Definition of p53 Overexpression and its Association with the Clinicopathological Features in Luminal/HER2-negative Breast Cancer

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Abstract. *Aims: A suitable cut-off value for p53 overexpression and its usefulness as a prognostic factor in luminal/HER2-negative breast cancer were evaluated. Patients and Methods: A retrospective analysis of 1,987 patients with luminal/HER2-negative breast cancer who underwent surgery between 2001 and 2009 was performed. Results: p53 expression ≥50% was present in 9% of the patients. Moreover, these patients had significantly lower estrogen/progesterone receptor-positive rates, higher Ki-67 values, larger tumors, disease-positive nodes, higher nuclear grade, and shorter disease-free survival than patients with p53 expression <50% (p<0.0001). Therefore, status of p53-positive cells ≥50% was classified as p53 overexpression. These findings indicate that p53 overexpression is associated with unfavorable characteristics and prognosis. Conclusion: The suitable cut-off value for p53 overexpression was determined to be 50%, and p53 overexpression appears to be a significant prognostic factor in patients with luminal/HER2-negative breast cancer.*

An immunopanel of estrogen receptor (ER), progesterone receptor (PgR), and HER2 is useful for the classification of breast cancer subtypes. Ki-67 has also become an important index to distinguish between luminal A and luminal B subtypes (1). Recently, p53 expression has also been proposed as a prognostic factor in breast cancer (2).

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Although p53 expression is associated with poor outcome in breast cancer, its utility as a prognostic marker has been controversial. To date, p53 assessment in breast cancer, especially in the most common type of hormone-sensitive breast cancer, has not been recommended for routine clinical use (3-5).

p53 expression status has been extensively used as a predictive factor for response to systemic therapy because tumor cells with non-functional p53 do not respond to systemic therapy due to a failure in apoptosis (3-10).

Many studies have reported that a suitable p53 cut-off point is 10% because *p53* mutations occur from this point (3-12). Furthermore, several other studies indicate that p53 overexpression is strongly correlated with *p53* mutation, because mutated p53 is more stable than wild-type p53 (13-16). To date, a suitable p53 cut-off point has not been established, therefore, the relationship of p53 with clinicopathological factors and prognosis of breast cancer remains largely unknown. Furthermore, the existing reports have included all types of breast cancer, and there are no reports, as far as we aware, that focused on just luminal/HER2-negative breast cancer.

In this study, we evaluated the suitable cut-off point for p53 and its association with clinicopathological and prognostic factors of breast cancer, in particular luminal/HER2-negative breast cancer.

Patients and Methods

Patients. A retrospective analysis of 2,873 patients with primary breast cancer who underwent surgery at our institution between 2001 and 2009 was performed. Among the 2,873 cases, 2,150 cases had tumors with luminal-type (1,113 cases: HER2-negative, low Ki-67 type; 874 cases: HER2-negative, high Ki-67 type; and 163 cases: HER2-positive), 275 cases had HER2-enriched, and 444 cases had triple-negative (TN) breast cancer, respectively. Luminal-type breast cancer was defined as that having ≥1% ER-positive rate regardless of PgR status. Expression of p53, HER2, Ki-67, and ER/PgR were determined using immunohistochemistry.

The characteristics of the 1,987 luminal/HER2-negative breast cancer cases are shown in Table I. Patient age ranged from 26 to 95 years (mean=56 years) and tumor diameter ranged from 0.1 to 17 cm (mean=2.0 cm). ER- and PgR-positive rates were 100% and 81.0%, respectively. A p53 expression rate of 50% or more was present in 9% of the patients.

The present study was approved by the Ethics Committee of Kumamoto City Hospital, and informed consent was obtained from all patients.

Histopathological examination. Histopathological examination was performed by two pathologists (T.Y. and A.N.) to determine the presence of lymph node metastasis, nuclear grade, ER/PgR status, Ki-67 labeling index, HER2 status, and p53 expression. ER, PgR, p53, Ki-67, and HER2 expression were determined using immunostaining as described previously (2, 8, 13). Nuclear grade was defined as an evaluation of the size and shape of the nucleus in tumor cells and the percentage of tumor cells that are in the process of dividing or growing. Cancers with low nuclear grade grow and spread less quickly than cancers with high nuclear grade. A value of ≥1% ER-positive cells was defined as ER-positivity. Ki-67 immunostaining (clone MIB-1; Dako Glostrup, Denmark) was used to assess proliferation rate. The fraction of proliferating cells was based on a count of at least 500 tumor cells. Ki-67 values were expressed as the percentage of positive cells in each case. Cases with ≥20% Ki-67 index were defined as positive and cases with <20% Ki-67 index were defined as negative (8,17). The expression of p53 and HER2 was immunohistochemically evaluated (LSAB method) using a mouse monoclonal antibody to p53 (Clone DO7; Dako) and the HercepTest (Dako), respectively. p53 values were expressed as the percentage of positive cells in each case and then the p53 staining pattern was divided into three groups: 2+ (homogeneous and diffuse staining, ≥50% of cancer cells), 1+ (heterogeneous or focal staining, 10 to 49% of cancer cells), and negative (focal staining, <10% of cancer cells) (Figure 1). The HER2 staining pattern was divided into four groups: 3+ (strong and diffuse staining ≥30%), 2+ (moderate and diffuse staining), 1+ (focal staining), and negative. HER2-positive cases were defined as 3+ or 2+ with FISH amplification ratio ≥2.2, and HER2-negative cases were defined as these found to be negative, 1+, or 2+ with FISH amplification ratio <2.2.

Adjuvant therapy. Most of the cases (88%) with luminal/HER2-negative tumors received endocrine therapy. One-fourth of the patients with luminal/HER2-negative tumors received chemotherapy and 22% received both endocrine and chemotherapy. On the other hand, most of the cases with TN and HER2-enriched tumors were treated with chemotherapy. About 60% of those with luminal/HER2-positive tumors were treated with chemotherapy. Anti-HER2 therapy with trastuzumab has been used in Japan since receiving approval in 2008 (Table I).

Statistical analysis. The mean values were analyzed using Student's *t*-test and analysis of variance (ANOVA). The significant differences of clinicopathological factors between two groups or among three groups were evaluated using the χ^2 test and Fisher's exact test. A two-sided *p*-value of <0.05 was considered statistically significant. The statistical analyses were performed with the SPSS version 18.0 statistical package. The Kaplan Meier test was used to calculate prognosis [cumulative disease-free survival (DFS) rate] and was tested with the log-rank procedure. The median observation period was 66 months from the start of treatment. Cox proportional hazards regression was used to calculate hazard ratios (HRs) and their associated 95% confidence intervals (95% CI) to estimate the suitable p53 cut-off point. Tumor size, nodal status, nuclear grade, ER and PgR-positive rates, HER2, Ki-67 labeling indices, 10% and 50% of p53 cut-off points were included in multivariate analysis.

Table I. *The characteristics of breast cancer cases*.

	All breast cancer 2,873 cases	Luminal/ HER2-negative 1,987 cases
Age (years)	56.3±12.9 (26–95)*	56.3±13.2 (26–95)*
Tumor size (cm)	2.21±1.69 (0.1–20.0)*	2.07±1.51 (0.1–17.0)*
Menopause		
Pre	1136 (40%)	829 (41%)
Post	1717	1149
Nodal status		
+	947 (33%)	833 (42%)
–	1849	1086
Nuclear grade		
3	498 (17%)	138 (7%)
1,2	2298	1798
ER		
+	2154 (75%)	1987 (100%)
–	719	0
PgR		
+	1747 (61%)	1618 (81%)
–	1124	369
HER2		
+	440 (15%)	0 (0 %)
–	2433	1987
Ki-67		
≥20%	1626 (57%)	874 (44%)
≤19%	1244	1114
p53		
≥50%	604 (21%)	174 (9%)
10%-49%	742	604
<10%	1523	1209
Surgery		
Bp	1681 (59%)	1210 (61%)
Bt	1092	708
Adjuvant therapy		
Hormonal only	558 (19%)	1302 (66%)
Hormonal + chemotherapy	1371	448
Chemotherapy	561	66
None	262	104

Bp, Breast-conserving surgery; Bt, mastectomy. *mean±SD (range).

Results

p53 Distribution. The distribution of p53 expression in 2,873 primary breast cancer cases had two peaks (0% to 20% and 70% to 100%). As shown in Figure 2 (left), tumors in most

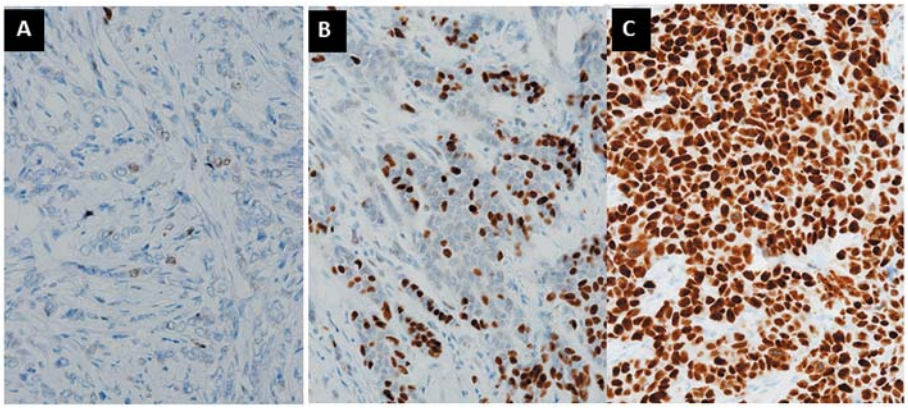

Figure 1. *Staining pattern of p53 protein. A: Negative (focal staining, fewer than 10% of cancer cells). B: 1+ (Heterogeneous or focal staining, 10 to 49% of cancer cells). C: 2+ (Homogeneous and diffuse staining, 50% or more of cancer cells).*

Table II. *Relationship of p53 expression (< 50% vs. ≥50%) with ER/PgR positivity and Ki-67 index.*

Breast cancer	Factor	p53 expression			R	*p*-Value
		<10%	10~49%	≥50%		
All	ER-positive rate (%)	84.3	78.6	24.5	–0.510	<0.0001
	PgR-positive rate (%)	65.8	62.5	21.7	–0.345	<0.0001
	Ki67-index	13.7	16.2	32.8	0.441	<0.0001
Luminal/HER2-negative	ER-positive rate (%)	85.6	86.9	75.9	–0.063	<0.0001
	PgR-positive rate (%)	54.5	59.1	38.5	–0.055	<0.0001
	Ki67-index	18.0	23.4	37.7	0.345	<0.0001

R, Correlation coefficient.

patients had a p53 expression of 1% to 10%. The proportions of patients with ≥10% and ≥50% p53 expression rates were 47% and 21%, respectively. Therefore, p53 expression was divided into the following three groups: <10%, 10% to 49%, and ≥50%.

The distribution of p53 expression in 1,987 luminal/HER2-negative breast cancer cases is also shown in Figure 2 (right), tumors in most patients had a p53 expression of <10%. The proportions of patients with ≥10% and ≥50% p53 expression rates were 30% and 9%, respectively. Most patients with luminal/HER2-negative breast cancer had tumor p53 expression <10%.

Cut-off point for p53 expression. Tumors in patients with ≥50% p53 expression had significantly lower ER/PgR-positive rates and higher Ki-67 labeling indices than those with <50% p53 expression, in the group overall and for those with luminal/HER2-negative breast cancer ($p<0.0001$; Table II). Furthermore, DFS was significantly shorter in patients with ≥50% p53 expression than in patients with <50% p53 expression in all patients and in those with luminal/HER2-negative breast cancer ($p<0.0001$; Figure 3a and b). The best cut-off point with the lowest *p*-value and highest HR was p53 expression of 50% in both the whole patients group and those with luminal/HER2-negative breast cancer (Table III). Therefore, the suitable cut-off point of p53 was found to be 50%, and we defined ≥50% p53 expression as p53 overexpression.

Clinicopathological factors and p53 overexpression. p53 overexpression was present in 9% of the patients with luminal/HER2-negative breast cancer. Table IV shows the relationship between p53 overexpression and clinicopathological factors in luminal/HER2-negative breast cancer. p53 overexpression was significantly correlated with larger tumors, disease-positive lymph nodes, higher nuclear grade, negative ER/PgR status and higher Ki-67 index ($p<0.0001$; Table IV).

p53 overexpression and prognosis of luminal/HER2-negative breast cancer. p53 overexpression was associated with a poorer prognosis in patients with luminal/HER2-negative

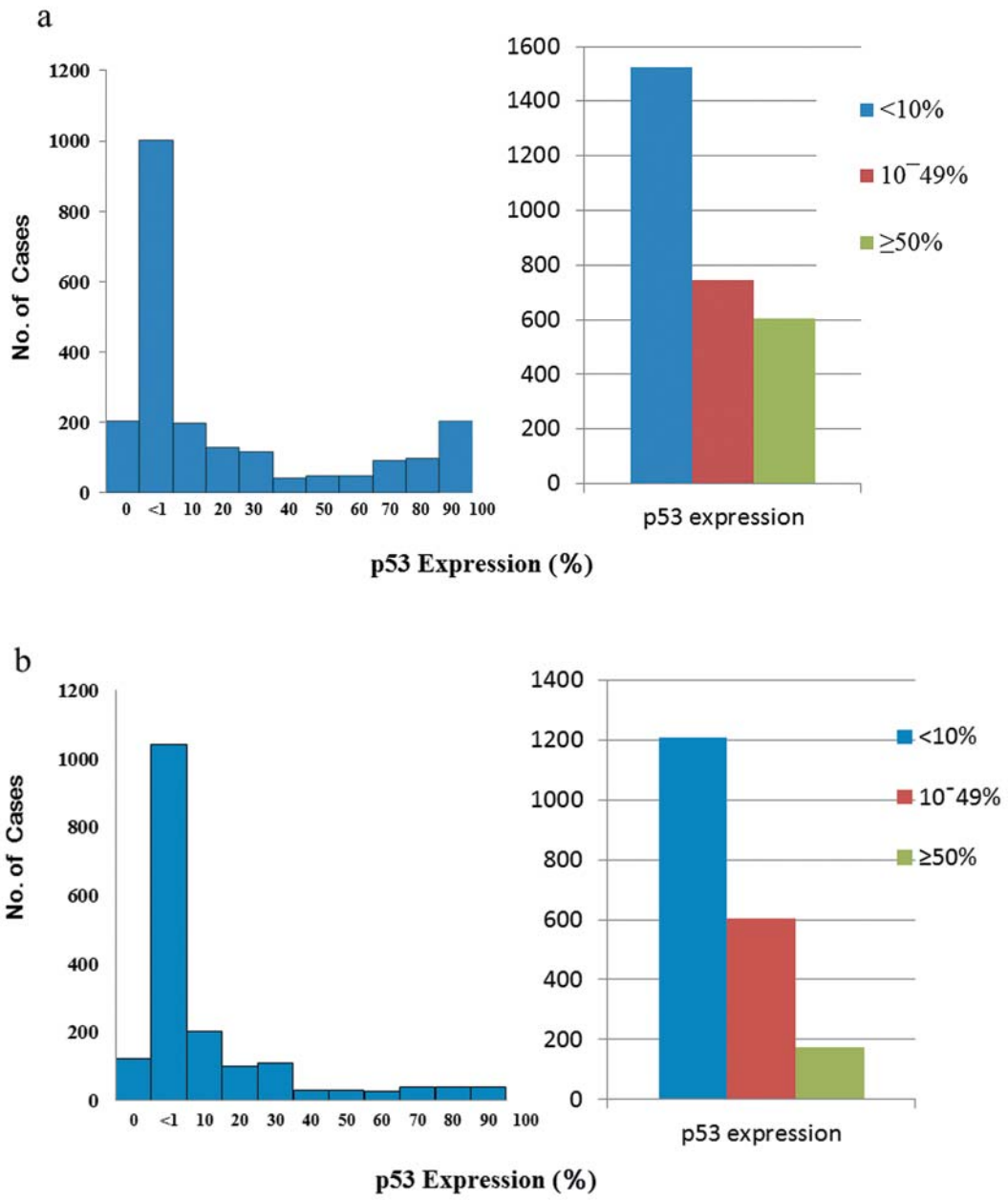

Figure 2. *The distribution of patients according to p53 expression for all breast cancer (a) and for luminal HER2-negative breast cancer (b) Right of each graph, the p53 expression divided into the three expression groups is shown. a: The distribution of p53 expression in 2,873 primary breast cancer cases had two peaks (0% to 20% and 70% to 100%). Most patients had tumor p53 expression of 1% to 10%. The proportions of patients with ≥10% and ≥50% p53 expression rates were 47% and 21%, respectively. b: The distribution of p53 expression in 1,987 luminal/HER2-negative breast cancer cases is shown. Most patients had a p53 expression of <10%. The proportion of patients with p53 expression rates of ≥10% and ≥50% were 30% and 9%, respectively. Most patients with luminal/HER2-negative breast cancer had a p53 expression of <10%.*

breast cancer. DFS was longer in patients with p53-negative tumors than those with p53-positive tumors (Figure 3). Patients with p53 overexpression had significantly lower (p<0.0001) DFS than those with negative p53 expression (Figure 3c).

Discussion

This study included almost 2,000 cases of luminal/HER2 negative-type breast cancer at a single institution to determine a suitable p53 cut-off point. Moreover, the relationship

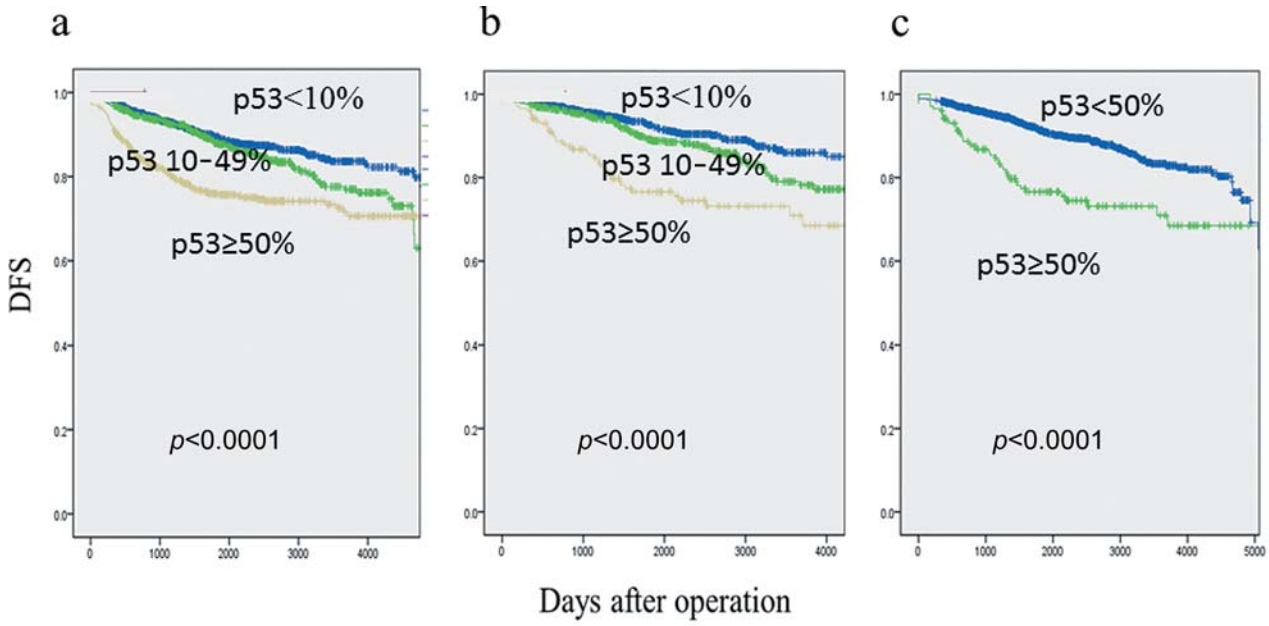

Figure 3. *Disease-free survival (DFS) in relation to p53 expression. DFS was significantly shorter in patients with p53 expression ≥50% than in patients with p53 expression <10% and 10–49% in breast cancer overall (a) and luminal HER2- negative breast cancer (b) (p<0.0001). (c) DFS in relation to p53 overexpression in luminal HER2-negative breast cancer. Patients with p53 expression ≥50% had significantly lower (p<0.0001) DFS than did patients with p53 expression of <50%.*

Table III. *Uni- and multivariate analysis for DFS using different p53 cut-off points.*

Breast cancer	p53 Cut-off point	Univariate analysis			Multivariate analysis		
		HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Whole	10%	1.70	1.40–2.07	<0.0001	1.30	1.03–1.66	0.029
	50%	2.05	1.67–2.51	<0.0001	1.74	1.36–2.23	<0.0001
Luminal/HER2 negative-type	10%	0.58	0.44–0.75	<0.0001	0.68	0.51–0.91	0.009
	50%	2.28	1.63–3.20	<0.0001	1.81	1.25–2.63	0.002

HR, Hazard ratio; CI, Confidence interval.

between p53 overexpression and clinicopathological factors that reflect prognosis were investigated.

Our data show that the suitable cut-off point for p53 was 50% in luminal/HER2-negative breast cancer, which was higher than previously reported (3-12). Based on our data, the p53 cut-off point of 50% was reasonable because p53 expression ≥50% was significantly associated with lower ER/PgR-positive rates and higher Ki-67 indices. Furthermore, DFS was significantly shorter in patients with p53 expression ≥50% than in patients with p53 expression <50%. p53 expression ≥50% was significantly correlated with larger tumors, disease positive nodes, higher nuclear grade, negative ER/PgR status and higher Ki-67. Using the p53 cut-off point of 50%, we found that p53 overexpression was associated with a poorer prognosis in patients with luminal/HER2-negative breast cancer.

We also investigated the role of p53 overexpression in each subtype of breast cancer. p53 overexpression plays an important role in luminal/HER2-negative and TN breast cancer, however, it does not in luminal/HER2-positive and HER2-enriched breast cancer. Furthermore, the distribution of p53 expression differs for each subtype. Most patients with luminal/HER2-negative breast cancer had a p53 expression <10%, while those with luminal/HER2-positive, HER2-enriched, TN breast cancer had two peaks of p53 expression <10% and ≥50% (data not shown).

Many studies use a p53 cut-off point of 10% based on immunohistochemical evaluation because of p53 mutations

Table IV. *p53 overexpression and clinicopathological factors in luminal/HER2-negative breast cancer.*

Factor	p53 overexpression		*p*-Value
	No (1813 cases)	Yes (174 cases)	
Tumor size (cm)	2.0±1.5 (0–15)	2.5±1.9 (0–17)	<0.001
Age (years)	56.3±13.3 (26–95)	56.2±12.3 (28–95)	0.919
Nodal status			
–	1208	93	
+	557 (31%)	76 (45%)	<0.0001
Nuclear grade			
1,2	1670	126	
3	94 (5%)	44 (26%)	<0.0001
ER			
–	0	0	
+	1813 (100%)	174 (100%)	*
PgR			
–	318	51	
+	1493 (82%)	123 (71%)	<0.0001
Ki67			
≤19%	1081	32	
≥20%	732 (40%)	142 (82%)	<0.0001
Adjuvant			
None	100	4	
Chemotherapy	55	11	
Hormonal	1231	69	
Chemo+hormonal	365 (20%)	83 (48%)	<0.0001
Intrinsic subtype			
Luminal/HER2(–)	1813 (91%)	174 (9%)	
Luminal/HER2(+)	103 (63%)	60 (37%)	
HER2-enriched	136 (49%)	139 (51%)	
Triple-negative	213 (48%)	231 (52%)	<0.0001

*mean± SD (range); chemo, chemotherapy; hormone, hormone therapy.

(3-12). Other reports defined p53 overexpression using the immunoreactive score (IRS) which is the product of the staining intensity and the percentage of positive cells (18). Marks *et al*. reported that p53 positivity was defined as a single malignant breast epithelial cell with positive nuclear staining for p53 (19). Martinazzi *et al*. reported that some nuclei with mutant p53 protein staining were considered positive (20).

Lara *et al*. reported that p53 expression based on a 10% cut-off point was not significantly associated with tumor size, ER status, or the number of positive nodes (11). Furthermore, p53 expression based on a 10% cut-off point was significantly associated with overall survival (OS) but not relapse-free survival. Martinazzi *et al*. reported that p53 expression, as determined by positive staining for mutant p53, was significantly correlated with negative ER/PgR status, large tumor size, high Ki-67, and high HER2 expression (20); however, the relationship between p53 expression and prognosis was not reported. Marks *et al*. found that p53 expression, defined as a single cancer cell with positive p53 staining, was significantly correlated with large tumor size and negative ER/PgR status, and was a prognostic indicator of OS and failure-free survival in early-stage breast cancer (19). However, these reports included all types of breast cancer, so we focused on luminal/HER2-negative breast cancer in this study. p53 expression was a significant prognostic factor in the present study when the ≥50% of p53 cut-off point was used instead of the previous cut-off point of 10% (3-12).

In our study, the p53 overexpression rate in luminal/HER2-negative breast cancer and in the breast cancer overall was 9% and 21%, respectively, using a p53 cut-off point of 50%. On the other hand, in the studies using a p53 cut-off point of 10%, the p53 overexpression rates were 15.5% (12) and 23 to 27% (11). Other p53 cut-off points were reported to be 13% (20) and 24%(19). Coutant *et al*. reported that the p53 mutation rate was 18.3% in patients with ER-positive tumor (21). Recently, Perou *et al*. reported that the p53 mutation rate was 12%, 29%, and 37% in luminal A, luminal B, and all subtypes of breast cancers, respectively (22). Furthermore, in our study, patients with p53 expression ≥50% had lower ($p<0.0001$) DFS than those with p53 expression ≥10% using Cox proportional hazards regression analysis. Therefore, we consider our 50% of p53 cut-off point to be reasonable because our p53 overexpression rate is in agreement with that of previous reports (11, 12, 19, 20) and the recent report by Perou *et al*. (22). In addition, a p53 cut-off point of 50% is reasonable, because this threshold defines more clearly the association of p53 with the clinicopathological features and prognosis of breast cancer (8, 13-16).

Several studies indicate that p53 overexpression is strongly correlated with *p53* mutation, because mutated p53 is more stable than wild-type p53 (13-16). Mutant p53 not only influences cell cycle checkpoint controls and apoptosis but also acquires new functions to drive cell migration, invasion, and metastasis (23). Therefore, the high expression of p53 in our study may reflect *p53* gene mutations more precisely.

A prognostic significance of p53 in breast cancer has been reported in several studies (3-6). Hasebe *et al*. reported that p53 expression is an important predictor of outcome in patients with invasive ductal carcinoma of the breast (3). Guarneri *et al*. also reported that p53 is a negative prognostic marker for DFS and OS in patients with Stage II or Stage III breast cancer (4). Kim *et al*. found that p53 overexpression is a poor prognostic factor in breast cancer patients treated with endocrine therapy and chemotherapy (5). Recently, Coutant *et al*. reported that p53 dysfunction is a predictor of poor clinical outcome in ER-positive cancers and a predictor of better prognosis in ER-negative cancers (21). Previous reports did not examine the relationship between p53 expression and ER-negative breast cancer.

In this study, we found that p53 overexpression, using a cut-off point of 50%, was a significant prognostic factor in luminal/HER2-negative breast cancer. Therefore, inclusion of p53 in breast cancer immunopanels would improve the prognostic evaluation of luminal/HER2-negative breast cancer.

Conflicts of Interest

The Authors made no disclosures and there are no conflict of interest.

References

1 Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes – dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol *22*: 1736-1747, 2011.

2 Millar EK, Graham PH, McNeil CM, Browne L, O'Toole SA, Boulghourjian A, Kearsley JH, Papadatos G, Delaney G, Fox C, Nasser E, Capp A and Sutherland RL: Prediction of outcome of early ER+ breast cancer is improved using a biomarker panel, which includes Ki-67 and p53. Br J Cancer *105*: 272-280, 2011.

3 Hasebe T, Iwasaki M, Akashi-Tanaka S, Hojo T, Shibata T, Sasajima Y, Kinoshita T and Tsuda H: p53 expression in tumor-stromal fibroblasts forming and not forming fibrotic foci in invasive ductal carcinoma of the breast. Mod Pathol *23*: 662-672, 2010.

4 Guarneri V, Barbieri E, Piacentini F, Giovannelli S, Ficarra G, Frassoldati A, Maiorana A, D'Amico R and Conte P: Predictive and prognostic role of p53 according to tumor phenotype in breast cancer patients treated with preop. erative chemotherapy: a single-institution analysis. Int J Biol Markers *25*: 104-111, 2010.

5 Kim HS, Yom CK, Kim HJ, Lee JW, Sohn JH, Kim JH, Park YL and Ahn SH: Overexpression of p53 is correlated with poor outcome in premenopausal women with breast cancer treated with tamoxifen after chemotherapy. Breast Cancer Res Treat *121*: 777-788, 2010.

6 Yamashita H, Toyama T, Nishio M, Ando Y, Hamaguchi M, Zhang Z, Kobayashi S, Fujii Y and Iwase H: p53 protein accumulation predicts resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer. Breast Cancer Res *8*: R48, 2006.

7 Berns EM, Foekens JA, Vossen R, Look MP, Devilee P, Henzen-Logmans SC, van Staveren IL, van Putten WL, Inganäs M, Meijer-van Gelder ME, Cornelisse C, Claassen CJ, Portengen H, Bakker B and Klijn JG: Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res *60*: 2155-2162, 2000.

8 Kai K, Nishimura R, Arima N, Miyayama H and Iwase H: p53 expression status is a significant molecular marker in predicting the time to endocrine therapy failure in recurrent breast cancer: a cohort study. Int J Clin Oncol *11*: 426-433, 2006.

9 Di Leo A, Tanner M, Desmedt C, Paesmans M, Cardoso F, Durbecq V, Chan S, Perren T, Aapro M, Sotiriou C, Piccart MJ, Larsimont D, Isola J; TAX 303 translational study team. p-53 gene mutations as a predictive marker in a population of advanced breast cancer patients randomly treated with doxorubicin or docetaxel in the context of a phase III clinical trial. Ann Oncol *18*: 997-1003, 2007.

10 Kandioler-Eckersberger D, Ludwig C, Rudas M, Kappel S, Janschek E, Wenzel C, Schlagbauer-Wadl H, Mittlböck M, Gnant M, Steger G and Jakesz R: TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. Clin Cancer Res *6*: 50-56, 2000.

11 Lara JF, Thor AD, Dressler LG, Broadwater G, Bleiweiss IJ, Edgerton S, Cowan D, Goldstein LJ, Martino S, Ingle JN, Henderson IC, Norton L, Winer EP, Hudis CA, Ellis MJ, Berry DA, Hayes DF; Cancer and Leukemia Group B. p53 Expression in node-positive breast cancer patients: results from the Cancer and Leukemia Group B 9344 Trial (159905). Clin Cancer Res *17*: 5170-5178, 2011.

12 Cattoretti G, Rilke F, Andreola S, D'Amato L and Delia D: P53 expression in breast cancer. Int J Cancer *41*: 178-183, 1988.

13 Bullock AN, Henckel J and Fersht AR: Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: definition of mutant states for rescue in cancer therapy. Oncogene *19*: 1245-1256, 2000.

14 Bullock AN and Fersht AR: Rescuing the function of mutant p53. Nat Rev Cancer *1*: 68-76, 2001.

15 Rodrigues NR, Rowan A, Smith ME, Kerr IB, Bodmer WF, Gannon JV and Lane DP: p53 mutations in colorectal cancer. Proc Natl Acad Sci USA *87*: 7555-7559, 1990.

16 Kaserer K, Schmaus J, Bethge U, Migschitz B, Fasching S, Walch A, Herbst F, Teleky B and Wrba F: Staining patterns of p53 immunohistochemistry and their biological significance in colorectal cancer. J Pathol *190*: 450-456, 2000.

17 Tashima R, Nishimura R and Arima N: Evaluation of an optimal cut-off point for the Ki-67 index as a prognostic factor in primary breast cancer. CTRC-AACR SAN ANTONIO BREAST CANCER SYMPOSIUM. San Antonio. 2012.

18 Friedrichs K, Gluba S, Eidtmann H and Jonat W: Overexpression of p53 and prognosis in breast cancer. Cancer *72*: 3641-3647, 1993.

19 Marks JR, Humphrey PA, Wu K, Berry D, Bandarenko N, Kerns BJ and Iglehart JD: Overexpression of p53 and HER-2/neu proteins as prognostic markers in early stage breast cancer. Ann Surg *219*: 332-341, 1994.

20 Martinazzi M, Crivelli F, Zampatti C and Martinazzi S: Relationship between p53 expression and other prognostic factors in human breast carcinoma. An immunohistochemical study. Am J Clin Pathol *100*: 213-217, 1993.

21 Coutant C, Rouzier R, Qi Y, Lehmann-Che J, Bianchini G, Iwamoto T, Hortobagyi GN, Symmans WF, Uzan S, Andre F, de Thé H and Pusztai L: Distinct p53 gene signatures are needed to predict prognosis and response to chemotherapy in ER-positive and ER-negative breast cancers. Clin Cancer Res *17*: 2591-2601, 2011.

22 Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature *490*: 61-70, 2012.

23 Muller PA, Vousden KH and Norman JC: p53 and its mutants in tumor cell migration and invasion. J Cell Biol *192*: 209-218, 2011.

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