Comparison of the Mutation Profiles of Triple-Negative Breast Cancers and Hormone Receptor-Positive/Human Epidermal Growth Factor Receptor 2-Negative Breast Cancers at the T2N0-1M0 Stage

Seungju Lee

Pusan National University Yangsan Hospital

Hyun Yul Kim

Pusan National University Yangsan Hospital

Youn Joo Jung

Pusan National University Yangsan Hospital

Hyun-June Paik

Pusan National University Yangsan Hospital

Chang Shin Jung

Pusan National University Yangsan Hospital

Jee Yeon Kim

Pusan National University Yangsan Hospital

Sun Min Lee

Pusan National University Yangsan Hospital

Seung Hwan Oh (✉ paracelsus@pusan.ac.kr)

Pusan National University Yangsan Hospital

Research Article

Keywords: Triple-negative breast cancer, TP53, PIK3CA

Posted Date: December 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-129609/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Triple-negative breast cancer (TNBC) has higher loco-regional recurrence and visceral metastasis compared to other breast cancer subtypes; however, little is known about the molecular pathogenesis and therapeutic targets of TNBC. Therefore, we compared the mutation profiles of early TNBC with those of hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2⁻) breast cancer using a customized next-generation sequencing capture panel.

Methods

DNA was obtained from the primary tumor tissues of 34 patients diagnosed with pT2N0-1M0 HR+/HER2⁻ breast cancer or TNBC. To enrich the 48 breast cancer-associated genes, 21,192 probes were designed using the SureSelect design tool. After library preparation using the SureSelect XT kit (Agilent), paired-end DNA sequencing was performed on a HiSeq platform (Illumina). The mean depth of the target regions was 1,766 (×). The subsequent output containing genetic variation was analyzed using a pipeline of bioinformatics tools. Significant mutations with allele frequencies of more than 30% were checked for their germline counterparts in the peripheral blood. Circulating cell-free nucleic acids were extracted and analyzed with a theascreen[®] PIK3CA RGQ PCR kit (QIAGEN).

Results

Significant mutations were found in *TP53*, *PIK3CA*, *AR*, *BRCA1*, *PTEN*, *BRCA2*, *BRIP2*, *KIT*, *MET*, *AKT1*, *ALK*, *BARD1*, *BRAF*, *CD274*, *ERBB2*, *FGFR1*, *IDH2*, *NOTCH1*, *RET*, and *STK11* (in descending order of occurrence). *TP53* mutations were identified in the TNBC group more frequently than in the HR+/HER2⁻ group ($p=0.003$). The presence of *TP53* mutations was associated with a higher tumor grade ($p=0.008$), p53 positivity ($p<0.0001$), and a higher (≥ 15) Ki-67 index ($p=0.004$). *PIK3CA* was the most frequently mutated gene in HR+/HER2⁻ breast cancer (8/22, 36.4%), but not in TNBC (1/12, 8.3%). However, circulating cell-free *PIK3CA* mutations were not detected in either group.

Conclusions

The *TP53* mutation is associated with higher tumor grade and Ki-67 expression in both groups, and with larger tumor size in TNBC, but not in HR+/HER2⁻ breast cancer. In the foundation of TP53 mutation, concomitant mutation numbers are proportional to tumor size, reflecting clonal progression. Breast cancer-associated mutations such as those in *TP53* and *PIK3CA* have different biological implications for the proliferation and clonal diversification of these two distinct groups of breast cancer.

Background

Triple-negative breast cancer (TNBC) has greater loco-regional recurrence and more distant metastasis compared to the other breast cancer subtypes [1–3]. Furthermore, TNBC lacks a targeted therapy because

of the absence of the hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2). Therefore, many researchers have tried to classify breast cancers according to molecular genetic events and sought to identify novel drug combinations by analyzing the accompanying gene mutations [4–7].

With the advent of next-generation sequencing (NGS), numerous breast cancer-associated mutations have been discovered. The most commonly mutated genes in primary breast cancers are tumor protein p53 (*TP53*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) [8]. Approximately 40% of HR-positive breast cancers harbor *PIK3CA* mutations. Recently, the PI3K inhibitor alpelisib with fulvestrant received Food and Drug Administration (FDA) approval for *PIK3CA*-mutated HR-positive (+)/HER2-negative (–) advanced breast cancer after prior endocrine therapy [9]. The FDA also approved companion diagnostic tests for *PIK3CA* mutations for specimens of either tumor tissue or plasma cell-free nucleic acids [10].

Therefore, we analyzed breast cancer-associated gene mutations in tissues of diagnosed TNBC and HR+/HER2– breast cancer using a customized NGS panel and ran the theascreen® *PIK3CA* RGQ PCR assay (QIAGEN) with cell-free nucleic acids extracted from *PIK3CA*-mutated patients. Finally, the clinicopathological parameters and accompanying gene mutations of relatively early stage TNBC and HR+/HER2– breast cancer were compared.

Methods

Patients

This study included 34 Korean patients who were diagnosed with pT2N0-1M0 HR+/HER2– or triple-negative primary invasive ductal cancer at Pusan National University Yangsan Hospital, Korea, in 2016. They were treated with surgery and adjuvant chemotherapy, radiation therapy, or endocrine therapy. We assessed the histology, tumor grade, tumor size, lymph node status, type of surgery, and status of chemotherapy, radiation therapy, and endocrine therapy. We used Allred scoring to grade the hormone receptor status, with scores of 3 to 8 considered positive. HER2 was analyzed immunohistochemically, and the results were scored on a scale of 0, 1+, 2+, and 3+. When the result was 2+ and considered equivocal, silver *in situ* hybridization was performed. TNBC included tumors that were negative for hormone receptors and HER2. This study was approved by the Institutional Review Board of Pusan National University Yangsan Hospital.

Tumor DNA extraction and customized targeted sequencing

DNA was extracted from frozen primary tumor tissues. Using the SureSelect design tool, 21,192 probes were designed to enrich 48 breast cancer-associated genes: *AKT1*, *ALK*, *AR*, *BARD1*, *BRAF*, *BRCA1*, *BRCA2*, *BRIP1*, *CCND1*, *CD274*, *CDK4*, *CDK6*, *CDKN2A*, *DDR2*, *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *ESR1*, *FGFR1*, *FGFR2*, *FGFR3*, *GNA11*, *GNAQ*, *HRAS*, *IDH1*, *IDH2*, *KIT*, *KRAS*, *MAP2K1*, *MET*, *MTOR*, *MYC*, *NOTCH1*, *NRAS*, *PALB2*, *PDGFRA*, *PIK3CA*, *PTEN*, *RAD51C*, *RAD51D*, *RAF1*, *RET*, *ROS1*, *SMO*, *STK11*, *TP53*, and *TSC1*. A DNA library was prepared using the SureSelect XT kit (Agilent Technologies Inc., Santa Clara, CA, USA), and paired-

end DNA sequencing was performed on the HiSeq platform (Illumina, San Diego, CA, USA). The mean depth of the target regions was 1,766 (×). The subsequent output containing genetic variants was analyzed using a pipeline of bioinformatics tools, including read mapping, variant calling, and annotation.

Circulating cell-free nucleic acid extraction from plasma and PIK3CA testing using the theascreen® PIK3CA RGQ PCR assay

Circulating cell-free nucleic acids were extracted with a QIAamp DSP Circulating Nucleic Acids kit (QIAGEN, Hilden, Germany) after double centrifugation from the plasma of 24 breast cancer patients including seven with PIK3CA mutations discovered by targeted sequencing. The quality of the extracted cell-free nucleic acids was checked using the 4150 TapeStation System (Agilent Technologies Inc.). The theascreen® PIK3CA RGQ PCR assay using real-time PCR (QIAGEN) was performed to detect C420R, E542K, E545A/D/G/K, Q546E/R, and H1047L/R/Y according to the manufacturer's instructions.

Sanger sequencing for determining germline/somatic mutations

All *TP53* and other pathogenic and likely pathogenic mutations with variant allele frequencies above 30% were checked for the presence of their germline counterparts by Sanger sequencing of available peripheral blood. We directly sequenced the genomic DNA using a BigDye™ Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3700 Genetic Analyzer (Applied Biosystems).

Statistical analysis

The tumor characteristics and gene mutations were compared using chi-square tests and Pearson correlations, and a *p*-value less than 0.05 was considered to denote significance. The data were analyzed using MedCalc (ver. 12.7.0.0, MedCalc Software, Ostend, Belgium).

Results

Patient characteristics

Table 1 shows the clinical and pathological characteristics of the enrolled breast cancer patients. All patients were diagnosed with invasive ductal cancer after surgery in 2016. Fourteen patients were diagnosed with pT2N0 and 20 with pT2N1. The median patient age was 50 (range 38–67) and 58 (range 37–75) years in the TNBC and HR+/HER2– groups, respectively. Although patient age did not differ significantly between groups, the age range in the TNBC group indicates more radiotherapy followed by breast-conserving surgery. Regarding the tumor grade, the majority of the TNBC patients had grade 3 tumors (11 patients, 91.7%), whereas 10 (45.5%) HR+/HER2– breast cancer patients had grade 3 tumors (*p* = 0.029). TNBC was closely related to p53 positivity and a high Ki-67 index (Table 1, Fig. 1). Conversely, lymphovascular invasion was more frequent in HR+/HER2– breast cancer. Loco-regional recurrence

occurred in two patients—one in each group. One patient with TNBC had lung and bone metastases during follow-up. Regarding primary tumor tissue mutations, nine patients had *PIK3CA* mutations and 14 had *TP53* mutations. As expected from the immunohistochemistry, TNBC was associated with *TP53* mutations detected by targeted sequencing (Table 1).

Table 1
Demographic and clinicopathological characteristics of the two
groups of breast cancer patients

Characteristics	HR+/HER2-	TNBC	<i>p</i> -value
Age at diagnosis (average)	58 yrs	50 yrs	0.286
Tumor size (cm)	2.8	3.0	0.163
N stage			0.966
N0	9	5	
N1	13	7	
No. of involved lymph nodes			0.594
0	9	5	
1	9	3	
2	2	3	
3	2	1	
Grade			0.029*
1	1	0	
2	11	1	
3	10	11	
p53			0.002*
negative	16	2	
positive	6	10	
Ki-67			0.001*
<15	12	0	
≥15	10	12	
Lymphovascular invasion			0.017
no	9	10	
yes	13	2	
Multiplicity			0.180
single	19	12	

* $p < 0.05$

Characteristics	HR+/ HER2–	TNBC	<i>p</i> -value
multiple	3	0	0.004*
Surgery			
breast-conserving surgery	9	11	0.005*
mastectomy	13	1	
Radiotherapy			0.180
yes	12	12	
no	10	0	0.003*
Adjuvant chemotherapy			
yes	19	12	0.077
no	3	0	
<i>TP53</i> mutation			0.077
negative	17	3	
positive	5	9	0.077
<i>PIK3CA</i> mutation			
negative	14	11	
positive	8	1	
* <i>p</i> < 0.05			

Comparison of the mutation profiles of primary breast cancer tissues between HR+/HER2– and TNBC (supplementary Table S1)

Significant [pathogenic, likely pathogenic, and variation of uncertain significance (VUS)] mutations were found in *TP53*, *PIK3CA*, *AR*, *BRCA1*, *PTEN*, *BRCA2*, *BRIP2*, *KIT*, *MET*, *AKT1*, *ALK*, *BARD1*, *BRAF*, *CD274*, *ERBB2*, *FGFR1*, *IDH2*, *NOTCH1*, *RET*, and *STK11* (Fig. 2). No significant variation in the *estrogen receptor alpha* (*ESR1*) gene was found in either group. Of the 34 breast cancer patients, 79% (27/34) harbored at least one pathogenic or likely pathogenic mutation.

PIK3CA mutations were detected in eight (36.4%) of 22 HR+/HER2– breast cancers and one (8.3%) of 12 TNBC. One HR+/HER2– patient had double *PIK3CA* mutations (*c.1357G > A* and *c.3140A > G*). *TP53* mutations were detected in five (22.7%) HR+/HER2– breast cancers and nine (75.0%) TNBC. One TNBC patient had a *TP53* missense mutation (*c.641A > G*) and a *PIK3CA* frame-shift mutation (*c.3627dupA*). All *TP53* mutations were confirmed as somatic mutations via direct sequencing of peripheral blood, regardless of their variant allele frequencies (range 14.41–86.58%). All three *BRCA2* mutations were

germline mutations, co-existed with somatic *PIK3CA*, *TP53*, and *ERBB2* mutations, and were found only in the HR+/HER2- group. However, only two of the four *BRCA1* mutations were germline mutations; one was a frame-shift mutation (*c.3627dupA*) that was also changed to a homozygous mutation in tumor tissue by somatic loss of heterozygosity (LOH).

We analyzed the relationship between the *TP53* and *PIK3CA* mutations based on tumor DNA and the patients' pathological parameters (Table 2). The presence of *TP53* mutations was associated with a higher tumor grade ($p = 0.008$), p53 positivity ($p < 0.001$), and a higher Ki-67 index ($p = 0.004$). The presence of *PIK3CA* mutations was associated with lymphovascular invasion ($p = 0.018$) and a lower Ki-67 index ($p = 0.022$).

Table 2
Pathological parameters according to mutation types.

Pathology	<i>TP53</i> mutation		<i>p</i> -value	<i>PIK3CA</i> mutation		<i>p</i> -value
	+	-		+	-	
Grade			0.008*			0.055
1	0	1		1	0	
2	1	11		5	7	
3	13	8		3	18	
Lymphovascular invasion			0.026*			0.018*
no	11	8		2	17	
yes	3	12		7	8	
Tissue p53			< 0.001*			0.082
negative	2	16		7	11	
positive	12	4		2	14	
Tissue Ki-67			0.004*			0.022*
<15	1	11		6	6	
≥15	13	9		3	19	
* $p < 0.05$						

Cell-free nucleic acid *PIK3CA* mutations from breast cancer patient plasma

Cell-free nucleic acid *PIK3CA* mutations were not detected in the 24 plasma samples from patients with breast cancers at these stages, although seven harbored *PIK3CA* mutations detected by targeted

sequencing.

Comparison of tumor size according to TP53 and accompanying mutations

Although every subject was classified as the same T2 stage (widest primary tumor size 2–5 cm), *TP53*-mutated tumors were smaller than *TP53*-non-mutated ones in the HR+/HER2– group; however, *TP53*-mutated tumors were relatively large in the TNBC group (Fig. 3A). In other words, the *TP53*-mutated TNBC tumors were larger than the *TP53*-mutated HR+/HER2– breast cancers ($p = 0.0493$) (Fig. 3A). Tumor size was correlated with the total number of significant mutations including *TP53* and other accompanying mutations in *TP53*-mutated breast cancers ($r = 0.7156$, $p = 0.004$) (Fig. 3B).

Discussion

TNBC is clinically aggressive with a shorter interval to recurrence and metastasis compared to HR + breast cancer [1–3, 11]. TNBC has no specific targeted therapy other than surgery, chemotherapy, and radiotherapy because it does not express HR or HER2. However, high-throughput molecular diagnostic technologies such as NGS and microarray analyses have expanded our knowledge of the genetic alterations in cancers and provided new insight into the molecular pathogenesis of cancers [8, 12, 13]. Using NGS, the most commonly mutated genes in primary breast cancer are *TP53* and *PIK3CA* [8]. The frequency of mutations in these genes depends on the breast cancer subtype. TNBC has a higher predominance of *TP53* mutations compared to luminal A breast cancer [5, 8, 14–16]. Similarly, we also found a higher incidence of *TP53* mutations in TNBC (75.0%) compared to HR+/HER2– breast cancer (22.7%) in sporadic breast cancers in ethnic Koreans. This was similar to previous findings that TNBC is associated with a higher incidence of *TP53* mutations (80%) than are luminal A breast cancers (12%) [8].

Regarding the clinicopathological implications of these two main breast cancer-associated genes, *TP53* mutations in primary tumor tissues were associated with p53 positivity ($p < 0.001$) and a higher Ki-67 index ($p = 0.004$), as expected. *TP53*-mutated breast cancers also had a higher tumor grade ($p = 0.008$), whereas *PIK3CA*-mutated breast cancers exhibited more lymphovascular invasion ($p = 0.008$).

All *TP53* and *PIK3CA* mutations were somatic mutations and were categorized as pathogenic or likely pathogenic variants. In addition, *AKT1*, *ALK*, *BRCA1*, *ERBB2*, and *PTEN* were somatically mutated genes and existed without additional pathogenic or likely pathogenic mutations. *AR*, *KIT*, and *STK11* were also somatically mutated genes, with co-existing pathogenic mutations. The other mutations were only VUS or germline mutations. All three *BRCA2* mutations were germline mutations, accompanied by additional pathogenic or likely pathogenic somatic mutations, and were found only in HR+/HER2– breast cancers. Four *BRCA1* mutations were found in both groups, one showed LOH of a germline mutation in TNBC and another was a somatic mutation in HR+/HER2– breast cancer. Although *BRCA1/2* mutations are strongly associated with TNBC and a high proportion of mutation carriers can develop TNBC, germline *BRCA1/2* mutations account for only 15.4% of TNBC [17, 18]. In this study, only two (16.7%) *BRCA1* mutations were found in sporadic TNBC, and one of them was a germline mutation with LOH.

Both the TNBC and HR+/HER2- groups had no significant variants of the *ESR1* gene, which are generally found in advanced breast cancer patients treated with endocrine therapy [19]. We monitored patients for a median of 43.5 (range 10–50) months. The follow-up period was short, although recurrences or metastases of TNBC are known to occur within 2–3 years. We had a limited ability to identify the relationship between gene mutations and prognosis because the study subjects had relatively early breast cancer diagnosed as pT2N0-1M0. Recent studies have revealed the relationship between gene mutations and prognosis through analyses of circulating tumor DNA (ctDNA) in advanced breast cancers [20–22]. Therefore, we analyzed circulating cell-free nucleic acids in preserved blood plasma with the theascreen® PIK3CA RGQ PCR kit (QIAGEN). Circulating cell-free *PIK3CA* mutations were absent in either groups, which might have been due to the relatively early stage of disease because this diagnostic assay is approved only for advanced disease. We think that the usefulness of theascreen® *PIK3CA* mutation detection with cell-free nucleic acids at the early stage is still questionable.

Somatic mutations of *TP53*, *PIK3CA*, and *PTEN* are considered founder mutations in primary breast cancers and additional mutations occur in genes involved in other pathways, such as those controlling cell shape and the cytoskeleton [23]. In TNBC, *TP53* mutations and methylation of the *BRCA1* promoter region are early oncogenic events, and additional structural and epigenetic events occur during clonal progression, resulting in the amplification of oncogenes such as *MYC*, *NOTCH2*, and *NOTCH3*, the hampering of tumor suppressor genes, including *RB1*, *PTEN*, and *KMT2C*, and the enhancement of the *TGFA* oncogene [24]. In this study, the *TP53* mutation was predominant in TNBC, and the tumor size seemed to be proportional to the total number of significant mutations in *TP53*-mutated breast cancer, although the cancers were at the same tumor stage (T2, 2–5 cm). Therefore, we inferred that tumor size reflects clonal progression and diversification at earlier tumor stages in *TP53*-mutated breast cancers.

Conclusion

The *TP53* mutation is more frequent in TNBC than in HR+/HER2- cancer. It is associated with a higher tumor grade and greater Ki-67 expression in both groups, and with a larger tumor size in TNBC, but not in HR+/HER2- breast cancer. With *TP53* mutation, concomitant mutation numbers are proportional to tumor size, reflecting clonal progression. Breast cancer-associated mutations such as those in *TP53* and *PIK3CA* have different biological implications for the proliferation and clonal diversification of these two distinct groups of breast cancer.

Abbreviations

TNBC: Triple-negative breast cancer

HR+/HER2-: hormone receptor-positive/human epidermal growth factor receptor 2-negative

NGS: next-generation sequencing

TP53: tumor protein p53

PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

FDA: Food and Drug Administration

VUS: variation of uncertain significance

LOH: loss of heterozygosity

Declarations

Availability of data and materials

The authors confirm that all data supporting the findings of this study are available within the article and its supplementary materials.

Ethics declarations

Ethics approval and consent to participate

All tested patients provided a signed informed consent form prior to gene mutation tests and permission for the anonymous use of their data for research purpose. This study was reviewed and approved by the Institutional Review Board of Pusan National University Yangsan Hospital (04-2020-003). Bio-specimens and clinical data were obtained from the Institutional Biobank Project (OF-2020-01) in accordance with the approved research protocol. All work in this study has been performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

This study was supported by the Research Institute for the Convergence of Biomedical Science and Technology (30-2020-014), Pusan National University Yangsan Hospital.

Authors' contributions

S Lee and SH Oh drafted the manuscript. SH Oh performed analysis and interpretation of the variant data and prepared all supporting figures. S Lee and SM Lee designed the study, and analyzed the clinicopathological data of patients. HY Kim, YJ Jung, HJ Paik, and CS Jung provided the patient materials and treatment. JY Kim interpreted all the pathologic findings. All authors have read and approved the final manuscript.

Acknowledgments

The authors thank Yelin Ryu, MT in the Department of Laboratory Medicine, Pusan National University Yangsan Hospital for her technical assistance.

Supplementary Information

Additional file 1: Table S1.docx

Significant sequence variations detected in this study.

References

1. Ihemelandu CU, Naab TJ, Mezghebe HM, Makambi KH, Siram SM, Leffall LD, Jr., DeWitty RL, Jr., Frederick WA: Treatment and survival outcome for molecular breast cancer subtypes in black women. *Ann Surg* 2008, 247(3):463-469. <https://doi.org/10.1097/SLA.0b013e31815d744a>
2. Lowery AJ, Kell MR, Glynn RW, Kerin MJ, Sweeney KJ: Locoregional recurrence after breast cancer surgery: a systematic review by receptor phenotype. *Breast Cancer Res Treat* 2012, 133(3):831-841. <https://doi.org/10.1007/s10549-011-1891-6>
3. McGuire A, Lowery AJ, Kell MR, Kerin MJ, Sweeney KJ: Locoregional Recurrence Following Breast Cancer Surgery in the Trastuzumab Era: A Systematic Review by Subtype. *Ann Surg Oncol* 2017, 24(11):3124-3132. <https://doi.org/10.1245/s10434-017-6021-1>
4. Le Du F, Eckhardt BL, Lim B, Litton JK, Moulder S, Meric-Bernstam F, Gonzalez-Angulo AM, Ueno NT: Is the future of personalized therapy in triple-negative breast cancer based on molecular subtype? *Oncotarget* 2015, 6(15):12890-12908. <https://doi.org/10.18632/oncotarget.3849>
5. Marotti JD, de Abreu FB, Wells WA, Tsongalis GJ: Triple-Negative Breast Cancer: Next-Generation Sequencing for Target Identification. *Am J Pathol* 2017, 187(10):2133-2138. <https://doi.org/10.1016/j.ajpath.2017.05.018>
6. Quintero M, Adamoski D, Reis LMD, Ascencao CFR, Oliveira KRS, Goncalves KA, Dias MM, Carazzolle MF, Dias SMG: Guanylate-binding protein-1 is a potential new therapeutic target for triple-negative breast cancer. *BMC Cancer* 2017, 17(1):727. <https://doi.org/10.1186/s12885-017-3726-2>

7. Weisman PS, Ng CK, Brogi E, Eisenberg RE, Won HH, Piscuoglio S, De Filippo MR, Ioris R, Akram M, Norton L *et al*: Genetic alterations of triple negative breast cancer by targeted next-generation sequencing and correlation with tumor morphology. *Mod Pathol* 2016, 29(5):476-488.
<https://doi.org/10.1038/modpathol.2016.39>
8. Cancer Genome Atlas N: Comprehensive molecular portraits of human breast tumours. *Nature* 2012, 490(7418):61-70. <https://doi.org/10.1038/nature11412>
9. Copur MS: Alpelisib to treat breast cancer. *Drugs Today (Barc)* 2020, 56(6):357-363.
<https://doi.org/10.1358/dot.2020.56.6.3137526>
10. Martinez-Saez O, Chic N, Pascual T, Adamo B, Vidal M, Gonzalez-Farre B, Sanfeliu E, Schettini F, Conte B, Braso-Maristany F *et al*: Frequency and spectrum of PIK3CA somatic mutations in breast cancer. *Breast Cancer Res* 2020, 22(1):45. <https://doi.org/10.1186/s13058-020-01284-9>
11. Elias AD: Triple-negative breast cancer: a short review. *Am J Clin Oncol* 2010, 33(6):637-645.
<https://doi.org/10.1097/COC.0b013e3181b8afcf>
12. de Abreu FB, Peterson JD, Amos CI, Wells WA, Tsongalis GJ: Effective quality management practices in routine clinical next-generation sequencing. *Clin Chem Lab Med* 2016, 54(5):761-771.
<https://doi.org/10.1515/cclm-2015-1190>
13. Klijn C, Durinck S, Stawiski EW, Haverty PM, Jiang Z, Liu H, Degenhardt J, Mayba O, Gnad F, Liu J *et al*: A comprehensive transcriptional portrait of human cancer cell lines. *Nat Biotechnol* 2015, 33(3):306-312. <https://doi.org/10.1038/nbt.3080>
14. Carrick DM, Mehaffey MG, Sachs MC, Altekrose S, Camalier C, Chuaqui R, Cozen W, Das B, Hernandez BY, Lih CJ *et al*: Robustness of Next Generation Sequencing on Older Formalin-Fixed Paraffin-Embedded Tissue. *PLoS One* 2015, 10(7):e0127353.
<https://doi.org/10.1371/journal.pone.0127353>
15. Ellsworth DL, Turner CE, Ellsworth RE: A Review of the Hereditary Component of Triple Negative Breast Cancer: High- and Moderate-Penetrance Breast Cancer Genes, Low-Penetrance Loci, and the Role of Nontraditional Genetic Elements. *J Oncol* 2019, 2019:4382606.
<https://doi.org/10.1155/2019/4382606>
16. Shang M, Chang C, Pei Y, Guan Y, Chang J, Li H: Potential Management of Circulating Tumor DNA as a Biomarker in Triple-Negative Breast Cancer. *J Cancer* 2018, 9(24):4627-4634.
<https://doi.org/10.7150/jca.28458>
17. Greenup R, Buchanan A, Lorzio W, Rhoads K, Chan S, Leedom T, King R, McLennan J, Crawford B, Kelly Marcom P *et al*: Prevalence of BRCA mutations among women with triple-negative breast cancer (TNBC) in a genetic counseling cohort. *Ann Surg Oncol* 2013, 20(10):3254-3258.
<https://doi.org/10.1245/s10434-013-3205-1>
18. Newman LA, Reis-Filho JS, Morrow M, Carey LA, King TA: The 2014 Society of Surgical Oncology Susan G. Komen for the Cure Symposium: triple-negative breast cancer. *Ann Surg Oncol* 2015, 22(3):874-882. <https://doi.org/10.1245/s10434-014-4279-0>

19. Fribbens C, Garcia Murillas I, Beaney M, Hrebien S, O'Leary B, Kilburn L, Howarth K, Epstein M, Green E, Rosenfeld N *et al*: Tracking evolution of aromatase inhibitor resistance with circulating tumour DNA analysis in metastatic breast cancer. *Ann Oncol* 2018, 29(1):145-153.
<https://doi.org/10.1093/annonc/mdx483>
20. Abbosh C, Swanton C, Birkbak NJ: Circulating tumour DNA analyses reveal novel resistance mechanisms to CDK inhibition in metastatic breast cancer. *Ann Oncol* 2018, 29(3):535-537.
<https://doi.org/10.1093/annonc/mdy017>
21. Kodahl AR, Ehmsen S, Pallisgaard N, Jylling AMB, Jensen JD, Laenkholm AV, Knoop AS, Ditzel HJ: Correlation between circulating cell-free PIK3CA tumor DNA levels and treatment response in patients with PIK3CA-mutated metastatic breast cancer. *Mol Oncol* 2018, 12(6):925-935.
<https://doi.org/10.1002/1878-0261.12305>
22. Lee JH, Jeong H, Choi JW, Oh HE, Kim YS: Liquid biopsy prediction of axillary lymph node metastasis, cancer recurrence, and patient survival in breast cancer: A meta-analysis. *Medicine (Baltimore)* 2018, 97(42):e12862. <https://doi.org/10.1097/MD.00000000000012862>
23. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G *et al*: The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 2012, 486(7403):395-399. <https://doi.org/10.1038/nature10933>
24. Kawazu M, Kojima S, Ueno T, Totoki Y, Nakamura H, Kunita A, Qu W, Yoshimura J, Soda M, Yasuda T *et al*: Integrative analysis of genomic alterations in triple-negative breast cancer in association with homologous recombination deficiency. *PLoS Genet* 2017, 13(6):e1006853.
<https://doi.org/10.1371/journal.pgen.1006853>

Figures

Figure 1

Comparison of the demographic and clinicopathological parameters between TNBC and HR+/HER2- breast cancer patients. Columns and error bars represent medians and 95% confidence intervals, respectively. *, $p < 0.05$; CA15-3, cancer antigen 15-3; CEA, carcinoembryonic antigen

Figure 2

Mutation profiles of the two types of breast cancer. Each row represents a gene and each column represents a subject in the two groups of breast cancer patients.

Figure 3

(a) Tumor size according to TP53 mutations in HR+/HER2- breast cancer and TNBC. In the HR+/HER2- group, TP53-mutated cancers were smaller, whereas TP53-mutated tumors were relatively larger in the TNBC group. TP53-mutated TNBC was larger than TP53-mutated HR+/HER2- breast cancers ($p=0.0493$).

(b) Tumor size according to the number of significant mutations in TP53-mutated breast cancer.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.docx](#)
- [TableS1.docx](#)