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P53 Overexpression in Ductal Carcinoma *in situ* of the Breast

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Abstract:

Background: Ductal carcinoma *in situ* (DCIS) is the most common type of non-invasive breast cancer. DCIS is not life threatening but can increase the risk of invasive ductal carcinoma (IDC) development. However, the role of P53 expression in the progression to invasiveness in DCIS is unclear. In this retrospective study, the differences in biological markers, including protein 53 (P53) expression, between DCIS and IDC ≤5 mm in diameter (T1a) were evaluated.

Methods: Among 2,497 primary breast cancer patients, who underwent initial surgery between 2001 and 2010, 267 (10.4%) patients were diagnosed with DCIS and 86 (3.4%) patients were diagnosed with T1a. P53 expression was evaluated using immunohistochemical analysis, and the distribution (<10%, <50%, and ≥50%) and the association with other biological markers was investigated in DCIS and T1a.

Results: Overexpression (≥ 50%) of P53 was seen in 13% of patients with DCIS and in 24% of patients with T1a, constituting a significant difference (p=0.001). P53 overexpression was significantly associated with higher nuclear grade, lower rates of estrogen receptor (ER)/progesterone receptor (PgR) positivity, higher rates of epidermal growth factor receptor 2 (HER2) positivity, and higher values of nuclear protein Ki-67 in patients with DCIS. Recurrence rates did not differ significantly between DCIS and T1a. Logistic regression analysis of factors associated with invasiveness revealed that P53 overexpression was one of the significant factors, and ER negativity was found to be a significant independent factor in multivariate analysis.

Conclusion: P53 overexpression reflected the aggressiveness and correlated with the invasiveness in DCIS. These findings suggest that P53 may play an important role in invasion of DCIS in the breast.

Keywords: P53 overexpression; DCIS; Invasiveness

Introduction

Ductal carcinoma *in situ* (DCIS) is the most common type of non-invasive breast cancer, in which cancer cells do not grow beyond the basal membrane of the duct [1]. DCIS is associated with a low risk of recurrence; however, it has been considered a precursor of invasive ductal carcinoma (IDC). Since the introduction of mammography in breast cancer screening, increasing numbers of cases of DCIS are now being identified and the frequency has increased to 15–20% [2]. In patients treated with breast-conserving surgery, half of the DCIS cases with ipsilateral breast tumor recurrence (IBTR) were observed to have invasive components. Moreover, a few cases with DCIS had distant metastases [3-6]. It is still unclear how to differentiate cases of DCIS with high risk of recurrence from those cases with originally low risk of recurrence. Histopathological factors [7,8] including nuclear grade, the presence of necrosis, tumor size, and surgical margin status were reported to be related to the risk of IBTR in DCIS. Surgical margin status was considered the most important factor for IBTR among patients with DCIS treated with breast-conserving surgery [8]. In our institute, intraoperative pathological examination is performed during primary surgery, to monitor surgical margin status with an aim to

achieve negative margin status. This system eliminates the risk of leaving cancer cells behind during primary surgery. Furthermore, several biological markers including human epidermal growth factor-2 (HER2), estrogen receptor (ER), Ki-67, and P53 have also been reported to be related to the risk of IBTR in DCIS [4-6,9-11].

Among these biological markers, P53 overexpression was introduced as a predictor of IBTR in patients with DCIS [9,12]. P53 has been widely applied as a prognostic marker in invasive breast cancer, and its overexpression, 70% or 80% of them reported as mutant, has been correlated with poor prognosis [13-16]. Recently, Muller et al. reported that mutant p53 could acquire new functions to drive cell migration and invasion [17]. However, few reports have investigated the role of P53 expression in DCIS of the breast [3,10,18]. The role of P53 overexpression remains unclear in DCIS of the breast. To date, several reports have described the biomarker profiles of patients with DCIS and the correlations among markers such as HER2, Ki-67, P53, and hormone receptors [3,10,18].

In this study, we evaluated the clinicopathological significance of P53 for local invasion in DCIS and the use of several biomarkers in detecting DCIS associated with a high risk of recurrence.

Materials and Methods

Patients

Among 2,497 primary breast cancer patients who underwent initial surgery between 2001 and 2010, 267 patients (10.4%) with DCIS and 86 (3.4%) patients with IDC ≤ 5 mm in diameter (T1a) were enrolled in this retrospective study. The age of the patients ranged from 25 to 86 years (mean, 54.0 years) in patients with DCIS and from 30 to 80 years (mean, 56.0 years) in patients with T1a. Of the 267 patients with DCIS and the 86 patients with T1a, 228 (85%) and 57 (66%) patients were ER-positive, 210 (79%) and 48 (56%) patients were progesterone receptor (PgR)-positive, 51 (19%) and 32 (37%) patients were HER2-positive, and 71 (27%) and 39 (45%) cases were Ki-67-positive (≥ 20%), respectively. Of the cases of P53 overexpression, 35 (13%) were of DCIS and 21 (24%) were of T1a. Breast-conserving surgeries were performed for 160 patients with DCIS (60%) and 56 patients with T1a (65%). All the cases received postoperative radiotherapy (Table 1).

We used to take the pharmacological anamnesis of the patients for standard therapy of breast cancer before surgical removal, such as the history of other cancer, concomitant medical disease like diabetes mellitus or asthma.

Treatment and follow-up

For patients with IDC, postsurgical follow-up examinations were performed every 3 months until 3 years after surgery, every 3–6 months until 3–5 years after surgery, and every 6–12 months up to 5–10 years after surgery. Once a year, the patients underwent chest radiography, mammography, tumor marker tests, and abdominal ultrasonography; computed tomography was performed after consultation with the patients. In cases of DCIS, the patients were followed-up for 10 years with annual mammography.

Histopathological examination

Histopathological examination was performed by 2 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 [13-15,16,19]. ER and PgR positivity was defined as the presence of ≥1% positive cells. Ki-67 immunostaining (clone MIB-1; Dako, Glostrup, Denmark) was used to assess the proliferation rate. The fraction of proliferating cells was based on a count of at least 500 tumor cells in the area including the hot spot. Ki-67 values were expressed as the percentage of positive cells in each case. Cases with a Ki-67 index ≥ 20% were defined as positive, and cases with a Ki-67 index <20% were defined as negative. The expression of P53 and HER2 was evaluated with immunohistochemical analysis (labeled streptavidin-biotin method) by using a mouse monoclonal anti-P53 antibody (clone DO7; Dako) and the rabbit monoclonal antibody (4B5, Ventana), respectively. The P53 staining pattern was classified into 3 groups: 2+ (homogeneous and diffuse staining, ≥50% of cancer cells), 1+ (heterogeneous or focal staining, 10–49% of cancer cells), and negative (focal staining, <10% of cancer cells) (Figure 1). P53 overexpression was defined as P53 expression in ≥ 50% of cancer cells [14]. The HER2 staining pattern was divided into 4 groups: 3+, strong and diffuse staining in ≥ 30% of cancer cells; 2+, moderate and diffuse staining; 1+, focal staining; and negative. HER2-positive cases were defined as 3+ or 2+ with a fluorescence *in situ* hybridization (FISH)

amplification ratio ≥ 2.2, and HER2-negative cases were defined as negative, 1+, or 2+ with a FISH amplification ratio <2.2. The association between surgical margin status and P53 overexpression was also investigated. Positive surgical margin status was defined as cancer cells ≤ 5 mm from the edge of the excised specimen.

Factors	Category	DCIS 267 cases	T1a 86 cases	Pvalue (χ2 test)
Age (years) (range)		56.9 ± 13.7 (25~87)	58.4 ± 12.8 (32~84)	
Menopausal status	pre	100 (38%)	32 (37%)	
	post	164	54	
Nuclear grade	3	19 (7%)	13 (15%)	0.001
	1,2	239	72	
ER	+	228 (85%)	57 (66%)	0.001
	-	37	28	
PgR	+	210 (79%)	48 (56%)	<0.0001
	-	55	37	
HER2	+	51 (19%)	32 (37%)	0.006
	-	213	54	
Ki-67	+	71 (27%)	39 (45%)	0.002
	-	193	47	
p53 overexpression	+	35 (13%)	21 (24%)	0.001
	-	228	65	
Surgery	Bp	160 (60%)	56 (65%)	0.222
	Bt	103	28	
	Unknown	4	2	
Adjuvant therapy	None	182 (68%)	34 (40%)	<0.0001
	Chemotherapy	7	9	
	Endocrine therapy	72 (27%)	39 (45%)	
	Chemo-endocrine therapy	3	3	
Recurrence	with	3*	1*	
site	Ipsilateral breast	3*	1*	

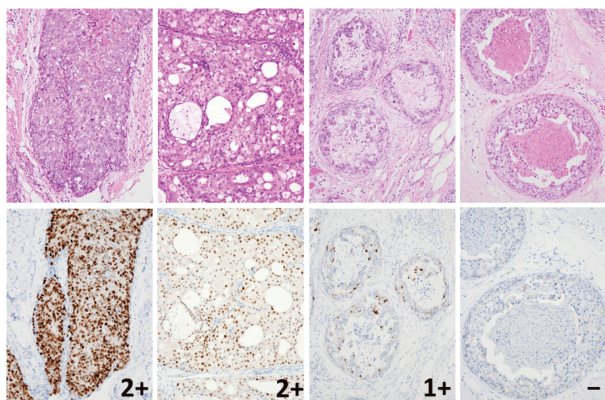**Table 1:** Background clinicopathological factors in patients with IDC (T1a) and DCIS.

Figure 1a: H.E.staining and P53 immunostaining pattern in DCIS. (upper) H.E.staining (lower) P53 staining corresponding to upper H.E.staining. The P53 immunostaining pattern was classified into 3 groups: 2+ (homogeneous and diffuse staining, ≥ 50% of cancer cells), 1+ (heterogeneous or focal staining, 10–49% of cancer cells), and negative (focal staining, <10% of cancer cells).
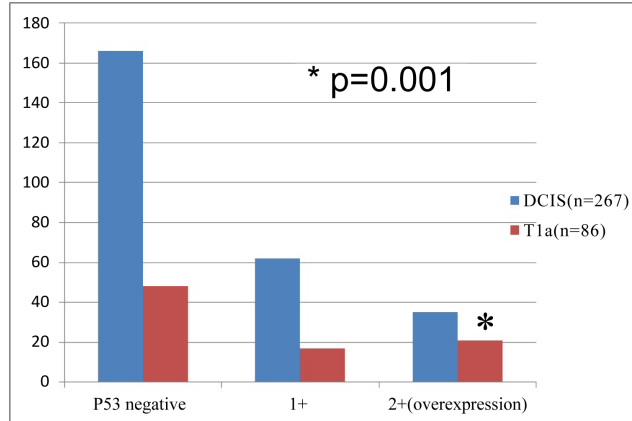

Figure 1b: P53 distribution in patients with DCI and T1a. P53 negativity, 1+ expression, and 2+ expression (overexpression) were observed in 166 cases (62%), 62 cases (23%), and 35 cases (13%) of patients with DCIS and in 48 cases (56%), 17 cases (20%), and 21 cases (24%) of patients with T1a, respectively. The P53 overexpression rate was significantly higher in patients with T1a (p=0.001).

Statistical analysis

The mean values were analyzed using the Student t-test and analysis of variance. Differences in clinicopathological factors between 2 groups or among 3 groups were evaluated using the χ2 test and Fisher exact test. A 2-sided p value of <0.05 was considered statistically significant. Logistic regression analysis was used to estimate suitable factors for DCIS with invasion. Nuclear grade, ER-positive rate, HER2 status, Ki-67 index, and P53 overexpression were included in

multivariate analysis. Statistical analyses were performed using the SPSS version 16.0 statistical package. The median observation period was 75 months from the initiation of treatment.

Results

Distribution of p53 expression in DCIS and T1a

Distribution of P53 expression in DCIS and T1a was investigated. The cases with P53 negativity, 1+ expression, and 2+ expression (overexpression) were observed in 166 cases (62%), 62 cases (23%), and 35 cases (13%) of patients with DCIS and in 48 cases (56%), 17 cases (20%), and 21 cases (24%) of patients with T1a, respectively. The P53 overexpression rate was significantly higher inT1a (p=0.001).

Comparison of clinicopathological factors between IDC (T1a) and DCIS

A comparison of clinicopathological factors, including P53 expression, between IDC (T1a) and DCIS is shown in Table 1. Nuclear grade, HER2, Ki-67, and P53 overexpression were significantly higher in IDC (T1a) than in DCIS (p=0.001, p=0.006, p=0.002, and p=0.001, respectively), while ER and PgR were significantly lower in IDC (T1a) than in DCIS (p=0.001 and p<0.0001, respectively). Three cases of DCIS and 1 case of T1a showed recurrence. Moreover, all cases had IBTR with invasive components.

Multivariate analysis of factors for invasion in DCIS and T1a tumors revealed that nuclear grade, ER positivity rates, HER2 status, Ki-67 index, and P53 overexpression were significant in univariate analysis (p=0.033, p<0.0001, p=0.001, p=0.002, p=0.016, respectively). Among these factors, ER was the independent significant factor for invasiveness using multivariate analysis (p=0.041) (Table 2).

Factors	Category	Univariate analysis		Multivariate analysis	
		Odds ratio	Pvalue	Odds ratio	Pvalue
Nuclear grade	3 vs 2.1	0.440	0.033	1.107	0.825
ER	+ vs -	3.027	<0.0001	2.148	0.041
HER2	+ vs -	0.404	0.001	0.656	0.247
Ki-67	+ vs -	0.443	0.002	0.647	0.197
p53 overexpression	+ vs -	0.475	0.016	1.062	0.883

Table 2: Multivariate analysis of factors for invasion in DCIS and T1a tumors.

Clinicopathological factors and P53 overexpression in DCIS and T1a

Overexpression of P53 was observed in 13% of the patients with DCIS. The relationship between P53 overexpression and clinicopathological factors in patients with DCIS and T1a was also investigated (Table 3a and 3b). P53 overexpression was found to significantly correlate with higher nuclear grade, negative ER/PgR status, higher HER2 status, and higher Ki-67 index (p<0.0001).

Association between the surgical margin status and P53 overexpression

In the cases with DCIS, 160 patients (60%) received breast-conserving surgery. HER2 status, Ki-67 labeling index and P53 overexpression was significantly associated with positive surgical margin status, among them P53 overexpression was found to be an independent factor (p=0.002) (Table 4). However, there were no cases with exposed cancer cells.

Factors	Category	p53 expression			P value
		Negative (n=166)	1+ (n=62)	Overexpression (n=35)	
Age (years)	Mean ± SD (range)	58.5 ± 14.0 (25~87)	54.4 ± 13.1 (26~81)	58.3 ± 12.8 (32~84)	0.135
Nuclear grade	1,2	157	58	21	
	3	4	2	13 (38%)	<0.0001
ER	-	11	5	21 (60%)	
	+	155	57	14	<0.0001
PgR	-	19	10	26 (74%)	
	+	147	52	9	<0.0001
HER2	-	149	49	13	
	+	16	12	22 (63%)	<0.0001
Ki-67	-	141	44	8	
	+	25	18	24 (69%)	<0.0001
Surgery	Bp	90	42	26 (74%)	
	Bt	74	19	9 (26%)	0.219
Adjuvant therapy	None	113	41	26 (74%)	
	Chemotherapy	0	1	5	
	Endocrine therapy	49	19	4	
	Chemo-endocrine therapy	3	0	0	
Subtype	Luminal A-like	125	38	4 (11%)	
	Luminal B-like	18	10	6(17%)	
	HER2	3	3	18 (51%)	
	Luminal/ HER2	13	9	4 (11%)	
	Triple Negative	7	2	3 (9%)	

Table 3a: p53 expression and clinicopathological factors in patients with DCIS.

Factors	Category	p53 expression			P value
		Negative (n=48)	1+ (n=17)	Overexpression (n=21)	
Age (years)	Mean ± SD (range)	56.3 ± 12.4 (33~80)	59.6 ± 12.8 (44~80)	54.5 ± 12.3 (36~75)	0.454
Nuclear grade	1,2	43	14	15	
	3	4	3	6 (29%)	0.100
ER	-	9	4	15 (71%)	
	+	38	13	6	0.001
PgR	-	12	10	15 (71%)	
	+	35	7	6	0.004
HER2	-	36	10	8	
	+	12	7	13 (62%)	0.013
Ki-67	-	38	7	2	
	+	10	10	19 (91%)	<0.0001
Surgery	Bp	35	11	10 (48%)	
	Bt	12	6	10 (48%)	0.263
Adjuvant therapy	None	18	4	12 (57%)	
	Chemotherapy	1	3	5	
	Endocrine therapy	26	9	4	
	Chemo-endocrine therapy	2	1	0	
Subtype	Luminal A-like	27	6	0 (0%)	
	Luminal B-like	5	3	3(14%)	
	HER2	6	3	9 (43%)	
	Luminal/ HER2	6	4	4 (11%)	
	Triple Negative	7	2	3 (9%)	

Table 3b: p53 expression and clinicopathological factors in patients with T1a.

Discussion

In this study, 267 patients with DCIS were analyzed to evaluate the clinical significance of P53 expression. Of the 267 patients with DCIS, 3 patients (1.1%) had IBTR with invasive components. This recurrence rate was substantially lower than that in previous reports [20,21]. Surgical margin status was considered an important factor for local recurrence among patients with DCIS treated with breast-preserving surgery [8]. Moreover, half of the recurrent cases were reported to be invasive cancer [8]. The rates of positive surgical margins were higher

in patients with P53 overexpression. However, there were no cases with exposed cancer cells due to intraoperative examination of margin status. P53 status should be considered in the decision regarding the surgical procedure, whether there is a need to perform breast-conserving surgery.

Factors	Category	Univariate analysis		Multivariate analysis	
		Odds ratio	Pvalue	Odds ratio	Pvalue
Nuclear grade	3 vs 2.1	1.636	0.652	7.798	0.101
ER	+ vs -	1.832	0.295	0.085	0.100
PgR	+ vs -	2.234	0.121	2.049	0.572
HER2	+ vs -	0.358	0.034	0.329	0.112
Ki-67	+ vs -	0.296	0.007	0.532	0.271
P53 overexpression	+ vs -	0.155	<0.0001	0.077	0.002

Table 4: Univariate and Multivariate analysis for the surgical margin status among 160 (60%) patients with DCIS who received breast-conserving surgery.

Several studies have reported on the predictive markers for microinvasion in DCIS. Nofech-Mozes et al. [4,6] reported that HER2 was the predictive factor for DCIS microinvasion, and Okumura et al. [5] reported that survivin expression was an independent factor associated with invasion. In this study, the significant factors for invasiveness of DCIS were nuclear grade, ER and PgR, HER2 status, Ki-67 index, and P53 overexpression according to the results of the univariate analysis, while multivariate analysis revealed that ER negativity was an independent significant factor. Some investigators have reported that ER-positive breast cancer patients can experience loss of or a reduction in ERα, which is associated with P53 overexpression [22-24]. Furthermore, Dumpy reported that estrogen and progesterone induce persistent increases in p53-dependent apoptosis and suppress mammary tumors in BALB/c-Trp53+/- mice [25]. P53 overexpression was significantly associated with ER negativity in this study. P53 overexpression might correlate with DCIS invasion via the ER to some extent.

The vascular role of estrogens in different kind of vascular district was reported in several references [26-28]. Ciccone MM et al. reported that estrogen increased cerebral perfusions [26], reduced the vascular effects of isometric muscle contraction by modulating the vasomotor tone of peripheral vessels in relation to exercise [27], and increased ophthalmic artery perfusion in healthy postmenopausal women [28]. This is really important when considering the probability for DCIS breast cancer to give invasion or metastasis.

In terms of the rate of P53 overexpression, DCIS tumors had a positive rate of 13% and T1a had a positive rate of 24% using the cut-off point of 50%. Nofech-Mozes et al. [4] reported the rate of P53 overexpression in DCIS as 22%, and Rajan et al. [12] reported it to be18%, which were both higher than the rate found in this study. Several other studies have investigated the role of P53 overexpression in DCIS. To date, 4 studies have examined the prognostic value of biological markers in DCIS. Ringberg et al. [11] reported that high levels of Ki-67, P53, and bcl-2 are significantly (or almost significantly) associated with disease recurrence in DCIS. Perin et al. [10] found no

significant association between a wide variety of biological markers, including ER, PgR, HER2, and P53, and the rate of disease recurrence in 49 patients with DCIS who received mastectomy or breast-conserving surgery with or without radiotherapy. de Roos et al. [9] reported that P53 is a common predictor of local recurrence in DCIS. Rajan et al. [12] reported that P53 is associated with comedo status, high nuclear grade, and high mitotic index in DCIS, and therefore represents a new parameter to evaluate the cellular biology and prognosis of DCIS. The current study did not find any significant impact of P53 on survival because of the low rates of recurrence.

In conclusion, P53 overexpression reflected the aggressiveness and correlated with the invasiveness in DCIS. These findings suggest that P53 may play an important role in the invasion of DCIS in the breast.

Conclusion

The rate of P53 overexpression was significantly higher in IDC (T1a) than in DCIS, as revealed by the results of univariate analysis, and was significantly associated with positive surgical margin status. Patients with P53 overexpression had significantly lower ER/PgR-positive rates, higher nuclear grade, higher HER2 status, and higher Ki-67 labeling indices in DCIS.

A limitation of this retrospective study is researcher bias that may occur when selecting the patients and treatment method for analysis. In order to deal with this potential problem, multivariable analysis was utilized to control for any confounding variables.

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