Somatic Mutations of TP53 Identified by Targeted Next-Generation Sequencing Are Poor Prognostic Factors for Primary Operable Breast Cancer: A Single-Center Study

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ABSTRACT

Few studies have reported on the clinical utility of targeted next-generation sequencing (NGS) for breast cancer in Korea. We retrospectively reviewed the targeted NGS data of 219 patients with breast cancer who underwent surgical resection between August 2018 and April 2021. Here, we described the mutational profiles of breast cancer and examined their prognostic implications. The most frequently mutated gene was PIK3CA (n = 97/219, 44.3%), followed by TP53 (n = 79/219, 36.1%), AKT1 (n = 23/219, 10.5%), and GATA3 (n = 20/219, 9.1%). TP53 mutations were associated with aggressive histologic features. We followed up for 31 (range, 1–39) months and observed 11 (5.0%) recurrences: nine were TP53 mutant and two were TP53 wild-type. Multivariable analysis revealed that TP53 mutation was an independent prognostic factor for recurrence (p = 0.012). Although no drug is currently available for TP53 mutations, it is valuable to know the mutational status of TP53 for the precise management of breast cancer.

Keywords: Breast Neoplasms; Disease-Free Survival; Genes, p53; High-Throughput Nucleotide Sequencing; Mutation

INTRODUCTION

Molecular studies have shown that breast cancer is a heterogeneous disease. Each breast cancer subtype has diverse biologic characteristics [1]. The development of next-generation sequencing (NGS) has reduced the time and cost of genomic analyses. Targeted NGS enables genomic analysis and is readily available in clinical settings. Several platforms for targeted NGS have been commercialized and used in clinical practice.

Since 2017, targeted NGS for solid cancers has been partially covered by the national health insurance in Korea [2]. However, the mutational profiles of Korean patients with breast cancer have been poorly characterized. Moreover, the clinical utility of targeted NGS for breast cancer in Korea remains underexplored. In this study, we described the mutational profiles of breast cancer and examined their prognostic implications.
primary operable breast cancer remains unclear [3]. To fill the gap between widespread clinical use and unproven clinical benefits, we aimed to describe our experience with targeted NGS for primary operable breast cancer.

**METHODS**

**Patient selection and data acquisition**

Targeted NGS was initiated at our institution in August 2018. We retrospectively reviewed the electronic medical records of patients with breast cancer who underwent surgical resection between August 2018 and April 2021. We included patients with stage I–III breast cancer and collected the available NGS data. Patients who underwent neoadjuvant chemotherapy and those with recurrent tumors, distant metastasis, and occult breast cancer were excluded from the study. We collected clinicopathological data, including age at diagnosis, menopausal status, body mass index, histologic subtype, nuclear grade, histologic grade, lymphovascular invasion, lymph node metastases, and immunohistochemical staining. Pathological data included estrogen receptor (ER), progesterone receptor, human epithelial growth factor receptor 2 (HER2), and Ki-67 index. According to the Saint Gallen consensus [4], we classified the patients into five subtypes: luminal A, luminal B/HER2-negative, luminal B/HER2-positive, HER2-enriched, and triple-negative breast cancers. We adopted a cut-off value of Ki-67 as 20% for distinguishing between the luminal A and luminal B/HER2-negative subtypes.

**NGS protocol**

All NGS procedures were performed in accordance with the institutional protocols. Formalin-fixed paraffin-embedded tissue blocks were dissected to 10-µm thickness. Tumor areas with high cellularity were selected and manually dissected for further analysis. The DNA was extracted and purified in a standard manner.

For sequencing, we used MiSeqDx (Illumina, San Diego, CA, USA), according to the manufacturer’s protocol. We selected 50 cancer-related genes and customized a pan-cancer panel. The genes included in the NGS panel were as follows: AKT1, ALK, APC, ARID1A, ATRX, BRAF, BRCAl, BRCa2, CDH1, CDK4, CDK6, CDKN2A, CTNNB1, EGFR, HER2, ERBB3, ERBB4, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FOXA1, GATA3, H3F3A, IDH1, IDH2, KIT, KRAS, MAP2K1, MET, MLH1, MTOR, MYC, MYCN, NRAS, PDGFA, PIK3CA, PTEN, RBI, RELA, RET, RHOA, RICTOR, ROS1, SMAD4, SMARCB1, SMO, STK11, and TP53. All coding exons of the genes were included in the panel.

Each variant was compared with known mutations stored in web-based databases, such as COSMIC (https://cancer.sanger.ac.uk/cosmic), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and OncoKB (https://www.oncokb.org/). According to the guidelines [5], the variants were classified into three groups: tier 1, variants with strong clinical significance; tier 2, variants with potential clinical significance; and tier 3, variants with unknown clinical significance. The variants of tiers 1 and 2 were included in the analysis.

**Statistical analysis**

Comparisons between categorical variables were performed using the χ² test or Fisher’s exact test. Comparisons between continuous variables were performed using the Student’s t-test. Disease-free survival (DFS) was compared using the Kaplan-Meier product limit method and log-rank test. The Cox proportional hazards model was used for the multivariable analysis.
All statistical analyses were performed using SPSS (version 27.0; IBM Corporation, Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

This study was reviewed and approved by the Institutional Review Board of Hallym University Sacred Heart Hospital (IRB number: 2019-05-009). The requirement for informed consent was waived due to the retrospective study design.

## RESULTS

### Clinicopathological characteristics

A total of 801 patients underwent surgery for breast cancer between August 2018 and April 2021. Among 258 patients who underwent NGS for breast cancer, 219 were included in the analysis (Supplementary Figure 1). \( TP53 \) mutations were significantly associated with aggressive histologic features, such as high nuclear grade, high histologic grade, and high Ki-67 index (Table 1). Adjuvant chemotherapy showed no significant differences between \( TP53 \) wild-type and mutant tumors (Supplementary Table 1).

| Variables                        | \( TP53 \) wild-type (n = 140) | \( TP53 \) mutant (n = 79) | \( p \)     |
|----------------------------------|-------------------------------|---------------------------|------------|
| Age (yr)                         | 55.09 ± 11.56                 | 54.77 ± 12.82             | 0.857      |
| Menopausal status                |                               |                           | 0.158      |
| Premenopausal                    | 66 (47.1)                     | 30 (38.0)                 |            |
| Postmenopausal                   | 72 (51.4)                     | 45 (57.0)                 |            |
| Perimenopausal                   | 2 (1.4)                       | 4 (5.1)                   |            |
| BMI (kg/m\(^2\))                | 24.49 ± 3.91                  | 25.03 ± 4.52              | 0.375      |
| Operation                        |                               |                           | 0.501      |
| BCS                              | 100 (71.4)                    | 53 (67.1)                 |            |
| TM                               | 40 (28.6)                     | 26 (32.9)                 |            |
| T stage                          |                               |                           | 0.029      |
| 1                                | 79 (56.4)                     | 30 (38.0)                 |            |
| 2                                | 49 (35.0)                     | 42 (53.2)                 |            |
| 3                                | 10 (7.1)                      | 4 (5.1)                   |            |
| 4                                | 2 (1.4)                       | 3 (3.8)                   |            |
| N stage                          |                               |                           | 0.024      |
| 0                                | 86 (61.4)                     | 39 (49.4)                 |            |
| 1                                | 27 (19.3)                     | 14 (17.7)                 |            |
| 2                                | 15 (10.7)                     | 21 (26.6)                 |            |
| 3                                | 12 (8.6)                      | 5 (6.3)                   |            |
| Nuclear grade                    |                               |                           | < 0.001    |
| 1                                | 19 (13.9)                     | 2 (2.5)                   |            |
| 2                                | 78 (56.9)                     | 17 (21.5)                 |            |
| 3                                | 40 (29.2)                     | 60 (75.9)                 |            |
| Histologic grade                 |                               |                           | < 0.001    |
| 1                                | 38 (27.9)                     | 5 (6.3)                   |            |
| 2                                | 72 (52.9)                     | 21 (26.6)                 |            |
| 3                                | 26 (19.1)                     | 53 (67.1)                 |            |
| Lymphovascular invasion          |                               |                           | 0.041      |
| Absent                           | 92 (67.2)                     | 42 (53.2)                 |            |
| Present                          | 45 (32.8)                     | 37 (46.8)                 |            |
| Ki-67 index                      | 20.06 ± 16.15                 | 40.98 ± 16.69             | < 0.001    |

Values are expressed as mean ± standard deviation or number (%). BMI = body mass index; BCS = breast conserving surgery; TM = total mastectomy.
The most commonly mutated gene was PIK3CA in 97 (44.3%) patients, followed by TP53 in 79 (36.1%), AKT1 in 23 (10.5%), and GATA3 in 20 (9.1%). The less commonly mutated genes included PTEN, CDH1, BRCA2, HER2, and BRCA1 mutations in 15 (6.8%), 11 (5.0%), 9 (4.1%), 5 (2.3%), and 4 (1.8%) patients, respectively.

Each breast cancer subtype exhibited different mutational characteristics (Table 2). PIK3CA mutations were more commonly found in the luminal A subtype (n = 55/83, 66.3%) than in the luminal B/HER2-negative (12/38, 31.6%), luminal B/HER2-positive (11/35, 31.4%), HER2-enriched (10/22, 45.5%), or triple-negative (9/41, 22.0%) subtypes (p < 0.001). TP53 mutations were more commonly found in the HER2-enriched (17/22, 77.3%) and triple-negative (9/41, 22.0%) subtypes than in the luminal B/HER2-positive (18/35, 51.4%), luminal B/HER2-negative (11/35, 31.4%), and luminal A (5/83, 6.0%) subtypes (p < 0.001). GATA3 mutations were exclusively found in luminal breast cancers (p = 0.023). BRCA1 mutations were more likely to be found in the triple-negative subtype (p = 0.024).

Survival analysis
We compared survival outcomes between wild-type and mutant tumors. Overall, there were 11 (5.0%) recurrences during 31 (range, 1–39) months of follow-up. TP53 mutations were significantly associated with worse short-term DFS (Figure 1). For the other genes, there were no significant differences in survival (Supplementary Figure 2).

Multivariable analysis was performed to further validate the prognostic implications of TP53 mutations (Supplementary Table 2). Univariable analysis revealed that high nodal stage, high nuclear grade, high histologic grade, ER negativity, and TP53 mutations were associated with recurrence. Multivariable analysis showed that TP53 mutations were independently associated with short-term DFS in breast cancer (hazard ratio, 7.23; 95% confidence interval, 1.55–33.77; p = 0.012).

DISCUSSION
We showed that somatic mutations of TP53, which can be identified by targeted NGS, are poor prognostic factors for breast cancer in a curative setting. Among the various genetic alterations, only TP53 mutations were associated with poor short-term DFS in patients with primary operable breast cancer. TP53 mutations are associated with poor prognostic factors,
such as high histologic grade, high Ki-67 index, and non-luminal subtype. Multivariable analysis showed that TP53 mutations were independent prognostic factors for recurrence.

TP53 mutations are driver mutations in various cancer types. TP53 mutations are closely related to aggressive histologic features and poor survival in breast cancer [6-8]. Paired analysis of primary breast tumors and metastatic samples showed that TP53 mutations were more commonly identified in metastatic samples [9,10]. However, it is unclear whether TP53 mutations are predictive factors. TP53 mutations were not predictive factors in a randomized controlled trial comparing taxane versus non-taxane neoadjuvant chemotherapy [11]. In hormone receptor-positive breast cancers, TP53 mutations were associated with resistance to hormonal treatment [12,13]. Another study suggested that tamoxifen is effective against breast cancer with wild-type TP53 [14]. There are no drugs available that target TP53 mutations; however, such drugs are currently under investigation [15-17].

The most commonly mutated gene in our study was PIK3CA. PIK3CA mutations were commonly identified in the luminal A subtype and were associated with indolent histologic features in our cohort. However, we were unable to demonstrate their prognostic value. In contrast to early breast cancer, PIK3CA mutations are poor prognostic factors for advanced breast cancer [12,18]. Drugs that target the PI3K/Akt/mTOR pathway are available for treating metastatic breast cancer [18-20]. Notably, alpelisib is an oral PI3Kα inhibitor that has recently been approved for hormone receptor-positive, HER2-negative metastatic breast cancers.

Although various genetic alterations can be identified through targeted NGS, there is insufficient evidence to guide treatment through NGS. All patients in our study received standard treatments. Only two genetic alterations, BRCA1 and BRCA2 mutations, can guide the surgical treatment of breast cancer. Mutations in BRCA1 or BRCA2 can cause hereditary breast and ovarian cancer syndromes. For patients with pathogenic germline mutations of BRCA1 or BRCA2, risk-reducing mastectomy may be recommended. We identified four (1.8%) BRCA1 and nine (4.1%) BRCA2 mutations. Among the three patients who underwent the germline BRCA test, two were confirmed to have germline BRCA mutations, and the other

Figure 1. Kaplan-Meier survival analysis of the study patients. TP53 mutations were associated with poor short-term DFS of primary operable breast cancer patients ($p = 0.001$). DFS = disease-free survival.
patient was confirmed to have a somatic mutation (Supplementary Table 3). The results of this study are consistent with those of a previous study that demonstrated that approximately one-third of BRCA mutations are of somatic origin [21]. Genetic counseling should be performed before initiating targeted NGS, and germline testing should be performed for all patients with BRCA mutations.

Some of the identified mutations have been associated with treatment resistance. We identified a patient with an ESR1 (p.Y537C) mutation located in the ligand-binding domain of ESR1 [22]. ESR1 mutations have rarely been identified in treatment-naive breast cancers. In metastatic cohorts, ESR1 mutations have been identified in up to 25% of cases [12,23,24]. Activating mutations in HER2 can be identified by NGS. HER2 mutations are not detectable by immunohistochemistry and are resistant to trastuzumab [25,26]. In our cohort, there were five cases of HER2 mutations. One case was a pleomorphic lobular carcinoma for which the pan-HER inhibitor neratinib could be applied [26].

Our study had several limitations. We only included patients with primary operable breast cancer for whom investigational drugs were not applicable. Due to the short observation period, we could not observe any late recurrences. Long-term follow-up is required to observe the recurrence of ER-positive breast cancer. Because our gene panel included only 50 genes, some important genetic alterations could not be identified. Despite these limitations, we demonstrated that TP53 mutations identified using targeted NGS can serve as independent prognostic markers for primary operable breast cancer. This study provides valuable real-world data on the genomic profiles of breast cancer in Korea.

In conclusion, targeted NGS can be used to identify genetic alterations that may serve as prognostic factors for primary operable breast cancer. Knowledge of the TP53 mutational status is valuable for the precise management of breast cancer and for designing clinical trials. Although there are no clinically available drugs that target TP53 mutations, such drugs are currently being investigated.

SUPPLEMENTARY MATERIALS

Supplementary Table 1
Adjuvant chemotherapeutic regimens recommended by the clinicians

Click here to view

Supplementary Table 2
Factors associated with the short-term DFS

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Supplementary Table 3
Clinical characteristics of the patients with BRCA mutations

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Supplementary Figure 1
Flow diagram of the study design.

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Supplementary Figure 2
Kaplan-Meier survival analysis of the patients with mutations other than TP53. There are no significant differences in (A) PIK3CA mutations (n = 97), (B) AKT1 mutations (n = 23), (C) GATA3 mutations (n = 20), (D) PTEN mutations (n = 15), (E) CDH1 mutations (n = 11), and BRCA2 mutations (n = 9).

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