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PIK3CA mutations in Peruvian patients with HER2-amplified and triple negative non-metastatic breast cancers

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PURPOSE: To determine the frequency of PIK3CA mutations in a Peruvian cohort with HER2-amplified and triple negative breast cancers (TNBC).

METHODS: We analyzed two cohorts of 134 primary non-metastatic breast cancer patients from Peru. Cohorts consisted of 51 hormone receptors (+)/HER2-amplified breast tumor patients surgically resected as first treatment included in the ALTTO trial (ALTTO cohort) and 81 TNBC patients with residual disease after neoadjuvant treatment (neoadjuvant cohort). Genomic DNA was extracted from paraffin-embedded tumor samples. Samples from the ALTTO and neoadjuvant cohorts were taken at biopsies and from residual tumors, respectively. PIK3CA mutations were detected by sequencing DNA fragments obtained by PCR amplification of exons and their flanking introns. All of the detected PIK3CA mutations were confirmed in a second independent run of sample testing.

RESULTS: PIK3CA mutations were present in 21/134 cases (15.7%). Mutations in exon 9 and 20 were present in 10/134 (7.5%) and 11/134 (8.2%), respectively. No cases had mutations in both exons. Mutations in exon 9 consisted of E545A (seven cases), E545K (two cases) and E545Q (one case); while in exon 20, mutations consisted of H1047R (10 cases) and H1047L (one case). Compared to TNBC patients, HER2-amplified patients were more likely to have PIK3CA mutated (23% vs 9.6%; $P = 0.034$). There were no associations between mutational status of PIK3CA with estrogen receptor status ($P = 0.731$), progesterone receptor status ($P = 0.921$), age ($P = 0.646$), nodal status ($P = 0.240$) or histological grade ($P = 1.00$). No significant associations were found between PIK3CA mutational status and clinicopathological features.

CONCLUSIONS: We found a similar frequency of PIK3CA mutations to that reported in other series. Although we did not include HR+/HER2 patients, those with HER2-amplified tumors were more likely to present PIK3CA mutations compared to patients with triple negative tumors.

KEYWORDS: Breast cancer; PIK3CA; HER2; Triple negative

Breast cancer (BC) is the most frequent cancer among women around the globe and the second most frequent malignancy in Peruvian women (age-standardized rate incidence of 34 per 100,000 women each year).[1] Latinas represent the largest (14% of US population), youngest and fast-est-growing minority in the U.S. and around 10% of developing countries.[2] It appears that BC is less prevalent but demonstrates higher mortality in Latinas than in Caucasian women.[3–5]

BC is a genetically heterogeneous disease and has significant variability among different ethnic and racial

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groups with respect to incidence, clinical characteristics, and prognosis.[6] Socio-economic and cultural factors are among the reasons underlying these differences. Little information is available for the Latina population.

HER2 is a molecule with the ability to define BC behavior and can predict response to anti-HER2 treatment. Its activation and dimerization leads to downstream activation of many signaling molecules, including phosphoinositide 3-kinase (PI3K). The PI3K is a cell pathway related to the regulation of cell signaling, growth, proliferation, and apoptosis. This pathway activates the serine/threonine kinase AKT which in turn activates phosphorylates and regulates mTOR.[7] Several preclinical studies suggest that HER2-independent activation of the PI3K-AKT pathway may be related to trastuzumab resistance.[8]

Preclinical and tumor tissue analysis have indicated PI3K-AKT pathway is frequently activated in BC; and its activation has been associated with aggressive features like high histological grade, basal-like and HER2 phenotypes, as well as poor clinical outcome. Most of the mutations in this pathway (80%) happen at three hotspots of the gene PIK3CA: two in exon 9 and one in exon 20. These mutations are found in 28–40% of BC tumors, and His1047Arg is the most common mutation. However, their relation to specific phenotypes or prognosis in BC is unclear.[9]

The relevance of this pathway has increased due to the recently demonstrated benefit of PI3K pathway modulators in hormone-sensible BC, and to evidence suggesting these drugs could improve trastuzumab activity in HER2-positive. Further, recent information indicates that PIK3CA mutation status could predict response to PI3K modulators.[9–11]

A study by Wu et al. in Los Angeles, US, found PI3K-AKT pathway activation in >70% in HER2(+) tumors from African-American and Latina BC patients, compared to <45% reported for HER2(+) tumors from Japanese and Swedish women.[12] Liedtke et al. evaluated PIK3CA mutations in 140 stage II–III BC cases, including 50 Hispanic cases (mostly from Peru and Mexico). The study found mutations in 10 cases (six in exon 9 and four in exon 20) from the 50 Hispanic patients, 10 (six in exon 9 and four in exon 20) from 74 Caucasian patients and two (only in exon 20) from 13 Black patients.[13]

This information suggests PIK3CA mutation rates could be higher in a Latina population. We were therefore determined to evaluate for the first-time the prevalence of PIK3CA mutations in a Peruvian HER2-amplified (HER2-amp) and triple-negative

BC series, and to compare it with data from other world regions.

METHODS

Patients and samples

We analyzed two cohort samples of 134 primary non-metastatic breast cancers diagnosed and treated at the Instituto Nacional de Enfermedades Neoplásicas (Lima, Peru). The first cohort (ALTTO cohort) consisted of 51 patients with ER+ and/or PR+ and HER2+ tumors surgically resected as first treatment and who were enrolled in the ALTTO trial. The second cohort consisted of 83 patients with triple negative breast cancer with residual tumor after the neoadjuvant treatment. The samples were examined histologically and were considered suitable for this study if the proportion of tumor cells exceeded 50% with sufficient cellularity as was proven by evaluation of tumor samples stained by hematoxylin and eosine.

Evaluation of ER, PgR, and HER2 status

Estrogen Receptor (ER), Progesterone Receptor (PgR) and HER2 were evaluated by immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded (FFPE) breast tumor samples with the follow primary antibodies: Anti-Estrogen Receptor ID5 clone (Dako, Carpinteria, CA); Anti-Progesterone Receptor PGRG36 clone (Dako, Carpinteria, CA); Anti-HER2, clone A0485 (Dako, Carpinteria, CA). Antigen retrieval for ER and PR was done with citrate buffer (pH 7.5) with heat for 15 min; while for HER2, enzymatic digestion with Trypsin was performed at 37 °C for 20 min. Steps for IHC were taken according to the manufacturer's instructions. A tumor was considered ER or PR positive if there was presence of nuclear staining in ≥1% of tumor cells; and HER2 was positive if 3 was scored by immunohistochemistry. A blinded review was conducted by one pathologist (FD).

Additional confirmation by fluorescent in situ hybridization (FISH) was done in a centralized laboratory in the ALTTO trial context (ALTTO Cohort) with the conditions described in Ref.14. The HER2 FISH evaluation in the neoadjuvant cohort was performed at the Vanderbilt-Ingram Cancer Center on FFPE tissues using the PathVysion HER-2 DNA Probe Kit (PathVysion Kit, Abbott Molecular, Des Planes, IL, USA) utilizing the Vysis® LSI® HER-2/neu 17q11.2–12 SpectrumOrange™ and Vysis CEP 17 (17p11.1–q11.1 Spectrum Green Alpha Satellite DNA) probes. Images were visualized on a Fluorescence Olympus BX60 Microscope and

analyzed using the Genus™ for Genetic Image Analysis software, version 3.6 as described in Ref. 15.

PIK3CA mutation screening
Analysis of the ALTTO cohort
FFPE tissue samples containing the rich-tumor region were identified by microscopy and the corresponding region was micro-dissected. Genomic DNAs were extracted from the tissue samples using the DNeasy tissue kit (Qiagen, Valencia, CA). The sample was incubated overnight with Proteinase K at 56 °C. The DNA was bound to silica columns, washed and eluted in 30 µl of nuclease-free water according to manufacturer's instructions. PIK3CA mutations were detected by screening DNA fragments obtained by PCR amplification of exons 9 and 20, and their flanking introns.

(PIK3CA-Exon9, Forward: GAGGGGAAAAA TATGACAAAG, Reverse: GAGATCAGCCAAA TTCAGTTA; PIK3CA-Exon20, Forward: TGAG CAAGAGGCTTTGGAGT, Reverse: GGTCTTT GCCTGCTGAGAGT). The amplified products were sequenced on an ABI Prism 3130 automated DNA sequencer (Applied Biosystems, Foster City, CA), and the sequences of PIK3CA sequencing profiles were compared with the corresponding reference sequence (NM_006218). All of the detected PIK3CA mutations were confirmed in a second independent run of sample testing.

Analysis of the neoadjuvant cohort
Samples were evaluated at Foundation Medicine Inc. facilities using a high throughput next-generation sequencing protocol. Briefly, DNA was extracted from 40 µm of unstained FFPE sections, and then purified with the Promega® Maxwell 16 Tissue LEV DNA kit; 50–200 ng of dsDNA were fragmented to ∼200 bp by sonication before purification with AMPure XP beads (Agencourt). SPRI purification and subsequent library construction with the NEBNext kits (E6040S, NEB) containing mixes for end repair, dA addition and ligation, were performed in 96-well plates (Eppendorf) on a Bravo Benchbot (Agilent). Indexed (6-bp barcodes) sequencing libraries were PCR amplified with HiFi™ (Kapa) for 10 cycles, 1.8X SPRI purified and quantified. Paired end sequencing was performed using the HiSeq2000 (Illumina). Sequence data from genomic DNA were mapped to the reference human genome (hg19) using the BWA aligner. PCR duplicate read removal and sequence metric collection were performed using Picard (http://picard.sourceforge.net)

and SAMtools.[16] Local alignment optimization was performed using GATK.[17]

Statistical analysis
Relationships between PIK3CA mutation status and clinical, histological, and biological parameters were estimated with the chi-squared test. Differences between the mutated and non-mutated populations were judged significant at confidence levels of greater than 95% ($P < 0.05$).

Ethical considerations
Patients in the ALTTO cohort (INEN 07-18) were informed in the ALTTO trial context and signed an informed consent stating that their tumor samples might be used for scientific purposes and that they had the opportunity to decline at any time. Patients on the neoadjuvant cohort were under an institutionally approved protocol (INEN 10-018).

RESULTS

Patient characteristics
ALTTO cohort
Fifty-one patients had breast conserving surgery or modified radical mastectomy as first treatment. All patients received adjuvant chemotherapy with anthracyclines and taxanes. Forty-one patients (80.4%) received adjuvant radiation. Patients included in the ALTTO study were treated with adjuvant trastuzumab with or without lapatinib.

Neoadjuvant cohort
This cohort comprised 81 patients with TNBC determined by immunohistochemistry with residual disease after anthracycline-based neoadjuvant chemotherapy. After FISH confirmation, eight patients had HER2 amplification.

PIK3CA status
PIK3CA mutations were present in 21 cases (15.7%). Mutations in exon 9 and 20 were present in 10 (7.5%) and 11 (8.2%) cases, respectively. No case had mutations in both exons or coexistence of two or more mutations. Mutations in exon 9 were E545A (seven cases), E545K (two cases) and E545Q (one case); while in exon 20, mutations were H1047R (10 cases) and H1047L (one case) (see Table 1).

HER2-amp patients were more likely to have PIK3CA mutations (23% vs 9.6%; $P = 0.034$). Differences between these two groups were in the exon 9 ($P = 0.023$). There were no associations between

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Table 1. PIK3CA mutation profiles.

Exon	Nucleotide change	Amino acid substitution	HER2-amp n (%)	TNBC n (%)	Total n (%)
9	A1634C	E545A	7 (11.5)	0	7 (5.2)
9	G1633A	E545K	1 (1.6)	1 (1.4)	2 (1.5)
9	G1633C	E545Q	0	1 (1.4)	1 (0.7)
20	A3140G	H1047R	6 (9.8)	4 (5.5)	10 (7.5)
20	A3140T	H1047L	0	1 (1.4)	1 (0.7)

mutational status of PIK3CA with estrogen receptor status ($P = 0.717$), progesterone receptor status ($P = 1.000$), age ($P = 0.808$) nodal status ($P = 0.432$) or histological grade ($P = 0.457$). No significant associations were found between PIK3CA mutational status and clinicopathological features according to HER2+ or TNBC subgroups (Table 2).

DISCUSSION

This study represents the first detailed analysis of PI3K mutations in a well-defined cohort of Hispanic patients. In this report we show an overall frequency of 15.7% of PIK3CA mutations in breast cancer tumors in a group of patients with HER2-amp and TNBC. Although the coexistence of two distinct PI3KCA mutations in the same sample has previously been reported, all mutations detected in our patients were unique and there was no coexistence of two or more mutations.

HER2amp tumors were more likely to have PIK3CA mutations than TNBC, and the significant difference was in the distribution in mutations in exon 9. Although there is no consensus about a predisposition of PIK3CA mutation by breast cancer phenotypes, several reports describe that 12–25% of HER2+ tumors had mutations in PI3KCA, and that TNBC harbors low rates of PI3KCA mutations. Cizkova et al. reported that PIK3CA mutations in 452 patients were more common in HR positive tumors (39%) and HER2-enriched tumors (25%) than in basal-like tumors (13%).[18] The data of the Cancer Genome Atlas Network of 507 patients evaluated with breast cancer showed a frequency of mutations of PIK3CA in 45% in luminal A, 29% in luminal B, 39% in HER2-enriched and 9% in basal-like tumors.[19]

Jensen et al. evaluated 240 HER2-positive early BC cases treated with adjuvant chemotherapy and trastuzumab and found PIK3CA mutations in 26% of patients and whose survival rates had worse outcome.[20] A retrospective analysis of PIK3CA mutations in 107 HER2-positive metastatic breast cancer detected a 22% rate of mutations with a correlation with worse TTP (median 7.6 vs 11.3 months) and OS (median 20.1 vs 41.0 months, $P = 0.046$).[21]

A previous report by Cizkova et al. describes more favorable metastases-free survival (MFS) in patients with PIK3CA mutations (five-year MFS of 81.0% versus 69.6% and 15-year MFS of 65.8% versus 53.4% for PIK3CA mutated vs wild type, respectively). Although some reports do not find prognostic relevance for the presence of PIK3CA mutation, it has been described as a PIK3CA mutation-associated gene expression signature predicting favorable survival in ER-positive breast cancer.[18,22]

Recently, Baselga et al. evaluated the role of PIK3CA mutations in approximately 800 naive HER2-positive metastatic BC cases included in the phase III clinical trial CLEOPATRA (trastuzumab with or without pertuzumab). They found that these mutations were associated with worse prognosis ($P = 0001$) and without difference in relation to the treatment arm.[23]

Analysis of the predictive value of PIK3CA mutations on anti-HER2 agents was performed in a biomarker analysis of around 200 patients included in the phase II study of neoadjuvant pertuzumab and trastuzumab, with findings of PIK3CA mutations in 24% of patients and not associated with pCR.[24]

There is also evidence showing that tumors with PI3K/AKT pathway activation including PTEN loss or PIK3CA mutation or both are less sensitive to trastuzumab treatment.[21] Interestingly, this resistance appears to be reversed by mammalian target of rapamycin (mTOR) or PI3K inhibitors.[25] A final validation of PIK3CA mutation as an independent

Table 2. Relationship between *PIK3CA* mutation status and standard clinical, pathological and biological features of breast cancer.

Patient features	*n*	Exon 9	Exon 20	Any exon 19 or 20	*P*[a]
Age in years					
<50	74 (55.2%)	5 (6.8%)	7 (9.6%)	12 (16.2%)	0.847
≥50	60 (44.8%)	5 (8.3%)	4 (6.8%)	9 (15%)	
Histological grade[b]					
II	14 (26.4%)	3 (21.4%)	0	3 (21.4%)	1
III	39 (73.6%)	4 (10.3%)	4 (10.3%)	8 (20.5%)	
Lymph node status[b]					
0	16 (30.2%)	2 (12.5%)	2 (12.5%)	4 (25%)	0.240
1–3	16 (30.2%)	3 (18.8%)	2 (12.5%)	5 (31.3%)	
>3	21 (39.6%)	2 (9.5%)	0	2 (9.5%)	
Nodal involvement after chemo[c]					
Positive	25 (31.6%)	1 (4.0%)	3 (12.0%)	4 (16%)	0.453
Negative	54 (68.4%)	1 (1.9%)	4 (7.4%)	5 (9.3%)	
NA	2				
Tumor size[b]					
<2 cm	10 (22.2%)	2 (20%)	1 (10%)	3 (30%)	0.393
>2 cm	35 (77.8%)	4 (11.4%)	2 (5.7%)	6 (17.1%)	
Not evaluable	8	1			
ER status					
Negative	117 (75.5%)	8 (6.8%)	10 (8.5%)	18 (15.4%)	0.731
Positive	17 (24.5%)	2 (11.8%)	1 (5.9%)	3 (17.6%)	
PR status					
Negative	122 (91.1%)	9 (7.4%)	10 (8.2%)	19 (15.6%)	0.921
Positive	12 (9.0%)	1 (8.3%)	1 (8.3%)	2 (16.7%)	
Phenotype					
TNBC	73 (75.5%)	2 (2.7%)	5 (6.8%)	7 (9.6%)	0.034
HER2-amp	61 (24.5%)	8 (13.1%)	6 (9.8%)	14 (23.0%)	

[a]*P*-value for the Chi-square or Fisher test. [b]For the ALTTO cohort.
[c]For the neoadjuvant cohort.

predictor of the response to trastuzumab treatment in ERBB2+ breast cancer needs a prospective randomized study.

Our results describe the presence of PIK3CA mutation in Peruvian women with breast cancer. Due to the prospective nature of our study, and a short period of follow-up, no conclusion on survival could be obtained. However, our data show that the association of PIK3CA mutations with the phenotype is present across different ethnic groups. Previous genomic analyses were performed almost entirely on populations of European descent. It is possible that specific differences in the genomic architecture of certain populations may influence the outcome of BC. The type and frequency of PI3K mutations in other subtypes of BC in this Hispanic population is yet to be evaluated. Moreover, studies that assess the prognostic and predictive value of alterations in PI3K pathways in different ethnic groups deserve further analysis (see Table 3).

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Table 3. Diverse mutations in diverse ethnic groups.

	N	Any exon (%)	Exon 9	Exon 20	Subset
Present study (Hispanic)	134	20.7	E545A, E545K, E545Q	H1047R, H1047L	HER2amp (23% mut); TNBC (9.6% mut)
Liedtke et al.[13]	140	16	E542K, E542V, E545K, Q546R	H1047R, H1047T, G1049R	CS II-III, all subtypes ER+ (19.2% mut); PR+ (20.6% mut); HER2+ (13.3% mut)
Asian	3	33			
Black	13	15			
Hispanic	50	20			
Caucasian	74	14			
Gori et al.[26] (Caucasian)	45	12.2	D549N	H1048Y, A987V, M1004I, H1048Y, L1026P	HER2+ Met
Jensen et al.[20] (Caucasian)	240	26	E542K, E545K, Q536K	H1047R, H1047L	HER2+ early stage
Stemke-Hale et al.[27] (Caucasian & Hispanic)	547	21.40	E542K, E545K, N345K, E418K, K11N	H1047R, H1047L, H1047Y, G1049R	All subtypes HR+(34.5% mut); HER2+(22.7% mut);TN (8.3%)
Cizkova et al.[18] (Caucasian)	151	33.4	E545A, Q546A, E542K, E545G, E545K, E545Q, N497S, Q546K, Q546R	N1044K, G1049R, T1052K, D1029H, H1047R, A1066V, M1043V	All subtypes Erα+(39.1% mut); PR (41.4% mut); HER2+ (24.7%)

Abbreviations: TNBC, triple negative breast cancer; amp, amplified; mut, mutated PIK3CA; met, metastatic; ER, estrogen receptor; PR, progesterone receptor.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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