Clinico-pathological Features of *PIK3CA* Mutation in HER2-Positive Breast Cancer of Indian Population

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Abstract

PIK3CA pathway is one of the important signaling pathways in cells, which is involved in cell proliferation, cell survival, motility, and growth. Mutation in *PIK3CA* gene negatively effects to anti-HER2 therapy in breast cancer patients. *PIK3CA* gene of HER2-positive breast cancers associated with reduced sensitivity to neoadjuvant therapy. In this study, we assessed the frequency of *PIK3CA* mutations and influence of *PIK3CA* mutations on patient survival in a series of HER2-positive breast cancer patients. *PIK3CA* mutations were assessed by pyrosequencing and next generation sequencing in 107 HER2-positive breast cancer patients of a tertiary Cancer Centre of India from Jan 2012 to Jun 2013 with minimum follow-up of 3 years. We found *PIK3CA* mutations in 26 tumors (24.2%) of which 5 were in exon 9, 20 were in exon 20, and 1 was in both exon 9 and 20. In exon 9, the mutation c.1634A>G was found in 4 cases and mutation c.1636C>A was found in 2 cases. In exon 20, the mutation c.3140A>G was found in 15 cases and c.3140A>T was found in 6 cases. The outcome between *PIK3CA* mutated versus *PIK3CA* wild type was significant showing *p* value 0.014. Overall survival of mutation and treatment with herceptin, mutation with other chemotherapy treatment in both early breast cancer (EBC), and locally advanced breast cancer (LABC) showed significant *p* value 0.037 and 0.044 respectively. In conclusion, we identified 24.2% somatic mutation of *PIK3CA* in HER2-positive breast cancer patients. *PIK3CA* mutation is significantly associated with ER-positive tumors. The frequency and distribution pattern reported in this study is similar to the global report. Overall survival of *PIK3CA* mutation is slightly lower but in patients who received herceptin with *PIK3CA* mutation showed better clinical outcome.

Keywords *PIK3CA* · HER2 · NGS · Breast cancer

Introduction

Breast cancer is the second most common cancer for women in the India. Researches have shown that about 20–30% of the breast cancer patients have HER2 amplification or overexpression that is associated with a more aggressive phenotype and decreased survival [1]. Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) is the gene located at chromosome 13 which encodes p110 α . The gene consists of 20 exons that code for a total of 1068 amino acids.

The *PIK3CA* gene is mutated frequently in common cancers, including carcinoma of breast, prostate, colon and endometrium [2]. Dysregulation of tyrosine kinase receptor (TKR)-phosphatidylinositol 3-kinase (PI3K) signaling pathways is frequent in human cancers. Among the most important molecular events downstream of TKR activation is PI3K activation, which catalyzes the phosphorylation of inositol lipids to phosphatidylinositol-3,4,5-triphosphate which in turn activates the serine/threonine kinase AKT and this regulates several signaling pathways controlling cell survival, apoptosis, proliferation, motility and adhesion [3]. The frequency of *PIK3CA* mutations in breast cancer cases ranges from 16.4 to 45% [4]. There are 3 mutation “hotspots” in the *PIK3CA* gene: E542K, E545K at exon 9 (helix domain), and H1047R at exon 20 (kinase domain). The 3 hotspots represent almost 80% of *PIK3CA* mutation and lead to constitutive PI3K activity by different mechanisms [5]. *PIK3CA* pathway plays an important role in cancer development and progression. Recent report suggests that *PIK3CA* pathway activation negatively

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affect the response to trastuzumab therapy. This observation was described on both retrospective and prospective patient series [6–8]. In this study, we assessed the frequency of *PIK3CA* mutations and influence of *PIK3CA* mutations on patient survival in a series of HER2-positive breast cancer patients.

Materials and Methods

One hundred seven consecutive cases of HER-2overexpressing/amplified breast cancer cases were studied in tertiary Cancer Centre from Jan 2012 to Jun 2013 with minimum follow-up of 3 years. All patients who came to our institution were informed that their tumor samples might be used for scientific purposes and had the opportunity to decline. This study was approved by the institutional ethics committee (Institutional Review Board, Rajiv Gandhi Cancer Institute).

Genomic DNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue chosen after examination of “H & E”-stained section for maximum viable tumor without pigment, necrosis or other fixation artifact.

Genomic DNA was extracted using QIAamp Mini Kit (Qiagen) according to the manufacturer’s instructions. The extracted DNA was stocked at -20°C for future analysis.

Pyrosequencing assay was developed to detect *PIK3CA* mutations, because pyrosequencing has been shown to be applicable to FFPE and more sensitive than Sanger dideoxy sequencing. The exon 9 PCR primers were: *PIK3CA* 9-F: 5'-biotin-AACAGCTCAAAGCAATTTCTACACG-3' and *PIK3CA* 9-R: 5'-ACCTGTGACTCCATAGAAAATCTTT-3'. The exon 20 PCR primers were: *PIK3CA* 20-F: 5'-biotin-CAAGAGGCTTTGGAGTATTTC-3' and *PIK3CA* 20-R: 5'-CAATCCATTTTGTGTGTC-3'. [9]

PCR was carried out using Pyromark mastermix. PCR conditions were as follows: 95°C for 15 min; 42 cycles of 95°C for 20 s, 53°C for 30 s, and 72°C for 20 s; and final extension at 72°C for 5 min. The PCR products were electrophoresed in an agarose gel to confirm successful amplification of the 81-bp (exon 9) and 74 bp (exon 20) products. The PCR products were then prepared with the Vaccum Prep workstation (Pyromark Q24 work station, Qiagen, German) according to following protocol: 10 μl of the amplicon, 1 μl streptavidin sepharose HP beads (GE Healthcare), 40 μl binding buffer, and 29 μl Nuclease free water were mixed for 15 min. The biotinylated amplicons were immobilized onto streptavidin Sepharose beads, washed by 70% ethanol, denatured by 0.2 mol NaOH, and washed by 10 M Tris Acetate using the Vaccum Prep workstation. The amplicons were transferred to a plate containing corresponding sequencing primer in 24.2 μl

Fig. 1 *PIK3CA* exon 9 Pyrograms (antisense strand). **a** Wild-type exon 9 by the 9-RS1 primer. **b** The c.1634A>G mutation (arrow) causes a shift in reading frame and results in a new peak at C (arrowhead). **c** The

c.1636C>A mutation (arrow) causes a shift in reading frame and results in a new peak at C (arrowhead)

Fig. 2 On analysis by Ion reporter software shows *PIK3CA* Exon 9 mutation (c.1634A>G) 37.77%

annealing buffer. For exon 9, the sequencing primer is *PIK3CA* 9-RS: 5'-CCATAGAAAATCTTTCTCCT-3'; for exon 20, the sequencing primer is *PIK3CA* 20-RS: 5'-GTTGTCCAGCCACCA-3' [6]. The plate was heated with samples at 80 °C for 2 min. Pyrosequencing was performed using Pyromark Q24 (Qiagen, German).

Next-generation sequencing (NGS) was done using Ion Ampliseq Cancer Hot Spot Panel V2 (4475346) which targets the 50 oncogenes of tumor suppressor genes. This panel includes 207 pairs of primers in a single tube. For library preparation, we used 10 ng of DNA and Ion Ampliseq Library Kit 2.0 (4480441). Ion Hi-Q One Touch2 (OT2) 200 template kits

Fig. 3 *PIK3CA* exon 20 pyrograms (antisense strand). (A) Wild-type exon 20. (D) The c.3140A > T mutation (arrow) causes a shift in reading frame and results in a new peak at G (arrowhead). (E) The c.3140A > G mutation (arrow) causes a shift in reading frame and results in a new peak at G (arrowhead)

Clinicopathologic character	Total population number (Percentage)	Mutated (%)	Wild type (%)	<i>p</i>
Total	107	26 (24.2)	81 (75.7)	
Histologic grade				
I	3 (2.8)	1 (3.8)	2 (2.4)	NS
II	35 (32.7)	8 (30.7)	27 (33.3)	
III	69 (64.4)	17 (65.3)	52 (64.1)	
Node status				
N0	41 (38.3)	5 (19.2)	36 (44.2)	NS
N+	66 (61.6)	18 (69.2)	48 (59.2)	
ER status				
Positive	53 (49.5)	10 (38.4)	43 (53)	0.018
Negative	54 (50.4)	8 (30.7)	46 (56.7)	
PR status				
Positive	31 (28.9)	9 (34.6)	22 (27.1)	NS
Negative	76 (71)	17 (65.3)	59 (72.8)	
Tumor size				
≤ 25	48 (44.8)	11 (42.3)	37 (45.6)	NS
> 25	54 (50.4)	4 (15.3)	50 (61.7)	

were used for emulsion PCR and Ion One Touch ES instrument was used for enrichment.

For sequencing, we used Ion PGM Hi-Q Sequencing kit using 316 chip and Ion torrent PGM Machine (Life Technologies). Ion express barcodes and Adapters from Life Technologies were used for sample barcoding during Library Preparation. All protocols were followed as recommended by the manufacturers without modification.

Statistical analyses were performed using the statistical packages SPSS (version 23.0 for Windows, IBM SPSS statistics). Relationships between *PIK3CA* mutation status and clinical, histological, and biological parameters were estimated with the chi-squared test. Survival distributions were estimated with the Kaplan-Meier method.

We examined *PIK3CA* mutation by pyrosequencing (Figs. 1 and 2) and next generation sequencing (Figs. 3 and 4) and mutations were found in 26 tumors (24.2%) of which 5 were in exon 9, 20 were in exon 20 and 1 was in both exon 9 and 20. In exon 9, the mutation c.1634A>G was found in 4 cases and mutation c.1636C>A was found in 2 cases. In exon 20, the mutation c.3140A>G was found in 15 cases and c.3140A>T was found in 6 cases. Out of 107 cases, 65 cases were early breast cancer (EBC) and 42 cases were locally advanced breast cancer (LABC). In cases of EBC, we found 14 (33.3%) cases were *PIK3CA* mutated and in LABC 12 (18.4%) were *PIK3CA* mutated Table 1.

No significant association between *PIK3CA* mutations and clinicopathological characteristics was found except ER status. Survival analysis found significantly lower overall survival ($p = 0.014$; Fig. 5a) in *PIK3CA*-mutated cases,

Fig. 4 On analysis by Ion reporter software shows *PIK3CA* Exon 20 mutation (c.3140A>G) 35%

Fig. 5 Overall survival of *PIK3CA* mutation

whereas *PIK3CA*-mutated cases who received Herceptin chemotherapy showed improved overall survival ($p = 0.002$; Fig. 5b) than *PIK3CA* wild type who received herceptin.

Discussion

PIK3CA is most frequently mutated oncogene in breast cancer. *PIK3CA* mutations were identified in 26 (24.2%) of 107 HER2-positive breast tumors, in keeping with results of the previous study, showing mutation rate 21.3% [10]. Exons 9 and 20 encode the helical and kinase domains of *PIK3CA*, respectively. We found a higher frequency of mutation in exon 20 (19.6%) compared to exon 9 (5.6%) similar to the study which shows predominance of exon 20 mutations in breast cancer has been reported [8]. We found one double mutation in exon 9 and exon 20. Double mutations in *PIK3CA* have been reported previously in gastric and breast cancers [11, 12] and it has been suggested these could indicate multiclonal tumors or a second hit in the alternate allele. In the present study, *PIK3CA*-mutated tumors showed poorer outcome than *PIK3CA* wild-type cases (Figure 3) similar to the study of M Cizkova et al. [10]. We found no significant association between *PIK3CA* mutations and important clinical or pathological features as in several studies [13]. As far as the prognostic significance of *PIK3CA* mutation is concerned, it is still debatable whether the presence of *PIK3CA* mutation is associated with good or poor clinical outcome. Some studies reported significantly longer metastasis-free survival and better clinical outcomes in patients with *PIK3CA* mutations [14, 15], while another series of studies reported lower survival and poor clinical outcomes [16, 17]. Our results state that *PIK3CA*-mutated cases who received herceptin shows improved clinical outcome in HER2-positive breast cancers than *PIK3CA* wild-type HER2-positive breast cancer.

Conclusion

In conclusion, we identified 24.2% somatic mutation of *PIK3CA* in HER2-positive breast cancer patients. *PIK3CA* mutation is significantly associated with ER-positive tumors. The frequency and distribution pattern reported in this study is similar to the global report. Overall survival of *PIK3CA* mutation is slightly lower but in patients who received Herceptin with *PIK3CA* mutation showed better clinical outcome. *PIK3CA* mutation seems to be potential to be used as an indicator of favorable prognosis with Herceptin. Our preliminary result needs to be confirmed by further study in larger series of patients with longer follow up period.

Compliance with Ethical Standards

Informed Consent Informed consent was obtained from the patient.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This study approved by the institutional ethics committee (Institutional Review Board, Rajiv Gandhi Cancer Institute). (RGCI ID-406/PA/AMH-09).

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