Original paper

Association of *PIK3CA* somatic mutations with clinicopathological parameters in breast cancer

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The enzyme phosphatidylinositide-3-kinase (PI3K) regulates cellular proliferation and apoptosis. Somatic mutations in the *PIK3CA* gene can accelerate these processes and significantly contribute to the development and progression of breast cancer. This study aimed to ascertain the *PIK3CA* gene mutations in breast cancer patients and investigate their correlation with certain clinicopathological characteristics.
We conducted a mutational investigation of the *PIK3CA* gene using next-generation sequencing (NGS) in a sample of 100 cases of primary breast cancer. We investigated the associations between *PIK3CA* mutations and clinicopathological characteristics. Our analysis revealed a mutation rate of 45% in the *PIK3CA* gene. The mutation frequencies for the three hotspot sites were 33.3% for E545K in exon 10, 26.7% for H1047R in exon 20, and 6.7% for E542K in exon 10. Of the 45 individuals with tumors carrying the *PIK3CA* mutation, 41 (91.2%) had only one mutation, while 4 (8.8%) had two. Pathogenic *PIK3CA* mutations were significantly correlated with tumor size ($p = 0.015$) and tumor location ($p = 0.017$).
Our study results demonstrated a significant association between tumor size, location, and presence of the *PIK3CA* mutation. We must validate these data in larger sample sizes.

Key words: breast cancer, PIK3CA, mutations, hotspot mutations, clinicopathological parameters.

Introduction

Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) is crucial in cellular signaling pathways. It encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K), an enzyme that regulates cell growth, proliferation, survival, and metabolism. The PI3K pathway is integral to many cellular processes, including insulin signaling, protein synthesis, and cytoskeletal rearrangement [1, 2].

However, mutations in the *PIK3CA* gene can lead to dysregulation of this pathway, contributing to various diseases, particularly cancer. Mutations in *PIK3CA* are frequently observed in many cancer types, including breast (approximately 40%), endometrial (approximately 53%), and cervical cancer (approximately 26%), among others [1]. These mutations can lead to constitutive activation of PI3K signaling, promoting uncontrolled cell growth and survival, hallmark features of cancer. Mutations in the *PIK3CA* gene can occur at various points along its sequence, resulting in different effects on protein function. Some mutations increase kinase activity, while others alter the protein's interaction with regulatory molecules, ultimately driving oncogenic processes [2].

The detection rate of *PIK3CA* somatic mutations in breast cancer is critical because these mutations can influence the biological characteristics and prognosis of breast cancer. *PIK3CA* mutations are detected in

approximately 40% of breast cancers. This makes it one of the most observed specific genetic alterations in breast cancer [1]. Mutations in the *PIK3CA* gene are predominantly found in the helical and kinase domains of the protein. Specifically, point mutations such as E545K, E542K, and H1047R enhance the catalytic activity of the PIK3CA protein, leading to excessive activation of cellular signaling pathways. These mutations can support a variety of biological processes associated with cancer, including cell growth, survival, and metastasis. Therefore, *PIK3CA* mutations can have a significant impact on breast cancer treatment and prognosis [3, 4].

This study aimed to determine the frequency of somatic mutations in *PIK3CA*, a commonly observed genetic alteration in breast cancer, and to investigate the relationship of these mutations with clinicopathological parameters such as age, tumor size, histological grade, hormone receptor status, and the presence of lymph node metastasis.

Material and methods

Case selection

The DNA profiles of 100 cases diagnosed with invasive breast carcinoma between 2019 and 2024 were analyzed by the Department of Pathology, Faculty of Medicine, Karadeniz Technical University, using next-generation sequencing (NGS). The cases included in the study were those diagnosed with invasive breast carcinoma in our department between 2019 and 2024, which had lymph nodes and/or other organ metastases, and for which the oncology clinic requested NGS testing to maintain further treatment options for these patients. A pathologist examined the formalin-fixed paraffin-embedded (FFPE) tissues of patients with invasive breast carcinoma and chose the most suitable block for the investigation. We verified instances of invasive breast cancer through histological examination and had sufficient clinical and pathological data available for the study. We acquired clinical data on the cases via the hospital system. The data comprised the patient's age and sex, tumor location (right or left side), tumor size, histological grade, lymph node involvement, expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER 2). The study excluded cases where the evaluation of DNA profiles revealed low DNA quality.

DNA extraction and *PIK3CA* mutation assay

We used the NGS method to examine *PIK3CA* somatic mutations molecularly in various cases. Our study evaluated not only hotspot mutations but also mutations in all exons of PIK3CA.

We utilized the NGS-DNA panels (QIAsec solid custom MSI panel, QIAact AIT DNA UMI panel, and AIT Basic QIAact Actionable Insights Tumor Panel) at different periods. We examined the following DNA mutations: *ALK*, *APC*, *ATM*, *BC0R*, *BRAF*, *BRCA1*, *BRCA2*, *CDKN2A*, *CTNNB1*, *CHEK2*, *DLX3*, *EGFR*, *ERBB2*, *ERBB3*, *ESR1*, *HRAS*, *IDH1*, *IDH2*, *KEAP1*, *KIT*, *KRAS*, *MET*, *MLH1*, *MSH2*, *MSH6*, *NFE2L2*, *NRAS*, *NTRK1*, *NTRK2*, *NTRK3*, *PDGFRA*, *PIK3CA*, *PIK3R1*, *PMS2*, *POLE*, *PTEN*, *RAF1*, *ROS1*, *RPL22*, *STK11*, *TERT*, *TP53*, and *WNT1*. We examined *BAT40(T)37*, *MONO-27(T)27*, *BAT26(A)27*, *NR24(T)23*, *BAT25(T)25*, *NR22(T)21*, *HSP110-T17(T)17*, *NR21(A)21* and *BAT34C4(A)18* loci for MSI detection.

We performed DNA isolation from the paraffin-embedded tumoral tissues of the cases using the QIAGEN GeneRead DNA/RNA FFPE Kit. The concentration of isolated DNA (Qubit 4) was sufficient for all cases, with a mean of 29 ng/μl. The gel running (Qiaxcel) method determined the quality of the DNA. We used the Illumina NovaSeq system to perform NGS. We performed bioinformatics analysis using the Qiagen Clinical Insight (QCI) Interpret and CLC Genomic Workbench interfaces.

In DNA-NGS studies performed with FFPE materials, we determine variants with a reading depth of 500X, an allele frequency of 5% or higher, and a quality score (QUAL) higher than 200. We grouped the detected variants according to the Tier classification, accepting those with clinical significance as pathogenic and likely pathogenic variants.

Statistical analysis

We inputted all the acquired data into the SPSS database. We performed a chi-square test to compare the *PIK3CA* mutation status and the occurrence of additional concomitant mutations acquired via NGS with age data. The evaluation results yielded the following descriptive statistics: the number and percentage represented categorical data, while the mean, minimum, and maximum values represented interval data. We conducted a statistical analysis of the distribution of interval data using the Kolmogorov-Smirnov test. We used Student's *t*-test to compare interval data between independent groups when the assumption of normal distribution was satisfied. If this criterion was not met, we used the Mann-Whitney *U* test instead. A statistical alpha significance level of $p < 0.05$ was used as the threshold for determining statistical significance.

Results

In our study, the *PIK3CA* mutation rate was 45%. The mutation rates for the three hotspot sites were 33.3% ($n = 15$) for E545K in exon 10, 26.7% ($n = 12$) for H1047R in exon 20, and 6.7% ($n = 3$) for E542K in exon 10 (Fig. 1). Aside from these hotspot *PIK3CA*

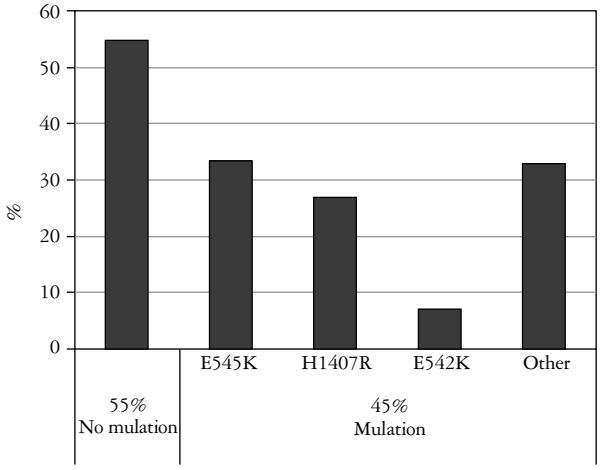

Fig. 1. Mutation rates of the *PIK3CA* NGS analysis

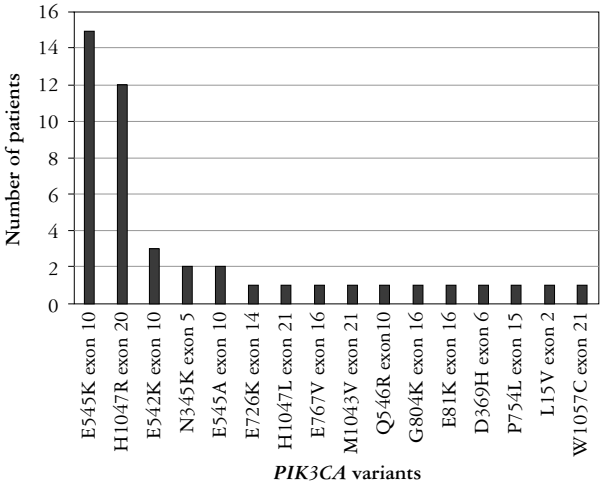

Fig. 2. Patient distribution of *PIK3CA* mutation variants

mutations, the status of other codons and exons where *PIK3CA* mutations were observed is specified in Figure 2. Among 45 patients with *PIK3CA* mutation tumors, 41 (91.2%) had a single mutation, and 4 (8.8%) had double mutations. These double mutations are:

- Codon M1043V exon 21 and codon E542K exon 10;
- Codon H1047R exon 21 and codon P539R exon 10;
- Codon E545K exon 10 and codon E726K exon 14;
- Codon E365K exon 6 and codon H1047R exon 21.

Of the 100 cases included in the study, only one was MSI-positive. In this case, a mutation in the *MSH2* gene was observed and classified as MSI-low. This case also had a *PIK3CA* codon E545K (exon 10) and an accompanying *PTEN* mutation.

Among the cases with double PIK3CA mutations, no additional mutation in a different gene was detected. However, among the 41 cases with a single *PIK3CA* mutation, accompanying mutations were detected in 11 cases for the *Tp53* gene and 6 cases for the *PTEN* gene. In our study, the most common accompanying mutation with the *PIK3CA* mutation was found to be the *Tp53* gene mutation (24.4%). Of the 45 cases with *PIK3CA* mutations, 6 (13.3%) had *PTEN* mutations. In contrast, among the 55 cases without *PIK3CA* mutations, 6 cases (10.9%) also had *PTEN* mutations. *PTEN* gene mutation levels in patients with *PIK3CA* mutations were higher than those without *PIK3CA* mutations.

We found the mean age of cases with the *PIK3CA* mutation was 55.1, while the mean age of cases without the *PIK3CA* mutation was 51.1. No statistically significant relationship was found between age and the presence of the *PIK3CA* mutation ($p = 0.126$).

Out of the cases, 49 were diagnosed with invasive breast cancer in the left breast and 51 in the right breast. Among the cases of invasive carcinoma in the left breast, 57.1% contained *PIK3CA* mutations, while 33.3% of the cases in the right breast had *PIK3CA* mutations. A statistically significantly higher rate of *PIK3CA* mutations was observed in cases of invasive carcinoma in the left breast ($p = 0.017$).

In cases with the *PIK3CA* mutation, the Ki-67 index range was 2% to 70%, with an average value of 22.4%. In cases without the *PIK3CA* mutation, the Ki-67 index range was 2% to 80%, with an average of 27.2%. There was no statistically significant relationship between the *PIK3CA* mutation and the Ki-67 index ($p = 0.191$).

PIK3CA mutations were more frequent in intermediately (34%) than well-differentiated (25%) tumors. In cases where the tumor size was more extensive than 2 cm, the *PIK3CA* mutation rate was statistically significantly higher ($p = 0.015$).

In ER-positive cases, the *PIK3CA* mutation was present in 48.7%, while in ER-negative ones, it was present in 33.4%. Among PR-positive cases, 49.3% had a *PIK3CA* mutation, whereas in PR-negative ones, it was present in 35.5%. The percentage of *PIK3CA* mutations in steroid hormone receptor-positive and HER2-negative cases was 48.8%, whereas in triple-negative breast cancer cases, the *PIK3CA* mutation rate was 26.7%. The *PIK3CA* mutation was detected at a higher rate in ER and PR-positive cases, i.e., steroid hormone-positive cases. However, no statistically significant relationship was found between ER, PR positivity, steroid hormone positivity, and the *PIK3CA* mutation (Table I).

Discussion

PIK3CA is one of the most commonly observed somatic mutations in invasive breast cancer cases. The most frequently mutated hotspot points are E542 K or E545 K in exon 9 and H1047R in exon 20. Samuels *et al*. first reported the *PIK3CA* mutation in human cancer, and since then, many studies have demonstrated the occurrence of *PIK3CA* gene mutations in breast cancer.

Table I. The association of *PIK3CA* mutation status with some clinical and histopathological parameters

Parameters	Number of patients	*PIK3CA* mutation, negative (*n*, %)	*PIK3CA* mutation positive (*n*, %)	*p*-value
Tumor size				
< 2 cm	25	19 (76)	6 (24)	0.015
≥ 2 cm	75	36 (48)	39 (52)	
Lymph node involvement				
Positive	15	10 (66.7)	5 (33.3)	0.325
Negative	85	45 (52.9)	40 (47.1)	
Histological grade				
G1	3	2 (66.7)	1 (33.3)	0.239
G2	62	30 (48.3)	32 (51.7)	
G3	35	23 (65.7)	12 (34.3)	
ER status				
Positive (≥ 1%)	76	39 (51.3)	37 (48.7)	0.188
Negative (< 1%)	24	16 (66.7)	8 (33.3)	
PR status				
Positive (≥ 1%)	69	35 (50.7)	34 (49.3)	0.2
Negative (< 1%)	31	20 (64.5)	11 (35.5)	
Her 2 status				
Positive	15	9 (60.0)	6 (40.0)	0.673
Negative	85	46 (54.1)	39 (45.9)	
SHR status				
Positive	80	41 (51.2)	39 (48.8)	0.089
Negative	20	14 (70.0)	6 (30.0)	
IHC types				
SHR (+), Her 2 (–)	80	41 (51.2)	39 (48.8)	
SHR (+), Her 2 (+)	6	3 (50.0)	3 (50.0)	0.241
SHR (–), Her 2 (+)	5	3 (60.0)	2 (40.0)	0.497
TNBC	15	11 (73.3)	4 (26.7)	0.521

ER – estrogen receptor, PR – progesterone receptor, Her 2 – human epidermal growth factor receptor 2, SHR – steroid hormone receptor, IHC – immunohistochemistry, TNBC – triple-negative breast cancer

The frequency of *PIK3CA* mutation was 46.5% in the Maruyama *et al*. [5] study and 34% in the Sudhakar *et al*. [6] study. The frequency of *PIK3CA* mutation was 42% in the Dirican *et al*. [7]. Our study detected the *PIK3CA* mutation in 45 of 100 breast cancer cases. In the present study, the frequency of *PIK3CA* mutations was 45%. The proportional difference between studies could be due to variations in the number of cases included in the study and geographical and racial differences.

The interesting finding from our study is that approximately 4% of all cases, or 8.8% of cases with *PIK3CA* mutations, had double *PIK3CA* mutations. In the study by Martinez *et al*. [8], two mutations were detected in 11.8% of cases, while three or more mutations were identified in 0.7% of cases. Preclinical studies revealed that hormone receptor-positive breast cancer cells with a double *PIK3CA* mutation exhibited higher PI3K activity and downstream signals than those with a single *PIK3CA* mutation. Both preclinical studies and early phase 1 trials have shown that breast cancer patients with double *PIK3CA* mutations exhibit a stronger response to PI3K alpha-specific inhibitors compared to those with single hotspot mutations [9]. As a result, detecting *PIK3CA* mutations, particularly double mutations, is critical for patient treatment.

Multiple studies suggest that the predominant *PIK3CA* mutation occurs in exon 9 in cases of invasive lobular carcinoma but in exon 20 in cases of invasive

ductal carcinoma. The elevated prevalence of invasive ductal carcinoma may be associated with the hotspot of this group, H1047R [10]. Our investigation included two cases of invasive lobular carcinoma and two mixed cases of carcinoma, specifically the coexistence of invasive ductal carcinoma and invasive lobular carcinoma. There were 96 cases left, all of which were invasive ductal carcinomas. A case of invasive lobular carcinoma exhibited a *PIK3CA* mutation, specifically the L15V exon 2 *PIK3CA* mutation. Two mixed carcinoma patients exhibited *PIK3CA* mutations, specifically the E545K mutation in exon 10 and the G804E mutation in exon 16. Patients with invasive ductal carcinoma who had a detectable *PIK3CA* mutation predominantly displayed the E545K exon 10 mutation. In the study by Dirican *et al*. [7], the most frequently detected *PIK3CA* mutation was the codon H1047R exon 20 mutation. The second most common mutation observed was the codon E545K mutation in exon 9. However, this study did not analyze *PIK3CA* mutations across all exons, focusing solely on the presence of mutations in exon 9 and exon 20. The *PIK3CA* mutation exhibits variations across different exons and is distinct among diverse histology types. Variations in distinct molecular processes, which activated PI3K kinase through various locations of the *PIK3CA* mutation, could account for the variability in outcomes, leading to diverse biological consequences. Since most of the cases we looked at were invasive ductal carcinoma, it would be advisable to conduct a more thorough study with more cases to find out why there is such variation between the different types of histology.

Aleskandarany *et al*. [11] found no statistically significant correlation between the *PIK3CA* mutation and other clinicopathological markers. The factors encompass histological grade, tumor size, Ki-67 index, lymphovascular invasion, and lymph node involvement. Lian *et al*. [12] found no statistically significant correlation between the *PIK3CA* mutation and factors such as age, histological grade, lymph node involvement, or ER/PR status. Our study showed no statistically significant correlation between age, histological grade, lymph node involvement, and ER/PR status. Our investigation revealed no significant correlation between the *PIK3CA* mutation and many histological characteristics. The parameters include histology grade, Ki-67 index, lymph node involvement, and ER/PR/Her2 status. Nevertheless, our investigation identified a statistically significant correlation between the size of the tumor and the presence of the *PIK3CA* mutation. We noted an elevated incidence of the *PIK3CA* mutation in situations where the tumor size was 2 cm or greater. Factors associated with sampling selection, such as age, histological subtype, and ethnic and regional disparities, may account for the observed diversity across different studies. Because of this, it is imperative to confirm the link between the clinical and pathological features of breast cancer and *PIK3CA* mutations in larger groups of patients and through more thorough studies.

Another interesting finding in our study is the statistically significant association between tumor localization and the *PIK3CA* mutation. Our study showed a higher rate of the *PIK3CA* mutation in cases of left breast cancer. Upon reviewing other studies in the literature, we found that none have included tumor localization in the analysis. However, the higher rate of PIK3CA mutation in tumors located on the left side could be relevant, despite its seemingly coincidental nature.

Reinhardt *et al*. [13] found that TNBC and HER2-positive cases have a lower rate of *PIK3CA* mutations and display a higher-risk biological behavior. In our study, we found a lower rate of *PIK3CA* mutation in HER2-positive and TNBC cases. Conversely, in ER- and PR-positive cases, the rate was higher. These findings raise the question why *PIK3CA* mutations are more frequently detected in ER-positive breast cancer cases. Some studies suggest that *PIK3CA* mutation-dependent *AKT* phosphorylation leads to ER activation. This results in estrogen-independent ER transcriptional activity, which in turn promotes the growth of ER-positive breast cancer. Therefore, mutated PI3K is likely to promote the development of ER-positive breast cancer. This phenomenon explains why tumors with *PIK3CA* mutations are often more well-differentiated and ER/PR-positive breast cancers are more common [14, 15].

Conclusions

Our analysis revealed a 45% *PIK3CA* mutation rate, with the most common mutations occurring at hotspot locations in exon 10 (E545K and E542K) and exon 20 (H1047R). Our investigation found a statistically significant association between the size of the tumor, its localization, and the presence of the *PIK3CA* mutation. We observed no significant correlation between the *PIK3CA* mutation and histopathological factors such as histological grade, age, lymph node involvement, and ER/PR/Her2 status. Additional research with a larger sample size is required to examine the correlation between clinicopathological characteristics and the *PIK3CA* mutation.

Disclosures

1. Institutional review board statement: The study was approved by the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (date: 28.03.2024, protocol number: 2024/66).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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