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Clinical Significance of p53 Overexpression in Terms of Prognosis and the Efficacy of Chemotherapy According to the Breast Cancer Subtypes

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Abstract

Background: The p53 tumor suppressor gene is important for cell cycle regulation and DNA repair. TP53 mutation can be detected by Immunohistochemistry (IHC) because of the prolonged stability of the p53 protein. The aim of this study was to evaluate the clinical significance of p53 protein status in terms of the Disease-Free Survival (DFS) rates and the efficacy of adjuvant chemotherapy (anthracycline +/- taxane) according to the breast cancer subtypes.

Methods: Primary invasive breast cancer patients (N=4,463) were enrolled in this retrospective study from January 2002 to December 2018. The IHC data (ER/PgR, HER2 and Ki-67) was used to determine the breast cancer subtypes. The Kaplan-Meier procedure and the log-rank test were used to calculate and analyze DFS. Univariate and multivariate analyses of the factors for DFS were performed using Cox's proportional hazard model.

Results: Eight hundred and eight (18.1%) out of 4,463 cases had p53 overexpression. A significant correlation was seen between the p53 overexpression and ER/PgR negativity, HER2 positivity, higher Ki-67 values, a higher nuclear grade, positive nodes and larger tumors. Moreover, a higher rate of p53 overexpression was significantly associated with HER2 and Triple Negative (TN) type cancers. A multivariate analysis found that p53 was a significant factor for DFS in only the luminal A/B types. Chemotherapy was not effective in improving DFS in relation to the p53 status in luminal A/B and HER2 types. However, TN type patients with p53 overexpression had a slightly worse DFS in cases that did not receive chemotherapy. Moreover, there was no difference in DFS in cases that received chemotherapy.

Conclusion: Luminal A/B subtypes with p53 overexpression had a higher grade of malignancy and a more unfavorable prognosis. However, the findings revealed that patients with TN subtype and p53 overexpression in adjuvant settings may benefit from chemotherapy.

Keywords: Breast cancer; Chemotherapy; Disease-free survival; ki-67; p53 overexpression; Subtype

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Introduction

Breast cancer is the most common cancer diagnosed among women in Japan and Globally [1]. Historically, breast cancer has been treated based on biomarkers such as the expression of Estrogen Receptor (ER), Progesterone Receptor (PgR), Ki-67 index values and the status of HER2 as assessed by Immunohistochemistry (IHC). More recently, breast cancer has been classified into intrinsic subtypes using IHC in practical medicine and adjuvant endocrine therapy, chemotherapy and anti-HER2 therapy are recommended according to the subtypes [2,3]. The goal of precision medicine is to give the most effective treatment for each patient with breast cancer. This involves getting the best results while avoiding unnecessary treatment, and to develop therapies to target specific tumors or specific cellular pathways. However, all treatments for breast cancer have a certain degree of risk and side effects that can be avoided by not using unnecessary treatments.

A mutation of the p53 gene (TP53) is often found across various cancer types. This gene encodes a tumor suppressor protein that regulates growth arrest, DNA repair and apoptosis [4,5]. Previous studies found that TP53 mutations were associated with poor survival in breast cancers [6,7]. TP53 mutations are found in about 30% of breast tumors and the rate, time of occurrence and

type of alterations are reported to be different among the molecular subtypes [8-12]. TP53 mutations are linked to increased mortality in cases with luminal B, HER2-enriched, and normal-like types, but not in cases with luminal A and basal-like types [12]. Moreover, the TP53 mutation contributes to a high rate of Complete Pathological Response (pCR) to dose-dense doxorubicin-cyclophosphamide chemotherapy in non-inflammatory locally advanced breast carcinomas [11]. However, the correlation between mutations in TP53 and the responsiveness to preoperative chemotherapy in breast cancer is still unclear [13-19]. The TP53 protein mutates and can be detected using Immunohistochemical (IHC) techniques because the altered gene is more stable and easier to analyze [20-23]. Therefore, p53 overexpression by IHC is considered a surrogate for the TP53 mutation status.

In this study, the p53 protein status was retrospectively investigated in primary invasive breast cancer cases and the clinical significance of p53 overexpression in relation to breast cancer subtypes was evaluated as a prognostic and predictive factor for the efficacy of adjuvant chemotherapy (anthracycline +/- taxane) in terms of Disease-Free Survival (DFS).

Patients and Methods

Patients

This retrospective study examined primary invasive breast cancer cases (n=4,463) from January 2002 to December 2018 at Kumamoto City Hospital and Kumamoto Shinto General Hospital. The study protocol was approved by the Institutional Review Board of Kumamoto Shinto General Hospital. The clinicopathological factors investigated were menopausal status, nodal status, tumor size, nuclear grade, ER/PgR and HER2 status, p53 overexpression, and the Ki-67 index value. Invasive breast cancer was divided into 5 subtypes according to the IHC data derived from ER/PgR, HER2 and the Ki-67 index values (cutoff point: 20%). Informed consent was obtained from all of the patients to participate in this study and to publish data and images related to the findings.

Histopathological examination

Evaluation of IHC for ER, PgR, HER2, p53 and Ki-67 was performed using an autostainer (Benchmark XT; Ventana Medical Systems, Inc., Tucson, USA) and the procedure followed the same guidelines as previously reported [24]. The primary antibodies used were ER (clone SP1; rabbit monoclonal), PgR (clone 1E2; rabbit monoclonal), HER2 (clone 4B5; rabbit monoclonal; all Ventana Medical Systems, Inc.), p53 (clone DO7; mouse monoclonal) and Ki-67 (clone MIB-1; mouse monoclonal; both Dako; Agilent Technologies, Inc., Santa Clara, CA, USA). The ER and PgR positive cell rates were determined using the ASCO/CAP recommendations (a value of ≥ 1% was considered positive). The positive nuclei for Ki-67 was calculated based on a count of at least 500 tumor cells in the hot spot and the value was represented as a percentage. The p53 overexpression was predetermined to be the number of cases with a positive cell count of ≥ 50% [25]. The HER2 status was dichotomized into positive and negative cases using IHC and the FISH test. HER2 positive was determined in cases with IHC3+ (strong and diffuse staining) or FISH amplified.

Breast cancer subtypes and adjuvant therapy

Breast cancer is classified by gene expression profile into subtypes consisting of two Hormone Receptor (HR)-positive types (luminal A and luminal B) and three HR-negative types (HER2 expressing, basal-like, and unclassified "normal-like"). In this study, HR positive (ER/PgR) and HER2 negative tumors with lower Ki-67 index values (<20%) were classified as luminal A type, those with higher Ki-67 index values (≥ 20%) as luminal B type, HR positive and HER2 positive tumors (HER2 IHC: 3+ or 2+ and FISH amplification ratio >2.0) as luminal HER2 type, HR negative and HER2 positive tumors as HER2 enriched, and HR negative and HER2 negative tumors as TN type.

Table 1: Patient Characteristics (n=4463).

Variables	Category	No. of Patients (%)
Menopausal status	pre	1597 (35.8)
	post	2866 (64.2)
ER	negative	903 (20.2)
	positive	3560 (79.8)
PgR	negative	1366 (30.6)
	positive	3097 (69.4)
Ki-67	<20%	1839 (41.2)
	20% - 49%	1814 (40.7)
	≥ 50%	809 (18.1)
	unknown	1
HER2	negative	3748 (84.0)
	positive	715 (16.0)
p53 Overexpression	With	808 (18.1)
	without	3655 (81.9)
Nuclear Grade	1	2071 (46.4)
	2	1270 (28.5)
	3	1024 (22.9)
	Unknown	98
Number of	0	2940 (65.9)
positive nodes	1-3	1085 (24,3)
	4-9	264 (5.9)
	≥ 10	101 (2.3)
	Unknown	73 (1.6)
Tumor size	≤ 2.0 cm	2908 (61.2)
	> 2.0 cm	1503 (33.7)
	Unknown	52
Adjuvant Therapy	none	483 (10.8)
	Chemotherapy	824 (18.5)
	Endocrine therapy	2363 (52.9)
	Chemo-endocrine	793 (17.8)
Age	Median (range)	57 years (24-94 years)
Follow-up period	Median	85 months
Total		4463

Most of the cases with luminal type tumors received endocrine therapy (tamoxifen or aromatase inhibitor) and most of the cases with triple negative and HER2 disease type were treated with chemotherapy (anthracycline containing regimen +/- taxane, and anti-HER2 therapy if HER2 positive). Anti-HER2 therapy (trastuzumab) was used in Japan after receiving approval in 2008.

Statistical analysis

The intergroup comparisons were done using the chi-square test

Table 2: Clinicopathological Factors and p53 Overexpression in Primary Invasive Breast Cancer.

Variables	Category	p53 Overexpression		Total	p value
		without	with		
Menopausal status	pre	1304	293 (18.3)	1597	
	post	2351	515 (18.0)	2866	0.39
ER	negative	452	451 (49.9)	903	
	positive	3203	357 (10.0)	3560	<0.0001
PgR	negative	832	534 (39.1)	1366	
	positive	2823	274 (8.8)	3097	<0.0001
Ki-67	< 20%	1766	73 (4.0)	1839	
	20% - 49%	1468	346 (19.1)	1814	<0.0001
	≥ 50%	421	388 (48.0)	809	
HER2	negative	3252	496 (13.2)	3748	<0.0001
	positive	403	312 (43.6)	715	
Nuclear Grade	1	1957	114 (5.5)	2071	
	2	1031	239 (18.8)	1270	<0.0001
	3	590	434 (42.4)	1024	
Number of positive nodes	0	2431	509 (17.3)	2940	
	1-3	873	212 (19.5)	1085	
	4-9	212	52 (19.7)	264	0.033
	≥ 10	73	28 (27.7)	101	
Tumor size	≤ 2.0 cm	2463	445 (15.3)	2908	
	> 2.0 cm	1160	343 (22.8)	1503	<0.0001
Adjuvant Therapy	none	376	107 (22.2)	483	
	Chemotherapy	421	403 (48.9)	824	
Endocrine therapy		2234	129 (5.5)	2363	<0.0001
Chemo-endocrine		624	169 (21.3)	793	

Table 3: Breast Cancer Subtypes and p53 Overexpression in Primary Invasive Breast Cancer.

Subtype	p53 Overexpression		Total	p value
	without	with		
Luminal A	1643	36 (2.1)	1679	
Luminal B	1342	208 (13.4)	1550	
Luminal HER2	238	125 (34.4)	363	<0.0001
HER2 Enriched	165	187 (53.1)	352	
Triple Negative	267	252 (48.6)	519	

and the Fisher's exact test (Table 2 and 3). Age and follow-up periods were determined using the Student's t-test. The Kaplan-Meier test was used to calculate cumulative Disease-Free Survival (DFS) and tested with the log rank procedure. The univariate and multivariate analyses for factors related to DFS were performed using the Cox proportional hazard model (SPSS version 21). The median follow-up period was 85.0 months.

Results

Clinicopathological factors and p53 overexpression in primary invasive breast cancer

The p53 overexpression rate was 18.1% (n=808) in all of the cases (Table 1). The p53 overexpression significantly correlated with negative ER/PgR (p<0.0001), positive HER2 (p<0.0001), higher Ki-67 index values (p<0.0001), higher nuclear grade (p<0.0001), positive nodes (p=0.033) and larger tumors (p<0.0001) (Table 2). Patients with p53 overexpression received chemotherapy more often than those without overexpression in adjuvant settings (p<0.0001).

Breast cancer subtypes and p53 overexpression in primary invasive breast cancer

There were significantly higher rates of p53 overexpression in the HER2 enriched type (53.1%) and the TN type (48.6%), but the luminal A and B types had lower p53 overexpression rates (2.1% and 13.4%). There were significant differences among the subtypes (Table 3).

DFS according to p53 status in relation to subtypes

DFS was evaluated in relation to p53 overexpression according to breast cancer subtypes (Figure 1). Patients with p53 overexpression had poorer DFS than those without p53 overexpression in the luminal A and B subtypes (p<0.0001). However, there was no difference in luminal/HER2, HER2 enriched and TN subtypes.

Multivariate analysis of factors for DFS according to subtypes

A multivariate analysis found that nodal status was a significant factor for DFS among the subtypes. On the other hand, p53 overexpression was found to be a significant factor in only the A and B luminal subtypes. p53 overexpression was not predictive for DFS in HER2 enriched, luminal HER2 and TN subtypes.

DFS according to the p53 status in relation to chemotherapy

The efficacy of chemotherapy for DFS was investigated according to the subtypes and p53 status (Figure 2). In the luminal A type group, cases with p53 overexpression had worse DFS irrespective of chemotherapy and in the luminal B type group, cases with p53 overexpression had worse DFS even in cases with chemotherapy. There was no difference in DFS between chemotherapy and p53 status in the HER2 types. The p53 overexpression slightly correlated with a poorer DFS in TN cases without chemotherapy (p=0.068), and especially in the node negative cases (p=0.064). However, there was no difference in DFS in the cases with chemotherapy. These findings suggest that chemotherapy is effective in improving the DFS in TN cases with p53 overexpression.

Discussion

The purpose of this study was to investigate the clinical significance of p53 overexpression in primary breast cancer, especially in relation to breast cancer subtypes. Eighteen percent (18%) of the patients in this study had p53 overexpression which is lower than the findings in the literature. The p53 gene is found to have mutated in approximately 20% to 40% of all breast carcinoma cases depending on the tumor size and stage of the disease [8]. The frequencies in node-negative patients are considerably lower (15% to 18%) than in node-positive patients, and large tumors and tumors from patients with advanced disease have a higher frequency of mutations than small tumors [8]. In this study, the p53 overexpression rate was 15.3% in the smaller tumor (<2 cm) and 17.3% in the node-negative tumor. Moreover, two third of the patients had node negative and smaller tumors.

Some studies have found that abnormal p53 immunohistochemical expression, or p53 positive status, was associated with more aggressive tumor features, a higher tumor grade, negative ER/PgR status and the more aggressive basal subtype [26,27]. The findings in this study revealed that the p53 overexpression significantly correlated with

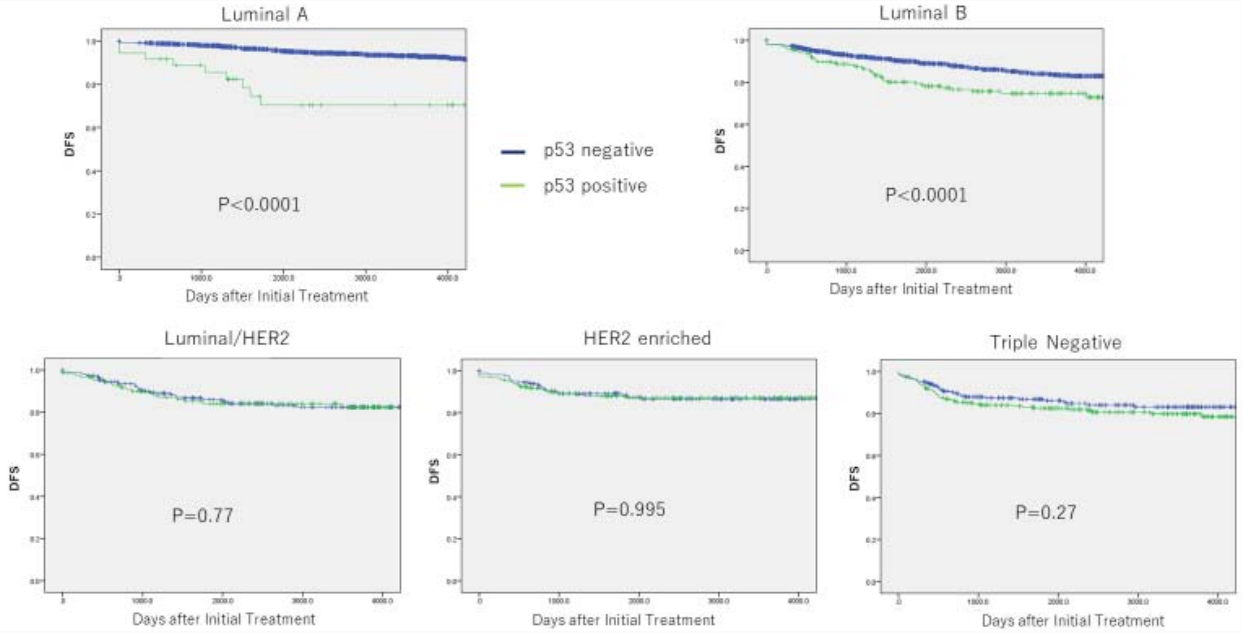

Figure 1: DFS According to p53 Status in Relation to Subtypes.
Patients with p53 overexpression had poorer DFS than those without p53 overexpression in the luminal A and B subtypes (p<0.0001). However, there was no difference in luminal/HER2, HER2 enriched and TN subtypes.

Table 4: Multivariate Analysis of Factors for DFS According to Subtypes.

Factors	Category	Luminal A		Luminal B		Triple Negative		HER2 Positive	
		p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)
Menopausal Status	post-/pre-	0.88	0.97 (0.63-1.48)	0.16	0.82 (0.61-1.09)	0.006	0.51 (0.31-0.83)	0.32	0.81 (0.54-1.23)
Tumour Size	≥ 2.0/<2.0 cm	<0.0001	2.63 (1.72-4.02)	<0.0001	2.07 (1.53-2.81)	<0.0001	3.67 (2.12-6.37)	0.14	1.68 (1.11-2.54)
Nodal Status	Positive/negative	<0.0001	3.98 (2.44-6.49)	<0.0001	2.17 (1.57-2.99)	<0.0001	4.51 (2.58-7.87)	<0.0001	3.21 (2.07-4.98)
p53 Over- expression	With/without	<0.0001	4.43 (2.12-9.25)	0.003	1.68 (1.19-2.36)	0.67	0.90 (0.55-1.47)	0.83	1.05 (0.69-1.58)
Adjuvant Chemo- therapy	With/without	0.001	2.19 (1.38-3.46)	<0.0001	1.89 (1.35-2.65)	0.97	0.98 (0.39-2.49)	0.49	0.82 (0.48-1.42)

negative ER/PgR, positive HER2, higher Ki-67 index values and nuclear grade. Moreover, the p53 status significantly correlated with the breast cancer subtypes. There were significantly higher rates of p53 overexpression in the HER2 enriched type and the TN type, but not in the luminal A and luminal B types. One study reported that the TP53 mutation is found in 17% of the luminal A type cases, 41% of the luminal B type cases, 50% of the HER2 cases, and 88% of the basal-like carcinoma cases [11]. Consequently, p53 is frequently expressed in high grade, aggressive and advanced breast cancer.

Moreover, the independent prognostic impact of IHC identified a correlation between the p53 mutation and DFS only in the luminal A/B subtypes. However, the p53 status did not correlate with DFS in TN and HER2 subtypes. The findings from a previous study revealed that the p53 status in combination with Ki-67 provided a better prognostic evaluation than using the Ki-67 index value as the only diagnostic indicator [28]. In this study, the luminal subtypes were divided into luminal A and luminal B according to the Ki-67 index values. Therefore, the p53 status can determine the prognosis of luminal A and B types irrespective of adjuvant chemotherapy. Pan Y et al. [29] found that p53, Ki-67 and family history were useful prognostic determinants in TN subtype. Although the p53 status was not a significant prognostic factor in TN and HER2 subtypes, p53 overexpression was a marginally significant predictive factor for chemotherapy benefit in terms of DFS in the TN subtype. Bertheau P et al. [11] found that TP53 mutated non inflammatory locally advanced breast carcinomas had a high rate of pCR to dose-dense doxorubicine/cyclophosphamide chemotherapy, while TP53 wild-type tumors never achieved complete response. From the phase II neoadjuvant GeparSixto trial, high TP53 mutation rates were seen in TN and HER2-positive breast cancer, but mutations did not predict the response to an intense neoadjuvant chemotherapy (anthracycline/taxane-based chemotherapy) in these two molecular breast cancer subtypes [30]. A recent study found that TP53 somatic mutations, particularly at codon p.R175H, are enriched in tumors with infiltrating immune cells and that missense mutations were linked to higher numbers of Tumor-Infiltrating Lymphocytes (TILs) in TN type [31]. The data suggests that TILs may be a viable biomarker for predicting the response to NAC in patients with TN and HER2 type tumors. Moreover, a decrease in the TILs count may also be an indicator for tumor recurrence [32]. These findings suggest that TP53 mutation may benefit from chemotherapy.

There are two possible limitations in this study. First, p53 IHC was used as a surrogate for mutation status. The detection of mutant TP53 using IHC is not an accurate substitute for complex mutations. Second, the subtypes were identified using IHC markers. However, the IHC method is cost efficient and does not need highly experienced technicians. In conclusion, the prognostic effect of p53 overexpression seems to be restricted to the luminal or ER positive subgroups, and

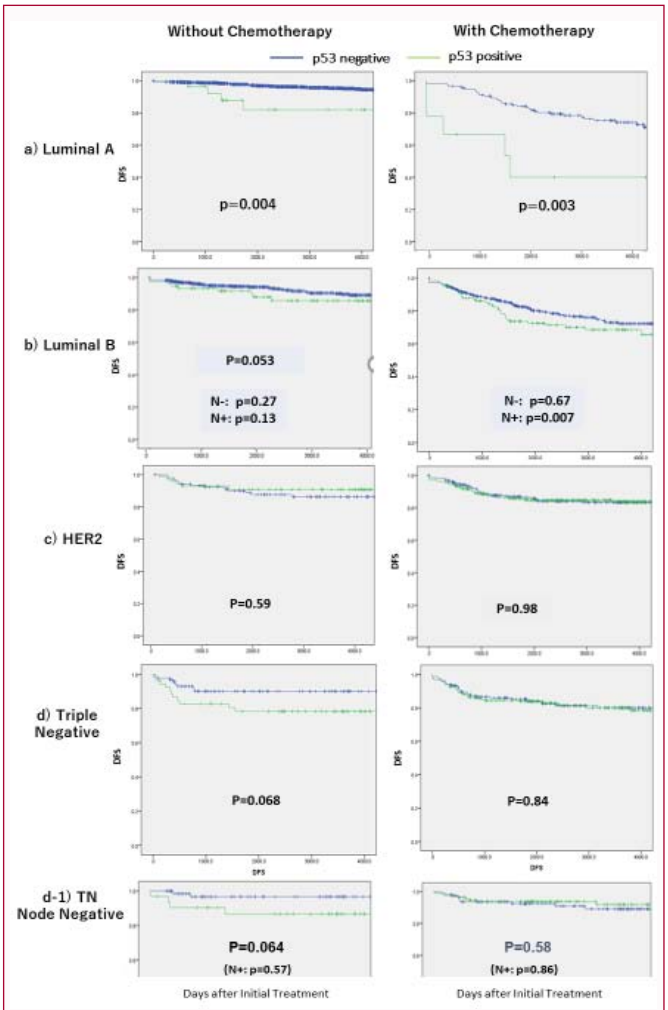

Figure 2: DFS According to the p53 Status in Relation to Chemotherapy. In the luminal A type group **(a)**, cases with p53 overexpression had worse DFS irrespective of chemotherapy and in the luminal B type group **(b)**, cases with p53 overexpression had worse DFS even in cases with chemotherapy. Chemotherapy did not affect the DFS in relation to p53 status in the HER2 types **(c)**. However, in the TN type **(d)**, patients with p53 overexpression had marginally worse DFS in cases without chemotherapy (p=0.068), especially in node negative cases (p=0.064). However, there was no difference in DFS in the cases with chemotherapy (d-1).

that chemotherapy cannot improve the prognosis of the luminal type cases with p53 overexpression. However, chemotherapy can improve the DFS in the TN cases with p53 overexpression. These findings suggest that the p53 status in breast cancer should be reconsidered. Further case-control or prospective cohort studies are needed to confirm these findings.

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Authors Contribution

Conceived and designed the experiments: RN Analyzed the data: RN, NA, TO, YO, MN and MF.

Contributed reagents/materials/analysis tools: RN, NA, TO, YO, MN and MF.

Wrote the manuscript: RN.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Kumamoto Shinto General Hospital (number: 2019-J06-007). All patients gave a written informed consent to participate in the study.

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