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KRAS, EGFR AND PIK3CA mutation in triple negative breast carcinomas

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ABSTRACT

Triple negative breast carcinoma is defined by the absence of oestrogen receptor (ER) and progesterone receptor (PR) expression and the lack of HER2 gene amplification. It comprise 10-17 % of all breast cancers. Though EGFR gene mutaion is rare in triple negative breast cancers, 47-70 % expressed EGFR protein. PIK3CA mutation is a frequent event in breast cancers. We aim to investigate EGFR, KRAS and PIK3CA mutation in triple negative breast carcinomas by pyrosequencing and compare the results with hormone receptor positive and HER2 overexpressed breast carcinomas which were identified by immunohistochemistry. No mutations in Exon 18, 19, 20 and 21 of EGFR gene were detected in all groups. KRAS mutation was identified in only one case. PIK3CA mutations were detected in 9 of TNBC (23.7 %), 5 of hormone receptors positive, HER2-negative tumors (50 %) In HER2-positive tumors, no mutations were detected in PIK3CA gene. Our results revealed that PIK3CA inhibitors might be a new candidate in the targeted treatment of triple negative breast cancers that were resistant to chemotherpy. These results should be supported by large series with survival analysis.

Key words: triple negative breast carcinoma, EGFR, KRAS, PIK3CA

INTRODUCTION

Breast cancer is the most cancer in women and constitutes 23 % of women cancers (1). Breast cancer has different subgroups according to different gene expression patterns that affect treatment and also tumor prognosis. The group called as triple negative involves breast carcinomas without estrogen receptor (ER), progesterone receptor (PR) expression and “human epidermal growth factor 2” (HER2) gene amplification. Triple negative breast cancer (TNBC) constitutes 10-17 % of all breast cancers. These tumors are mostly observed in women with BRCA1/2 mutation carriers at a younger age (<50 years), and are resistant to the chemotherapy used, have more aggressive clinical course and poor prognosis (2).

Genomic studies carried out in TNBCs aim to targeted treatments in these tumors. No specific molecular agent has been found in the treatment yet (3). In triple negative tumors, the overexpression of epidermal growth factor receptor and the hyperactivation of MEK/ERK signaling pathway are observed, and these types of tumors show sensitivity against DNA damaging agents (4). One of them is the platinum compounds. Another agent is poly (ADP) ribose polymerase I (PARP1) inhibitors. Cells with defects in BRCA genes provide their genomic integrity depending on PARP1. Therefore, PARP1 inhibition can lead to cell death (5). Also, the cell cycle regulator which is called as mTOR and the protein playing role in the lower stage of PIK3CA/PTEN/AKT signaling pathway are pointed as target in triple negative tumors. PTEN losses that lead to an increase in AKT and mTOR activation frequently take place in triple negative tumors. Therefore, agents such as everolimus (Novartis) and temsirolimus (Wyeth) from mTOR inhibitors have begun to be tested in these tumors (2).

Although activating mutations in the EGFR gene are rare in triple negative tumors, EGFR expression is observed in approximately 45-70% of them (6). Cetuximab (Merck Serono), a monoclonal antibody developed against EGFR, also has been tested in metastatic triple negative samples (7).

The signal transduction which begins by the binding of EGF protein to its receptor, EGFR, is transmitted to the nucleus through HRAS, NRAS and RRAS protein including KRAS, and proliferation and differentiation signals

have been given to cell. KRAS gene mutations that lead to continuous activation have been determined in different types of cancers. It affects the response to chemotherapy particularly in colon cancers, and the presence of the mutation is correlated with the resistance (8). Sanchez-Munoz et al scanned most common seven KRAS gene mutations by using real-time PCR method in 35 triple negative breast cancers, and observed no change (8).

Another target of research in recent years is the phosphoinositide-3 kinase (PIK3CA) pathway. PIK3CA signaling pathway is activated by binding of growth factors or ligands to receptor tyrosine kinases. There are two hot-spot regions for PIK3CA gene mutations: one of them; E545K and E542K in helical domain in exon 9, and the other one; H1047R amino acid changes in kinase domain in exon 20. The incidence of these mutations in breast cancers varies between 20-25 % by tumor subtypes. PIK3CA mutations lead to resistance to anti-HER2 agents used in treatment (9). Therefore, investigation of PIK3CA gene mutations is important in tumors without particular treatment protocol such as TNBCs.

In our study, we aimed to screen the EGFR (exon 18, 19, 20, 21), KRAS (codon 12, 13, 61) and PIK3CA (codon 540-546 in exon 9 and codon 1042-1049 in exon 20) gene mutations in 38 TNBC by pyrosequence method. Also for the comparison of results, the same mutations was investigated in 10 hormone receptor (HR) positive and HER-2 positive cases, 10 HR negative, HER-2 positive cases and 10 HR positive and HER-2 negative cases determined by immunohistochemistry.

MATERIALS AND METHODS

Tumor tissue selection

Invasive breast carcinomas which were diagnosed between 2006-2011 in our institution were used. The histopathological findings of these cases (tumor size, tumor type, tumor grade, lymph node involvement) were obtained from the Pathology archive. ER, PR and HER2 data which were determined by immunohistochemical method during the first diagnosis, were used. Thirty-eight invasive breast carcinomas evaluated as triple negative, 10 ER and/or PR positive, HER2 negative, 10 ER and/or PR positive, HER2 positive and 10 ER and/or PR negative, HER2 positive carcinomas were included in the study for molecular analysis. Tumors were classified as luminal A when ER and/or PR positive, HER2 negative; luminal B when ER and/or PR-positive, and HER2 positive; HER2 positive when ER and/or PR negative and HER2 positive.

DNA Isolation from Formalin Fixed Paraffin Embedded Tissues:

Ten micron thick section was directly taken from the tumor blocks with 80% and more tumor area and put into sterile 2 ml. centrifuge tube. For DNA isolation, DNA isolation kit (Qiagen) for the paraffin-embedded tissue was used. QIAmp DNA FFPE tissue kit (Qiagen, Hilden, Germany) deparaffinization of the tissue was ensured, and 180 µl ATL Buffer and 20 µl Proteinase K were added on the tissue in the centrifuge tube for lysis phase. It was left in incubation for 16-18 hours at 56 ^{0}C during the night. The following day, the centrifuge tubes containing the tissue lysis were hold for 15 minutes heat block adjusted to 95 ^{o}C. Irrigation solutions and subsequently elution solutions were added on the tissue lysis, and elution of DNA in the colon was ensured and DNA was stored at -20 ^{o}C.

Therascreen® KRAS Pyro® Kit was used to determine possible mutations in KRAS gene Codon 12, 13 and 61

The Therascreen® KRAS Pyro® Kit (QIAGEN) was used manufacturer's instructions. Briefly, 25 µl PCR reaction was made for both Codon 12/13 and Codon 61. For the PCR reaction, 5 ng DNA was used for per reactions. The target regions were amplified using following conditions; One 15-minutes cycle at 95 ^{o}C followed 42 cycles of, respectively, 20 seconds at 95 ^{o}C, 30 seconds at 50 ^{o}C, 20 seconds at 72 ^{o}C and finally cycle of 5 minutes at 72 ^{o}C. Unmethylated Control DNA was used as a positive control for PCR and sequencing reactions. The prepared PCR reaction was placed into the PCR device (2720 Thermal Cycler, Applied Biosystems). Subsequently, pyrosequencing analysis was performed for both Codon 12/13 and Codon 61.

Therascreen EGFR Pyro Kit was used to determine possible mutations in Exon 18, 19, 20 and 21 in EGFR gene.

The Therascreen® EGFR Pyro® Kit (QIAGEN) was used manufacturer's instructions. Briefly, genomic DNA was amplified in 4 separete PCR reactions with specific primers for each of EGFR hot spots regions which are exons 18 (codon 719), 19, 20 (codon 768 and 790) and 21 (codons 858-861). For the initial PCR reactions, 5 ng DNA was per reaction. The cycling parameters included an initial activations step for 15 minutes 95 ^{o}C, followed by 42 cycles of 20 seconds at 95 ^{o}C, 30 seconds at 50 ^{o}C, and 20 seconds at 72 ^{o}C, followed by final extension of 5 minutes at 72 ^{o}C. Unmethylated Control DNA was used as a positive control for PCR and sequencing reactions. The prepared PCR reaction was placed into the PCR device (2720 Thermal Cycler, Applied Biosystems). Subsequently,

pyrosequencing analysis was performed in 5 separate reactions (Codons 768 and 790 are sequenced in separate pyrosequencing reactions.)

Therascreen PIK3CA Pyro Kit was used to determine possible mutations in Codon 542-546 and Codon 1042-1049 in PIK3CA gene.

The Therascreen® PIK3CA Pyro® Kit (QIAGEN) was used manufacturer's instructions. Briefly, 25 µl PCR reaction was made for both Codon 542-546 and Codon 1042-1049. For the PCR reaction, 5 ng DNA was used for per reactions. The Codon 542-546 and Codon 1042-1049 were amplified using following conditions; One 15-minutes cycle at 95 ^{o}C followed 42 cycles of, respectively, 20 seconds at 95 ^{o}C, 30 seconds at 50 ^{o}C, 20 seconds at 72 ^{o}C and finally cycle of 5 minutes at 72 ^{o}C. Unmethylated Control DNA was used as a positive control for PCR and sequencing reactions. The prepared PCR reaction was placed into the PCR device (2720 Thermal Cycler, Applied Biosystems). Subsequently, pyrosequencing analysis was performed for both Codon 542-546 and Codon 1042-1049.

Pyrosequence Stage:

The pyrosequencing analysis was performed according to the manufacturer's recommendations for Pyromark Q24 KRAS, EGFR and PIK3CA v2.0 assays. 10 µl of biotin-labeled amplicons are immobilized on Streptavidin Sepharose® High Performance beads (GE Healthcare, Piscataway, NJ.). The purified amplicons attached to the beads were washed and denatured to single strands according to manufacturer instructions with the use of the PyroMark Q24 Vacuum Workstation (QIAGEN). Subsequently, the beads were added to the pyrosequencing primers. PyroMark Gold 96 Reagents (QIAGEN) containing enzyme and substrate mixture, dATP, dCTP, dGTP and dTTP were used. Pyrosequencing was performed on the PyroMark Q24 Instrument (QIAGEN). PyroMark Q24 version 2.0.6 software, which identifies the presence of a specific mutation and its percentage, was used for analyzing of pyrosequencing results. According to software, for the mutational analysis, a required peak heights for the "passed" quality is 30 relative light units (RLU) and for the "check" quality is 10 RLU. Samples with low mutation rate or with "check" quality peak heights were analyzed to a second round PCR reaction and pyrosequencing analysis with Unmethylated Control DNA.

RESULTS

Among 68 invasive breast carcinomas included in the study 39 were classified as ductal, 1 as lobular and 28 as other subtypes. Sixteen (42%) of triple negative invasive carcinomas were evaluated as Basal-like breast carcinomas that had positive staining with basal keratins CK 14 and CK5/6 and EGFR by immunohistochemical method. Five of the Basal-like carcinomas were diagnosed as Metaplastic (adenosquamous) carcinoma. Distribution of triple negative tumors according to subtypes is given in Table 1.

In the study, invasive breast carcinomas were classified by ER, PR positivity and HER2 expression determined by immunohistochemical method. They were classified as ER and/or PR positive, HER2 negative tumors Luminal A, ER and/or PR positive, HER2 positive tumors Luminal B, tumors with only HER2 expression and triple negative group with negative three indicator. HER2 positivity was performed according to "The ASCO / CAP HER2 Testing Guideline". The histopathological features of the tumors are summarized in table 2.

Table 1. Distribution of Triple Negative Breast Carcinomas by their histopathologic subtypes

Tumor type	***38 (%)***
Bazal like carcinoma	11 (29)
Metaplastc carcinoma	5 (3)
Invaziv ductal carcinoma	16 (42)
Clear cell carcinoma	1 (2)
Carcinoma with neuroendocrine features	1 (2)
Carcinoma with apocrinedifferentiation	1 (2)
Mucinous carcinoma	1 (2)
Polimorphous carcinoma	1 (2)

Table 2. Histopathological Features of the tumors

Histopathologic types (68)	N (%)
Ductal	39 (57.3)
Lobular	1 (1.5)
Other	28 (41.2)
Histologic grade	
1	11 (16.2)
2	19 (27.9)
3	38 (55.9)
Tumor size	
≤ 2cm	27 (39.7)
> 2cm	41 (60.3)
Lymph node metastasis	
pN0	32 (47.1)
pN1	13 (19.1)
pN2	12 (17.6)
pN3	9 (13.2)
PNx	2 (2.9)
ER and/or PR and HER2 Status	
Luminal A	10 (14.7)
Luminal B	10 (14.7)
HER 2 (+)	10 (14.7)
Triple negative	38 (55.9)

KRAS mutation was found only in one case in the triple negative group with the diagnosis of invasive apocrine carcinoma. G12D (Gly12Asp; GGT>GAT) mutation was observed in this tumor (Figure 1).

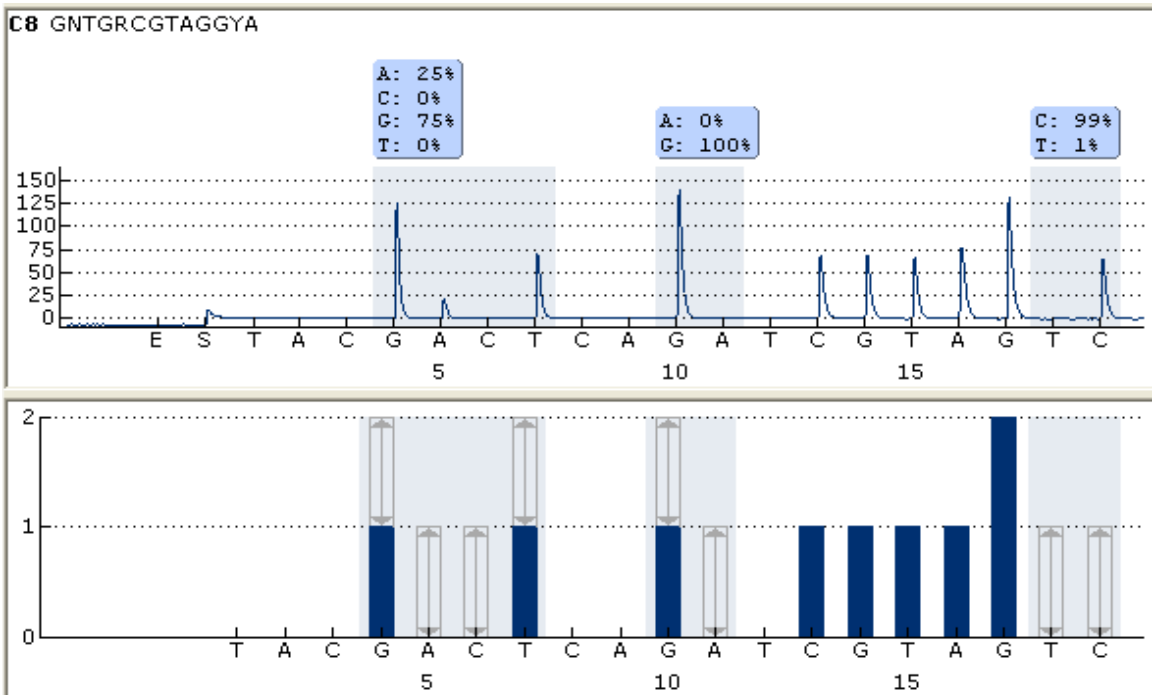


Figure 1: G12D (Gly12Asp; GGT>GAT) mutation in KRAS gene Codon 12

EGFR mutation was not observed in any group.

Although PIK3K mutation was not observed in HER2 positive tumors, it was observed in Luminal A, B groups and TN group. In 9 of triple negative carcinomas (2: E542K, 1: E54K, 5: H1042R and 1: M1043I mutation), in 3 of ER and / or PR positive also HER2 positive group (1: E545K, 2: H1047R mutation), in 5 of ER and / or PR positive, HER2negative group (2: E545K, 1: H1047L, 1: H1047R and, both E542K and M1043I in 1 tumor) (Table 3-5) (Figure 2-4).

Table 3 PIK3CA mutation in tumors with positive hormone and HER2 negative

	PIK3CA (540-546 kodonları)	**PIK3CA** (1042-1049 kodonları)
1	NEGATIVE	NEGATIVE
2	NEGATIVE	NEGATIVE
3	**codon 545: GAG>AAG: E545K (T:%25, C:%73)**	NEGATIVE
4	NEGATIVE	**codon 1047: CAT>CTT: H1047L (A:%25, T:%75)**
5	**codon 542: GAA>AAA: E542K (T:%23, C:%75)**	**codon 1043: CAT>TAT: M1043I (T:%21, C:%76)**
6	NEGATIVE	NEGATIVE
7	NEGATIVE	NEGATIVE
8	**Kodon 545: GAG>AAG: E545K (T:%12, C:%87)**	NEGATIVE
9	NEGATIVE	**Kodon 1047: CAT>CGT:H1047R (C:%28, T:%69)**
10	NEGATIVE	NEGATIVE

Table 4. PIK3CA PIK3K mutation in tumors with positive hormone receptors and HER2 positive

	PIK3CA (540-546 kodonları)	**PIK3CA** (1042-1049 kodonları)
1	NEGATIVE	NEGATIVE
2	NEGATIVE	NEGATIVE
3	NEGATIVE	NEGATIVE
4	NEGATIVE	NEGATIVE
5	**codon 545: GAG>AAG: E545K (T:%34, C:%64)**	NEGATIVE
6	NEGATIVE	**codon 1047: CAT>CGT: H1047R(C:%32, T:%68)**
7	NEGATIVE	**codon 1047: CAT>CGT: H1047R (C:%7, T:%93)**
8	NEGATIVE	NEGATIVE
9	NEGATIVE	NEGATIVE
10	NEGATIVE	NEGATIVE

Table 5. PIK3K mutations in triple negative tumors

	PIK3CA (540-546 kodonları)	**PIK3CA** (1042-1049 kodonları)
1	NEGATIVE	NEGATIVE
2	NEGATIVE	NEGATIVE
3	NEGATIVE	**codon 1043: CAT>TAT: M1043I (T:%8, C:%91)**
4	NEGATIVE	NEGATIVE
5	**Kodon 542: GAA>AAA: E542K (T:%12, C:%87)**	**NEGATİF**
6	NEGATIVE	NEGATIVE
7	NEGATIVE	NEGATIVE
8	NEGATIVE	NEGATIVE
9	**codon 542: GAA>AAA: E542K (T:%23, C:%76)**	NEGATIVE
10	NEGATIVE	**codon 1047: CAT>CGT:H1047R (C:%10, T:%89)**
11	NEGATIVE	NEGATİF
12	NEGATIVE	NEGATİF
13	NEGATIVE	NEGATIVE
14	NEGATIVE	NEGATIVE
15	NEGATIVE	NEGATIVE
16	NEGATIVE	NEGATIVE
17	NEGATIVE	**Kodon 1047: CAT>CGT: H1047R (C:%13, T:%87)**
18	NEGATIVE	NEGATIVE
19	NEGATIVE	NEGATIVE
20	NEGATIVE	**(codon 1047: CAT>CGT: H1047R (C:%58, T:%41)**
21	NEGATIVE	**codon 1047: CAT>CGT: H1047R (C:%26, T:%74)**
22	NEGATIVE	NEGATIVE
23	NEGATIVE	NEGATIVE
24	NEGATIVE	NEGATIVE
25	NEGATIVE	NEGATIVE
26	NEGATIVE	NEGATIVE
27	NEGATIVE	NEGATIVE
28	NEGATIVE	NEGATIVE
29	NEGATIVE	NEGATIVE
30	NEGATIVE	NEGATIVE
31	NEGATIVE	NEGATIVE
32	NEGATIVE	NEGATIVE
33	NEGATIVE	NEGATIVE
34	**codon 545: GAG>AAG: E545K (T:%49, C:%49)**	NEGATIVE
	PIK3CA (540-546 kodonları)	**PIK3CA** (1042-1049 kodonları)
35	NEGATIVE	NEGATIVE
36	NEGATIVE	NEGATIVE
37	NEGATIVE	**codon 1047: CAT>CGT: H1047R (C:%15, T:%85)**
38	NEGATIVE	N NEGATIVE

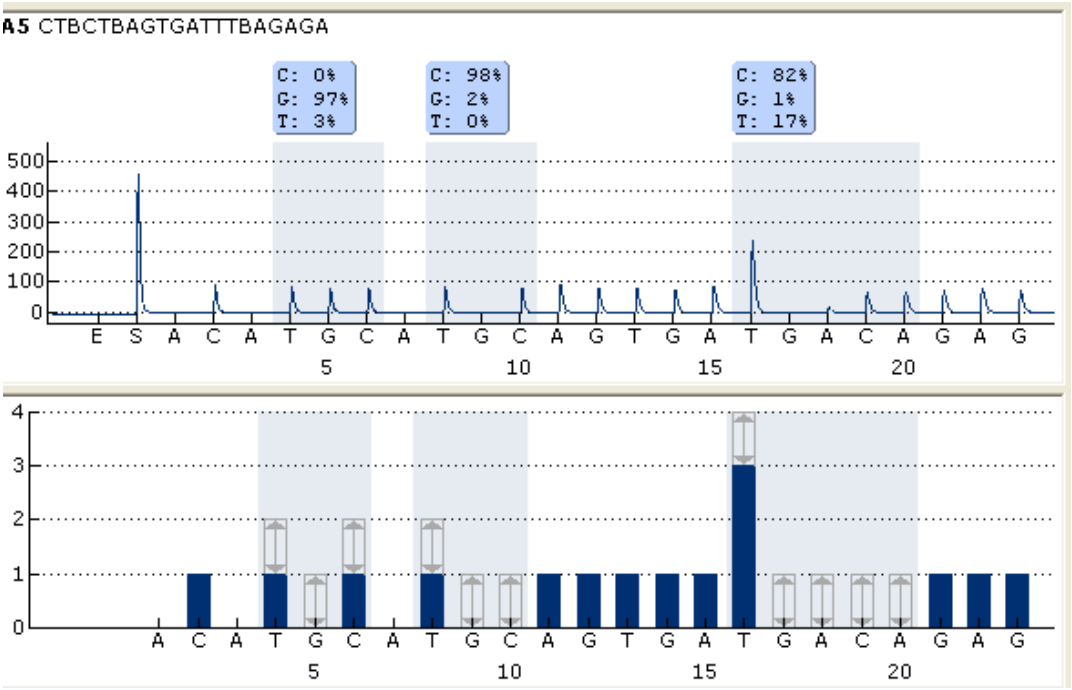
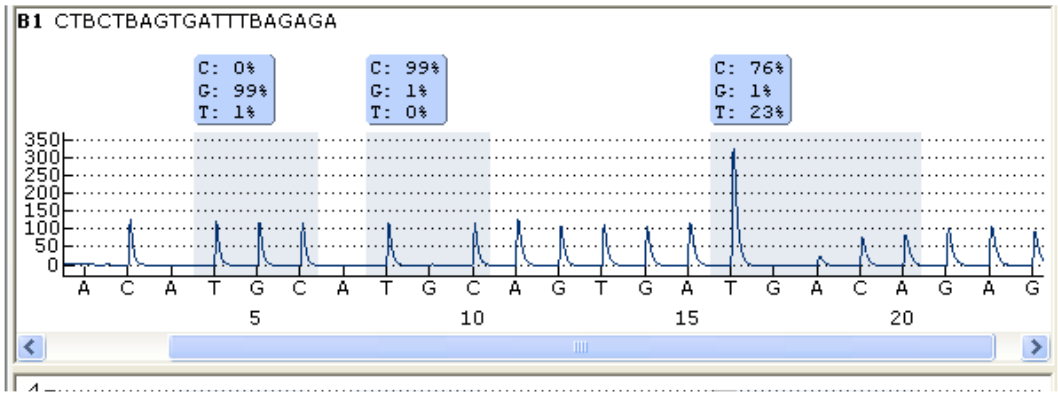


Figure 2: . PIK3CA mutation in triple negative breast cancer Codon 542: GAA>AAA: E542K (T: 11%, C: 87%)



Figuer 3: PIK3CA mutation in Luminal A breast cancer:E542K (GAA>AAA) (T:%23, C:%76)

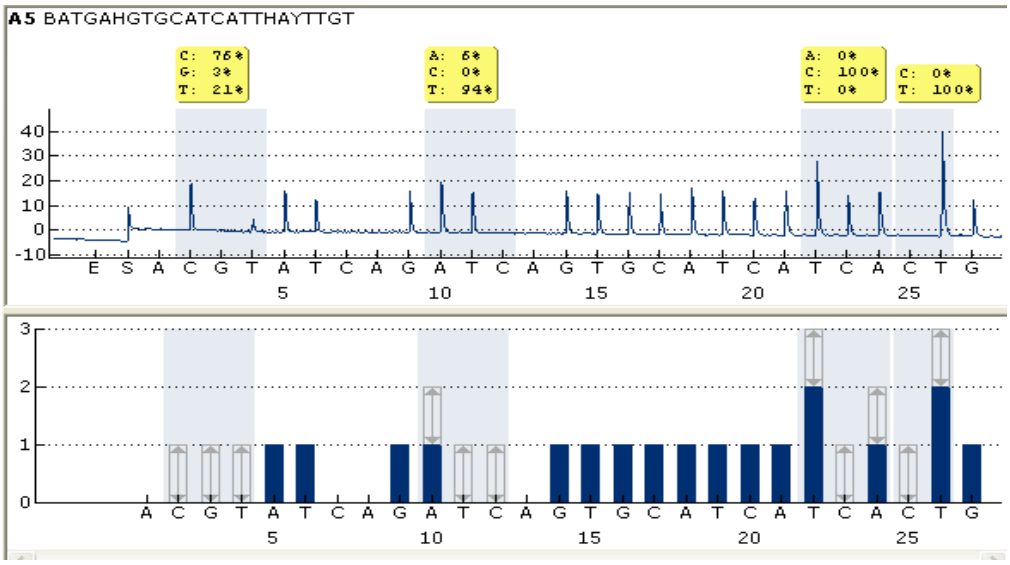


Figure 4: Double PIK3CA mutation in Luminal A breast cancer
M1043 (CAT>TAT) (T:%21, C:%76)

PIK3CA mutation was most commonly observed in hormone receptor positive group. Also, in triple negative tumors 23.7% mutation was detected. Statistically significant difference was observed between the incidences of PIK3CA mutation in low-grade (grade 1-2) and hormone receptor positive tumors and tumors with high-grade (grade3) and HER2 expression ($p< 0.05$) (Table 6).

Table 6. Correlation of clinicopathologic parameters with PIK3CA mutation

Histologic type (68)	**N (%)**	**PIK3CA MUTATION (%)**	***P****
Ductal	39 (57.3 %)	12 (30.8 %)	-
Lobular	1 (1.5 %)		
Other	28 (41.2 %)	5 (17.8 %)	
Histological grade			
1-2	30 (44.1 %)	12 (40.0 %)	*0.011*
3	38 (55.9 %)	5 (13.2 %)	
Tumor size			
? 2cm	27 (39.7 %)	10 (37.0 %)	*0.033*
> 2cm	41 (60.3 %)	6 (14.6 %)	
Lymph Node metastasis			
pN0- pN1	45 (66.2 %)	10 (22.2 %)	*1.000*
pN2- pN3	21 (30.9 %)	5 (23.8 %)	
Hormone receptor status/HER2 Status			
Luminal A-B	20 (29.4 %)	8 (40.0 %)	*0.056*
HER 2 (+)	10 (14.7 %)	0	
Triple negative	*38 (55.9 %)*	*9 (23.7 %)*	

DISCUSSION

Breast carcinomas involve a heterogeneous group tumor with different clinical behaviors and response to treatment. Breast tumors were grouped according to the similarities in gene expression profiles in the study of Perou et al for the first time (10). Although Basal-like carcinomas and triple negative tumors do not overlap one to one in this grouping, they have many similar molecular and morphological features. Their aggressive clinical behaviors and resistance to chemotherapy used are similar. TNBCs are seen by 15% (11). Also, TNBCs are heterogeneous group with different molecular features as well as responses to the treatment (12). The possibility of targeted therapy will be increased by determination of these molecular features in different subgroups of TNBCs.

In our study, with this purpose, EGFR, KRAS and PIK3CA mutations, previously determined in other carcinomas and some targeted therapy agents had been developed were examined in TNBCs.

KRAS mutation is a genetic change which is found in many cancers and has importance for the target treatment. In our study, G12D (Gly12Asp; GGT>GAT) mutation in KRAS gene codon 12 was observed in only one of the TNBCs. KRAS mutation was not detected in luminal A and luminal B type carcinomas. Sanchez et al did not detect

any mutations in their study of 35 TNBCs (8). KRAS mutation is rarely observed in breast carcinomas (13). In the study carried out by Santarpia et al on fine needle aspiration material of 267 stage I-III breast carcinomas, KRAS mutation was observed by 1.5 % (14). KRAS mutation was not found in the study of Grob et al in which HER2(exon18-23), EGFR(exon 18-21), KRAS (exon 2) and BRAF(exon 15) mutations were examined in 65 triple negative breast carcinomas by sequence analysis method (15). rs61764370 T>G variant allele in 3'UTR of KRAS gene has been recently reported in hereditary ovarian and breast cancer patients. Hollestelle et al showed this KRAS variant was more in BRCA1 carriers (23.5%). However, the effect of this finding on breast cancer development was not observed (16). In our study, patients were not investigated in terms of BRCA1 / 2 gene mutation. Also, Paranjabe et al examined the same KRAS variant incidence in 415 breast cancers including 140 TNBCs, and they detected the KRAS variant association in triple negative carcinomas in premenopausal women (17). In their study, Cerne et al reported that this variant was not associated with familial breast cancer, it was associated with HER2 positive and poorly differentiated carcinomas (18). In another study, KRAS codon 12 mutation was observed as 7.7 % in triple negative breast carcinomas, as 2 % in luminal A, as 20 % in luminal B and as 17.4 % in HER2 positive tumors of 116 breast cancer patients receiving neoadjuvant chemotherapy (19). In another study in which OncoCarta panel was used, KRAS mutation was observed only in one case (0.9 %) in triple negative tumors (20). In our study, in accordance with the previous studies, it was observed that KRAS mutation was a very rare molecular change in breast carcinomas.

EGFR protein over expression is found in TNBCs by immunohistochemical method (6). However, EGFR activating mutations were not detected in none of the cases in our study. Also in other studies, EGFR mutation was not shown or observed at very low rate in TNBCs (15,21,20,14). Only in the study of Teng et al, EGFR activating mutations were reported in 70 (11.4%) of 653 TNBCs (21). This difference was thought to be based on the population characteristics studied (22). In many studies investigating the EGFR gene amplification, it was detected especially in TNBCs (23,24-27). The increase in EGFR protein expression was based on the increase in gene copy number rather than the activated mutations. It was stated that EGFR inhibitors could be used in the targeted therapy in TNBCs depending on EGFR gene amplification (4). In the study investigating the EGFR protein over expression, gene copy number and mutation; 64 % protein overexpression, 33 % gene copy number increased and 3% EGFR gene mutation were reported in triple negative tumors. It was emphasized that EGFR gene amplification was associated with poor prognosis and could be used as predictive data in triple negative carcinomas (26,28). In our study, mutation of EGFR gene was not examined. However, based on the literature data, it is believed that the detection of EGFR gene amplification by using FISH analysis, will be useful in the targeted therapy of these tumors.

In our study, PIK3CA mutation was more frequently detected in breast carcinomas of luminal A and B type with small size and low histological grade. In triple negative carcinomas, mutation was detected in 9 of 38 tumors (23.7%). Mutation was not observed in any HER2 positive tumor group. Mutations in PIK3CA oncogene are the most commonly detected mutations in breast cancer (20-40 %) (29,30). PIK3CA mutation is reported to be associated with ER positivity in the studies (14, 29,31-33). In recent studies, Lehmann BD at al identified that a TNBC subtype that has a luminal

phenotype and expresses the androgen receptor (AR+) and TNBC cells derived from these luminal AR + tumors have high frequency PIK3CA mutations (34). Then they using AR + TNBC cell line and xenograft models they evaluated the effectiveness of phosphoinositide 3-kinase (PI3K) inhibitors, used alone or incombination with an AR antagonist, on tumor cell growth and viability Then they explored the combination of AR antagonism and PI3K inhibition and found an additive or synergistic effect on AR + TNBC cell growth (35)These results provide rationale for pre-selection of TNBC patients with a biomarker (AR expression) to investigate the use of AR antagonists in combination with PI3K/mTOR inhibitors. These data provide the design of a combination therapy, the first trial in which TNBC patients were divided based on a biomarker (AR expression) and then to investigate the use of AR antagonists in combination with PI3K/mTOR inhibitors.

In the study carried out by Aleskandarany et al, PIK3CA expression was observed to be associated with poor prognostic data such as high histological grade, large tumor size and lymphovascular invasion. These results are controversial to our finding of PIK3CA mutation in small size and low grade tumors. This was thought to depend on the the small number of our patients. The disease-free survival period was observed to be shorter in these patients (36-37).

In another study, p53 mutation was reported to be the most frequent in TNBCs, and secondly PIK3CA mutation with 10.7% (38). It is stated that PI3K inhibitors can also be used in TNBC in addition to chemotherapeutic agents known in the targeted therapy (37-39).

CONCLUSION

KRAS mutation was detected only in one triple negative carcinoma. EGFR mutation was not detected in any carcinoma in our study similar to other studies. PIK3CA mutation was most frequently observed in luminal-type breast carcinomas containing hormone receptor, and secondly it was observed less frequently in TNBCs. PIK3CA mutation was observed in none of the HER2 positive carcinomas. The addition of PI3CA inhibitors in the treatment of TNBCs will have a positive contribution in the TNBC cases with PIK3CA mutation. Future studies of large series with survival analysis should be carried out to support this finding.

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