therapies are brought into clinical trials, using primary tumor signatures for patient selection may not represent the signature status in the metastatic disease that is being treated and this may have significant outcome implications. Indeed, it may be necessary to develop approaches to assess predictive signatures present in metastatic disease to engender optimal outcomes. This could be through repeat biopsies, through novel molecular imaging technologies, or potentially through analysis of circulating DNA, microRNA, or tumor cells. Identification of robust biomarkers that can accurately assess driver aberrations and predict response to therapies and that can be used widely with low

interlaboratory variability is critical to successful delivery personalized cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported in part by SUC2-AACR-DT0209 01 (A.M. Gonzalez-Angulo, G.B. Mills, and F. Meric-Bernstam), National Cancer

Institute 1K23CA121994-01 (A.M. Gonzalez-Angulo), ASCO Career Development Award (A.M. Gonzalez-Angulo), KGKG081099 (A.M. Gonzalez-Angulo, K. Stemke-Hale, G.B. Mills), and National Cancer Institute through The University of Texas MD Anderson Cancer Center Support grant (P30 CA016672).

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.

Received December 20, 2010; revised March 11, 2011; accepted March 20, 2011; published OnlineFirst April 13, 2011.

References

- Li BD, Byskosh A, Molteni A, Duda RB. Estrogen and progesterone receptor concordance between primary and recurrent breast cancer. *J Surg Oncol* 1994;57:71-7.
- Simon R, Nocito A, Hübscher T, Torhorst J, Schraml P, Bubendorf L, et al. Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. *J Natl Cancer Inst* 2001;93:1141-6.
- Mittendorf EA, Wu Y, Scaltriti M, Meric-Bernstam F, Hunt KK, Dawood S, et al. Loss of HER2 amplification following trastuzumab-based neoadjuvant systemic therapy and survival outcomes. *Clin Cancer Res* 2009;15:7381-8.
- Liedtke C, Broglio K, Moulder S, Hsu L, Kau SW, Symmans WF, et al. Prognostic impact of discordance between triple receptor measurements in primary and recurrent breast cancer. *Ann Oncol* 2009;20:1953-8.
- Meric Bernstam F, Gonzalez-Angulo AM. Targeting the mTOR signaling network for cancer therapy. *J Clin Oncol* 2009;27:2278-87.
- Agarwal R, Carey M, Hennessy B, Mills GB. PI3K pathway-directed therapeutic strategies in cancer. *Curr Opin Investig Drugs* 2010;11:615-28.
- Perez-Tenorio G, Stal O. Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. *Br J Cancer* 2002;86:540-5.
- Miller TW, Perez-Torres M, Narasanna A, Guix M, Stål O, Pérez-Tenorio G, et al. Loss of Phosphatase and Tensin homologue deleted on chromosome 10 engages ErbB3 and insulin-like growth factor-I receptor signaling to promote antiestrogen resistance in breast cancer. *Cancer Res* 2009;69:4192-201.
- Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, 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.
- Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 2007;12:395-402.
- Akcakanat A, Sahin A, Shaye AN, Velasco MA, Meric-Bernstam F. Comparison of Akt/mTOR signaling in primary breast tumors and matched distant metastases. *Cancer* 2008;112:2352-58.
- Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998;11:155-68.
- Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 2010;28:2784-95.
- Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor2 testing in breast cancer. *J Clin Oncol* 2007;25:118-45.
- Vincent-Salomon A, Jouve M, Genin P, Fréneaux P, Sigal-Zafrani B, Cally M, et al. HER2 status in patients with breast carcinoma is not modified selectively by preoperative chemotherapy and is stable during the metastatic process. *Cancer* 2002;94:2169-73.
- Simon R, Nocito A, Hübscher T, Bucher C, Torhorst J, Schraml P, et al. Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. *J Natl Cancer Inst* 2001;93:1141-46.
- Gancberg D, Di Leo A, Cardoso F, Rouas G, Pedrocchi M, Paesmans M, et al. Comparison of HER-2 status between primary breast cancer and corresponding distant metastatic sites. *Ann Oncol* 2002;13:1036-43.
- Lacroix H, Iglehart JD, Skinner MA, Kraus MH. Overexpression of erbB-2 or EGF receptor proteins present in early stage mammary carcinoma is detected simultaneously and overexpression in primary and regional metastases. *Oncogene* 1989;4:145-51.
- Shimizu C, Fukutomi T, Tsuda H, Akashi-Tanaka S, Watanabe T, Nanasawa , et al. c-erbB-2 protein overexpression and p53 immunoreaction in primary and recurrent breast cancer tissues. *J Surg Oncol* 2000;73:17-20.
- Cardoso F, Di Leo A, Larsimont D, Gancberg D, Rouas G, Dolci S, et al. Evaluation of HER2, p53, bcl-2, topoisomerase II-alpha, heat shock proteins 27 and 70 in primary breast cancer and metastatic ipsilateral axillary lymph nodes. *Ann Oncol* 2001;12:6415-20.
- Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 2008;68:6084-91.
- Miron A, Varadi M, Carrasco D, Li H, Luongo L, Kim HJ, et al. PIK3CA mutations in *in situ* and invasive breast carcinomas. *Cancer Res* 2010;70:5674-8.
- Edgerton SM, Moore D II, Merkel D, Thor AD. erbB-2 (HER-2) and breast cancer progression. *Appl Immunohistochem Mol Morphol* 2003;11:214-21.
- Pertschuk LP, Axiotis CA, Feldman JG, Kim YD, Karavattayhayill SJ, Braithwaite L. Marked intratumoral heterogeneity of the proto-oncogene Her-2/neu determined by three different detection systems. *Breast J* 1999;5:369-74.
- Kerbel RS. Growth dominance of the metastatic cancer cell: cellular and molecular aspects. *Adv Cancer Res* 1990;55:87-132.
- Weigelt B, Glas AM, Wessels LF, Witteveen AT, Peterse JL, van't Veer LJ, et al. Gene expression profiles of primary breast tumors maintained in distant metastases. *Proc Natl Acad Sci U S A* 2003;100:15901-5.