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Expression of Cyclins in Ductal Hyperplasia, Atypical Ductal Hyperplasia and Ductal Carcinoma *in situ* of the Breast

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Abstract

Cyclin/cdc complexes are known to function in cell-cycle regulation. Cyclin D1/cdk4 and -6 complexes, which functions as a G1-S checkpoint and cyclin B1/cdc2 complexes, a G2-M checkpoint are essential for DNA synthesis and mitosis, respectively. Thus, dysregulated overexpression of cyclins appears to be involved in uncontrollable cell proliferation and early tumor development. We investigated the expression and proliferative index of cyclin D1 ($PI_{cyclin\ D1}$), cyclin B1 ($PI_{cyclin\ B1}$) and Ki-67 (PI_{Ki-67}) using immunohistochemical staining on 15 cases of ductal hyperplasia (DH), 26 cases of atypical ductal hyperplasia (ADH) and 43 cases of ductal carcinoma *in situ* (DCIS) of the breast in order to evaluate whether these cyclins are associated with abnormal cell proliferation and play a role in tumor development from ADH to carcinoma. Furthermore, we investigated whether the expression and proliferative index of the cyclins and Ki-67 are correlated with the histologic grade according to the Van Nuys classification and with the histologic subtype according to traditional classification. Finally, we estimated the correlation coefficient among $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$, PI_{Ki-67} and estrogen receptor in ADH and DCIS. The expression of cyclin D1 was detected in 39.5% of DCIS and 7.7% of ADH cases. In the DH cases, expression of cyclin D1 was not found. Expression of cyclin B1 was also detected in 69.7% of DCIS, 50.0% of ADH and 93.3% of the DH cases. The $PI_{cyclin\ D1}$ was significantly different among these three groups. Moreover, the $PI_{cyclin\ D1}$ and PI_{Ki-67} were differed significantly between the low grade DCIS and ADH cases. However, $PI_{cyclin\ B1}$ only appeared to be significantly different between the total DCIS and ADH. Results of the correlation coefficient among $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$ and PI_{Ki-67} were positively correlated with each other. No significant correlation was found between the expression of ER and cyclin D1 in ADH and DCIS. In summary, our results support the hypothesis that a cyclin D1 and cyclin B1 protein aberration, along with Ki-67, may act as a relatively early event in the tumor development from ADH to carcinoma.

Key Words: Cyclin D1, cyclin B1, Ki-67, atypical ductal hyperplasia, ductal carcinoma *in situ*, proliferative index

INTRODUCTION

Cyclin/cdc complexes are known to function in cell-cycle regulation. The cyclin D1/cdk4 and -6 complexes and cyclin B1/cdc2 complexes play a critical role in the timing of DNA synthesis and mitosis, respectively, in the normal cell cycle of mammalian cells.[1] The cyclin D1 gene, a member of the G1 cyclin family, is located on the long arm of the chromosome 11 band q13 and plays a major role in the normal cell cycle.[1] Cyclin D1 is first expressed in the cell cycle, rises, reaches a peak level in the late G1 phase and dissolves in the nucleus before S phase. Also, cyclin B1 acts as a cell cycle regulator combined with cdc2 and is expressed in the mitotic cycles during the transition from the G2 phase to the M phase.[1] Thus, dysregulated overexpression of cyclins as a result of gene amplification or chromosomal translocation might appear to be involved in uncontrollable cell proliferation and tumor development.

In infiltrating breast cancer, the overexpression and amplification of cyclin D1 has been studied by immunohistochemistry, polymerase chain reaction and *in situ* hybridization using paraffin embedded tumor tissues or tumor cell lines. The cyclin D1 gene is overexpressed from 28% to 80%[2-7] and amplified from 10% to 20% of primary breast cancer.[2,8] However, it has not been clarified whether cyclin D1 is cor-

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related with patient outcome. The overexpression of cyclin B1 in breast lesion has been rarely reported.[9-11] Morever, the overexpression of cyclins in precancerous lesion and early breast cancer has been infrequently studied.[3,4,12]

Therefore, in this study we evaluated whether cyclins are associated with abnormal cell proliferation and play a role in the development of tumors from atypical ductal hyperplasia (ADH) to carcinoma. Also, we evaluated whether the expression and proliferative index of cyclins and Ki-67 are correlated with the histologic grade according to Van Nuys classification and the histologic subtype according to traditional classification. Finally, we estimated the correlation coefficient among $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$, PI_{Ki-67} and estrogen receptor in ADH and DCIS.

MATERIALS AND METHODS

Case selection

A total of 84 cases of breast lesions including 15 cases of ductal hyperplasia (DH), 26 cases of atypical ductal hyperplasia (ADH) and 43 cases of ductal carcinoma *in situ* (DCIS) without microinvasion were selected. The fifteen cases of DH were composed of two cases of mild hyperplasia, 13 moderate or florid hyperplasia. The selected cases were obtained by excision or mastectomy. Both pathologic reports and histologic sections were reviewed. One representative formalin-fixed, paraffin-embedded block was chosen for study from each case and was examined microscopically for diagnostic confirmation.

Pathological examination

The pathological diagnosis of each case was evaluated independently by two pathologists and only cases with an agreed diagnosis were enrolled in the study. The diagnostic criteria of Tavassoli and Norris[13] was applied to the diagnosis of DCIS and ADH. The diagnostic criteria was as follows. First, ADH was diagnosed when the sum of the cross-sectional diameter of small ducts or ductules revealing nonnecrotic atypical proliferations with both architectural and cytologic features similar to those of low grade DCIS was less than 2 mm in size. Second, proliferations with high grade cytology with or without necrosis were qualified as DCIS, regardless of the size or quantity of epithelial proliferation. We classified DCIS by traditional classification based on the predominant architectural pattern occupying more than 75% of the tumor. If no predominant pattern was found, the cases were classified as mixed. Comedo DCIS was arbitrarily defined as DCIS having more than 50% of the cross sectional area of a duct involved by necrosis and surrounded by a solid arrangement of malignant cells. The subtype of DCIS was composed of 9 comedo, 6 solid, 20 cribriform, 4 micropapillary, 1 papillary and 3 mixed. Additionally, we classified DCIS by Van Nuys classification[14] using the combination of nuclear grade and presence of necrosis. Nuclear grade was defined as nuclear size, chromatin pattern, and presence of nucleoli and mitotic activity and divided into three grades. Lesions with high-grade nuclear features with or without necrosis were classified as the high-grade group. Lesions that have non-high grade nuclear features (nuclear grade 1 or 2) with no necrosis were classified as the low-grade group and lesions that have non-high grade nuclear features (nuclear grade 1 or 2) with necrosis were classified as the intermediate-grade group. The application of a grading system was based on the highest nuclear grade among the tumor tissue identified. According to Van Nuys classification, DCIS was composed of 14 low-grade, 14 intermediate-grade and 15 high-grade.

Immunohistochemical stainings

Immunohistochemical stainings were performed by the labelled streptavidin-biotin method using a DAKO LSAB kit (Dako, Carpinteria, CA, USA). One representative section was cut at 4 μm and was placed on poly-L-lysine coated slides. The slides were deparaffinized, rehydrated, immersed in 10 mM sodium citrate (pH 6.0), pretreated in a microwave oven for 10 minutes, and rinsed with TBS buffer (pH 7.6) for 10 minutes. After blocking with 3% hydrogen peroxide and with normal goat serum for 30 minutes, respectively, the slides were incubated at 4°C overnight with cyclin D1 (NCL-Cyclin D1-GM, Novocastra, Newcastle, UK, 1 : 40), cyclin B1 (NCL-Cyclin B1, Novocastra, Newcastle, UK, 1 : 30), Ki-67 (MIB-1, Immunotech, Marseille, France, 1 : 100) and estrogen receptor (ER, 1D5, DAKO, 1 : 40). Sequentially, biotinylated anti-mouse and rabbit IgG (DAKO) and a complex of peroxidase conjugated streptavidin (DAKO)

were added. The final reaction product were visualized with 3-amino-9-ethylcarbazole and light hematoxylin counterstain.

Interpretation of immunohistochemical stainings

Immunohistochemical stainings of cyclin D1, Ki-67 and ER were detected as positive when the nucleus of tumor cells were stained and cyclin B1 was detected as positive when the nucleus or cytoplasm of tumor cells were stained. The staining intensity of cyclin D1 was weak in ADH when compared to that of DCIS. All stained sections were surveyed microscopically at 100× for areas with highest density of positive tumor cells. At least, two or three fields in these areas were examined at 400× and a total of 500 tumor cells were counted in order to estimate the percentage of positive cells to cyclin D1, cyclin B1 and Ki-67. A proliferative index is the percentage of the tumor cells stained with the primary antibody.

Statistical analysis

Statistical analysis was performed using the MS Window-based SAS package 6.1. A one way ANOVA test was used to determine the significant differences of the proliferative indices of cyclin D1 ($PI_{cyclin\ D1}$), cyclin B1 ($PI_{cyclin\ B1}$) and Ki-67 ($PI_{Ki\text{-}67}$) according to the histologic subtype, histologic grade, and nuclear grade of DCIS as well as among the groups of DH, ADH and DCIS. The difference of the proliferative indices of cyclin D1, cyclin B1 and Ki-67 between the groups of ADH and total DCIS and between those of ADH and low grade DCIS were examined by t-test. The difference was considered significant when the p value was 0.05 or less. Additionally, the correlation coefficients among the $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$, $PI_{Ki\text{-}67}$ and ER were analyzed.

RESULTS

$PI_{cyclins}$ and $PI_{Ki\text{-}67}$ among the groups of DH, ADH and DCIS

The expression of cyclin D1 was detected in 39.5% of DCIS and 7.7% of ADH. However, in the DH cases, the expression of cyclin D1 was not found. Expression of cyclin B1 was also detected in 69.7% of DCIS, 50% of ADH and 93.3% of DH cases.

$PI_{cyclin\ D1}$ was significantly different among the groups of DH, ADH and DCIS (0 vs. 0.2 ± 0.9 vs. 2.7 ± 4.4, p-value <0.01). Although $PI_{cyclin\ B1}$ showed no difference among the three groups, there were significant differences between the total DCIS and ADH values (0.9 ± 1.2 vs. 0.5 ± 0.6, p-value <0.01). Also, the $PI_{Ki\text{-}67}$ of the total DCIS was two times higher than those of ADH (9.5 ± 8.5 vs. 4.3 ± 3.6, p-value <0.01). Moreover, all of the $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$ and $PI_{Ki\text{-}67}$ of low grade DCIS were higher than those of ADH. No significant differences in $PI_{cyclin\ B1}$ and $PI_{Ki\text{-}67}$ were found between DH and ADH.

Table 1. Proliferative Indices Analysis of Cyclin D1, Cyclin B1 and Ki-67 in Ductal Hyperplasia, Atypical Ductal Hyperplasia and Ductal Carcinoma *in Situ* of the Breast

	No	Cyclin D1		Cyclin B1		$PI_{Ki\text{-}67}$
		Positive (%)	$PI_{cyclin\ D1}$	Positive (%)	$PI_{cyclin\ B1}$	
DCIS total	43	17 (39.5)*	2.7±4.4*	30 (69.8)	0.9±1.2*	9.5±8.5*
High grade	15	7 (46.7)	3.0±3.9	11 (73.3)	1.3±1.5	13.6±9.9
Intermediate grade	14	5 (35.7)	2.4±4.3	11 (78.6)	0.6±0.5	5.9±3.3
Low grade	14	5 (35.7)†	2.5±5.1	8 (57.1)	0.9±1.4	9.6±8.6†
ADH	26	2 (7.7)	0.2±0.9†	13 (50.0)‡	0.5±0.6	4.2±3.5
DH	15	0 (0)	0	14 (93.3)	0.7±0.7	5.3±2.9

DCIS, ductal carcinoma *in situ*; ADH, atypical ductal hyperplasia; DH, ductal hyperplasia; $PI_{cyclin\ D1}$, proliferative index of cyclin D1; $PI_{cyclin\ B1}$, proliferative index of cyclin B1; $PI_{Ki\text{-}67}$, Ki-67 proliferative index.
* significantly different between DCIS and ADH (p-value <0.01).
† significantly different between low grade DCIS and ADH (p-value <0.05).
‡ significantly different between ADH and DH (p-value <0.01).

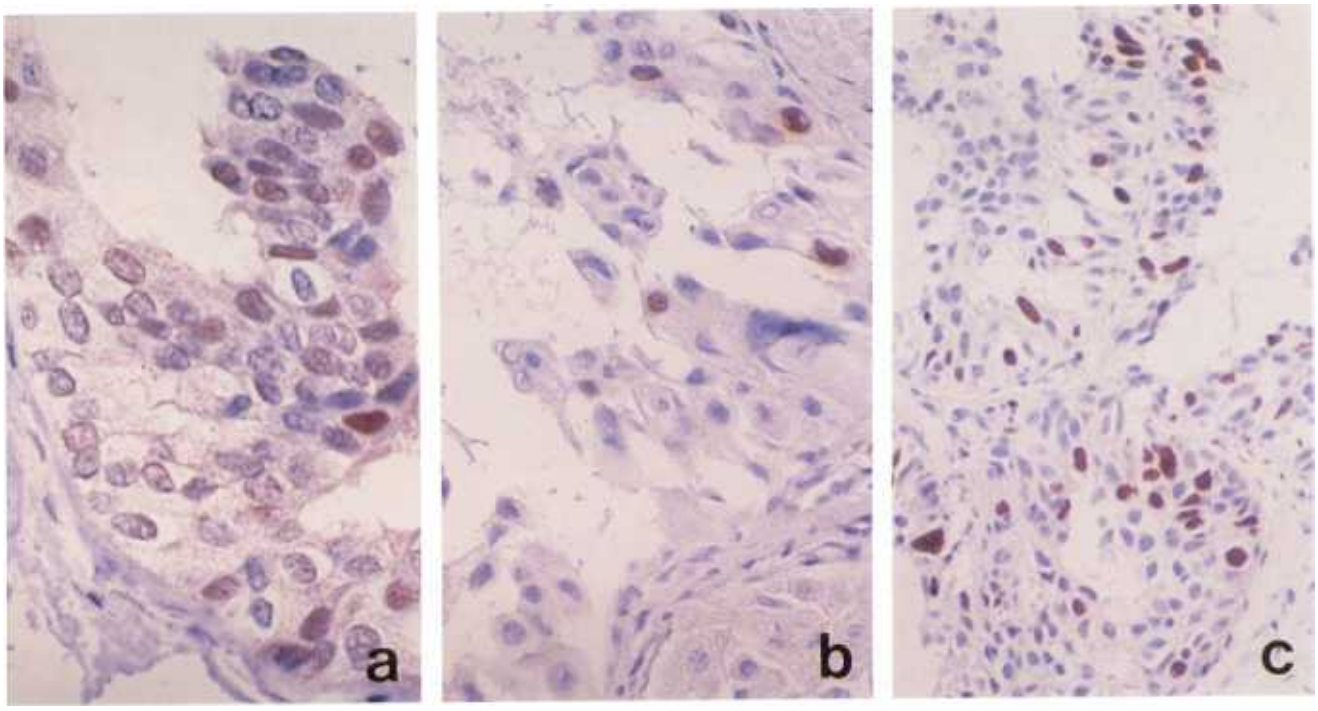

Fig. 1. Immunohistochemical detection of cyclin D1 (a), cyclin B1 (b) and Ki-67 (c) in ductal carcinoma in situ of the breast.

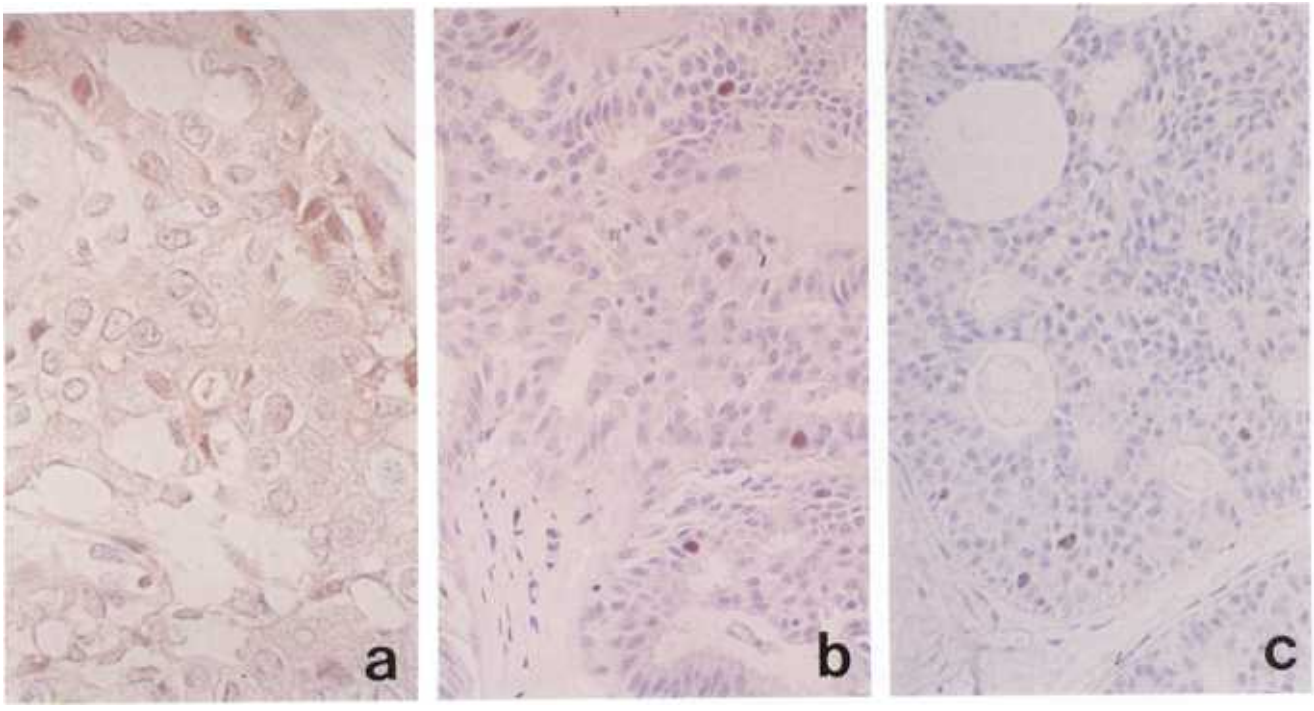

Fig. 2. Immunohistochemical detection of cyclin D1 (a), cyclin B1 (b) and Ki-67 (c) in atypical ductal hyperplasia of the breast.

These results reveal that cyclin D1, cyclin B1 and Ki-67 play a role in the early tumor development from ADH to DCIS (Table 1, Fig. 1 and 2).

$PI_{cyclins}$ and PI_{Ki-67} according to histologic grade, nuclear grade and histologic subtype of DCIS

According to the histologic grade of DCIS using the Van Nuys classification, the $PI_{cyclin\ D1}$ was 2.5±5.1 in the low-grade, 2.4±4.3 in intermediate-grade and 3.0±3.9 in high-grade DCIS. The $PI_{cyclin\ B1}$ was 0.9±1.4 in the low-grade, 0.6±0.5 in intermediate-grade and 1.3±1.5 in high-grade DCIS. There were no significant differences in $PI_{cyclin\ D1}$ and $PI_{cylin\ B1}$ according to the histologic grade of DCIS. However, high-grade DCIS tends toward a higher $PI_{cyclin\ D1}$ and $PI_{cyclin\ B1}$ than intermediate or low grade DCIS, but with no statistically significant difference. The PI_{Ki-67} according to the histologic grade of DCIS was 9.6±8.6 in low-grade, 5.9±3.3 in intermediate-grade and 13.6±9.9 in high-grade DCIS. The PI_{Ki-67} was significantly higher in the high-grade DCIS than in intermediate or low grade DCIS (Table 1).

The $PI_{cyclin\ D1}$ according to the nuclear grade of DCIS was 1.3±2.8 in nuclear grade 1, 5.1±7.0 in nuclear grade 2 and 3.0±3.8 in nuclear grade 3 and $PI_{cyclin\ B1}$ was 0.5±0.7 in nuclear grade 1, 1.4±1.5 in nuclear grde 2 and 1.3±1.4 in nuclear grade 3. No significant difference of $PI_{cyclin\ D1}$ and $PI_{cyclin\ B1}$ according to the nuclear grade of DCIS was noted. However, the PI_{Ki-67} was significantly increased according to the nuclear grade of DCIS. PI_{Ki-67} was 6.3±5.4 in nuclear grade 1, 9.3±9.1 in nuclear grade 2 and 13.6±9.9 in nuclear grade 3 (p-value $<$ 0.05, Table 2).

The $PI_{cyclin\ D1}$ according to the histologic subtype

Table 2. Proliferative Indices According to Nuclear Grade of Ductal Carcinoma *in Situ* of the Breast

	No.	Cyclin D1		Cyclin B1		PI_{Ki-67}
		Positive (%)	$PI_{cyclin\ D1}$	Positive (%)	$PI_{cyclin\ B1}$	
Nuclear grade 1	20	5 (25.0)	1.3±2.8	12 (60.0)	0.5±0.7	6.3±5.4
2	8	5 (62.5)	5.1±7.0	7 (87.5)	1.4±1.5	9.3±9.1
3	15	7 (46.7)	3.0±3.8	11 (73.3)	1.3±1.4	13.6±9.9
Total	43	17 (39.5)	2.6±4.4	30 (69.8)	0.9±1.2	9.5±8.5
p-value		0.14	0.10	0.33	0.10	0.03*

* p-value $<$ 0.05.

Table 3. Proliferative Indices According to Histologic Subtype of Ductal Carcinoma *in Situ* of the Breast

	No.	Cyclin D1		Cyclin B1		PI_{Ki-67}
		Positive (%)	$PI_{cyclin\ D1}$	Positive (%)	$PI_{cyclin\ B1}$	
Comedo	9	6 (66.7)	3.9±3.6	79 (77.8)	1.5±1.8	15.6±11.0
Noncomedo	34	11 (32.4)	2.3±4.5	23 (67.6)	0.7±1.0	7.8±6.9*
Solid	6	3 (50.0)	4.7±5.9	5 (83.3)	0.7±0.5	6.3±4.4
Cribriform	20	6 (30.0)	1.4±2.7	15 (75.0)	0.9±1.1	8.0±7.8
Micropapillary	4	2 (50.0)	5.7±8.8	1 (25.0)	0.4±0.9	6.9±3.1
Papillary	1	0 (0)	0	0 (0)	0	5.8±0
Mixed	3	0 (0)	0	2 (66.7)	0.8±0.8	12.3±11.3
Total	43	17 (39.5)	2.7±4.4	30 (69.8)	0.9±1.2	9.5±8.5

* significantly different between comedo and non-comedo ductal carcinoma *in situ* (p-value $<$ 0.05).

Table 4. Correlation Coefficient of Proliferative Indices in Atypical Ductal Hyperplasia and Ductal Carcinoma *in Situ* of the Breast

	$PI_{cyclin\ D1}$	$PI_{cyclin\ B1}$	ER
PI_{Ki-67}	0.35*	0.55*	NS
$PI_{cyclin\ B1}$	0.33*		NS
$PI_{cyclin\ D1}$			NS

* p-value $<$ 0.01, NS: not significant, ER: estrogen receptor.

of DCIS using the traditional classification was 3.9±3.6 in comedo, 4.7±5.9 in solid, 1.4±2.7 in cribriform, 5.7±8.8 in micropapillary DCIS. In the papillary and mixed DCIS, no cyclin D1 was expressed. The $PI_{cyclin\ B1}$ was 1.5±1.8 in comedo, 0.7±0.5 in solid, 0.9±1.1 in cribriform, 0.4±0.9 in micropapillary, and 0.8±0.8 in mixed DCIS. Papillary DCIS showed no expression of cyclin B1. The PI_{Ki-67} was 15.6±11.0 in comedo, 6.3±4.4 in solid, 8.0±7.8 in cribriform, 6.9±3.1 in micropapillary, 5.8±0 in papillary, and 12.3±11.3 in mixed DCIS. $PI_{cyclin\ D1}$, $PI_{cylin\ B1}$ and PI_{Ki-67} according to the histologic subtype were not significantly different. Among the histologic subtypes of DCIS, micropapillary DCIS showed the highest $PI_{cyclin\ D1}$ and comedo DCIS showed the highest $PI_{cyclin\ B1}$ and PI_{Ki-67}. When DCIS was divided into comedo and noncomedo DCIS, comedo DCIS revealed a higher $PI_{cyclin\ D1}$ and $PI_{cyclin\ B1}$ than noncomedo DCIS, but showed no significant difference. However, the PI_{Ki-67} was significantly different between them (p-value $<$ 0.01, Table 3).

Correlation coefficient of proliferative indices and expression of ER in ADH and DCIS

The results of establishing the correlation coefficient revealed that $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$ and PI_{Ki-67} were positively correlated. No significant correlation was found between the expression of ER and $PI_{cyclin\ D1}$ (Table 4). In cases of cyclin D1 and B1 coexpression, the PI_{Ki-67} was significantly higher than those in single expression or double negative expression of cyclin D1 and B1.

DISCUSSION

The purpose of this study was to determine whether cyclins are associated with abnormal cell proliferation and with the development of tumors from precancerous lesion to carcinoma. We demonstrated that $PI_{cylin\ D1}$ was increased significantly in the ascending order of ductal hyperplasia, atypical ductal hyperplasia and ductal carcinoma *in situ*. Between the groups of ADH and those of total DCIS, $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$ and PI_{Ki-67} were significantly different. However, even though the mean value of $PI_{cyclin\ B1}$ was 0.9±1.2 in DCIS and 0.5±0.6 in ADH, their mean value was significantly different. In low-grade DCIS, the values of $PI_{cyclin\ D1}$ and $PI_{cyclin\ B1}$ were higher than those of ADH, but the difference was not statistically significant. Morever, the PI_{Ki-67} was significantly different between the groups of ADH and those of low-grade DCIS. These results suggest that cyclin D1 along with cyclin B1 is associated with abnormal cell proliferation and serve major and complex roles in the early development of tumors.

The role of cyclin D1 in breast tumorigenesis is uncertain, although it is thought to be involved in the progression of breast carcinoma. Overexpression and amplification of cyclin D1 in breast cancer have recently been reported. Cyclin D1 protein overexpression was found along with a greater than threefold amplification of the cyclin D1 gene in breast cancers.[15,16] Throughout the progression of breast cancer from DCIS to invasive cancer, cyclin D1 immunostaining pattern was preserved.[3] Recently, experiments in normal human breast cell cultures as well as in experimental animal models suggest a critical role of cyclin D1 in mammary epithelial proliferation.[17]

The expression of cyclin in premalignant lesion and early breast cancer has been rarely reported.[3,4,12] According to Weinstat-Saslow et al., the overexpression of cyclin D mRNA using *in situ* hybridization can distinguish carcinoma with or without invasion from non-malignant lesions.[4] Increased levels of cyclin D1 mRNA were noted in 18% of ADH, whereas the levels of overexpression in low grade DCIS were 76%. Morever, Simpson et al.[12] revealed the overexpression of cyclin D1 in 50% of DCIS using fluorescence *in situ* hybridization. Our results showed the overexpression of cyclin D1 in 39.5% of DCIS and 7.7% of ADH. These findings concur with previous reports that cyclin D1 regulation may define a major transition from benign to commitment to carcinoma in human breast neoplasia.[4]

According to recent reports of a large series, cyclin D1 overexpression did not appear to provide significant prognostic value in invasive breast cancer.[16-19] In contrast, some reports proved that cyclin D1 overexpression was associated with a longer disease-free and overall survival period and a low rate of recurrence.[16,20] In addition, it has been reported that the expression of cyclin D1 in conjunction with epidermal growth factor receptor (EGFR) or retinoblastoma protein (pRb) had a significantly poorer prognosis in comparison to those with the expression of cyclin D1 alone.[5]

In contrast to knowledge regarding altered cyclin D1 expression in various kinds of breast lesions, cyclin B1 overexpression in breast cancer has been rarely studied.[9-11] Kawamoto et al. demonstrated significant differences in $PI_{cyclin\ B1}$ and PI_{cdc2} values between benign/premalignant lesions and breast carcinomas.[9] They proved that cyclin B1 and cdc2 expression play a consequential role in the malignant transformation of breast lesion.

We had expected that PI_{cyclin}, according to the histologic grade and the nuclear grade of DCIS, could be significantly increased. However, we were unable to find any significant difference of $PI_{cylin\ D1}$ and $PI_{cyclin\ B1}$ according to histologic grade or nuclear grade of DCIS. However, the $PI_{cyclin\ D1}$ and $PI_{cyclin\ B1}$ had a higher index in high-grade DCIS than in low-grade DCIS. Therefore, this may suggest that cyclins are associated with abnormal cell proliferation within the DCIS. In the expression of cyclin D1, according to histologic grade, it has been reported that high-grade DCIS had a higher expression rate (87%) when compared to low-grade DCIS (76%).[4] By contrast, Simpson et al. reported that low-grade DCIS had a higher mean percentage of positive nuclei of cyclin D1 overexpression (47%) than those of intermediate or high-grade DCIS (39%).[12]

The immunohistochemical staining pattern of cyclin D1 was shown in the nucleus and cytoplasm of some tumor cells due to the diffusion of staining in our cases. Some reports[2,18,20] have demonstrated that selected tumor cells were stained faintly in the cytoplasm. We interpreted the staining as positive only when the staining of the nucleus was stronger than that of cytoplasm. The immunohistochemical staining pattern of cyclin B1 showed that the cytoplasm of tumor cells was stained in the G2 phase and either the nucleus or cytoplasm was stained in the prophase, prometaphase and metaphase of the M phase.[11,21] Because cyclin B1/cdc2 complex shifts from the cytoplasm in the G2 phase to the nucleus in the M phase, either the nucleus or cytoplasm could be stained in the tumor tissues.[11] The high staining indices could be due to overexpression of the cyclins, amplification of the cyclin genes or the failure to appropriately degrade the cyclin messages in the cell cycle.[9] The intensity of the immunohistochemical staining of cyclin D1 is directly proportional to the degree of amplification and tumor differentiation.[4,16,22] More intense nuclear staining is correlated with poor differentiation, high grade cancer, increased level of CCND1 RNA expression and high amplification.[4,16,22]

In normal cells, growth factors and estrogens can increase the level of protein in early to mid G1, and thus stimulate proliferation. However, progress into the S-phase can be halted by inhibition of the cyclin D1/cdk/pRb complex by $p16_{ink4}$ and p21/WAF1.[23] Thus, the estrogen and cyclin D1/cdk complex may be associated with cellular proliferation in the cell cycle. Some previous reports[6,7,19,24,25] have demonstrated that the expression of cyclin D1 was positively correlated with the expression of estrogen receptor in breast cancer and Gillett et al.[22] have shown significant association between cyclin D1 and ER along with a response to tamoxifen in metastatic disease. By now, the relationship between cyclin D1 and ER suggests that antiestrogen exerts an antiproliferative action by blocking the entry of G1 into the S-phase through the cyclin D1/cdk4 and 6/pRb complex. Also, in cultures of normal breast cells, growth inhibition by antiestrogen and concurrent G1 arrest was preceded by a sharp decrease in CCND1 gene expression, suggesting a likely role for cyclin D1 in mediating many of the known hormonal effects on cell proliferation in breast epithelial cells.[18] However, some reports[5,26] have found no correlation between cyclin D1 and ER immunoreactivity in infiltrating ductal carcinoma. We have also found that the expression of ER showed no correlation with the expression or proliferative index of cyclin D1 and cyclin B1 in DCIS of the breast. In a large series of breast cancer, cancer with amplified foci were typically ER positive.[22] However, immunohistochemical detection of cyclin D1 protein does not always denote the presence of gene amplification.[26] Also, most of the previous reports[5,26] have studied infiltrating carcinoma, not early breast cancer including DCIS. To date, the

relationship between cyclin D1 and ER immunoreactivity in the DCIS and ADH of the breast has not been studied and understood. Therefore, more study is required to evaluate the relationship between ER immunoreactivity and cyclin D1 amplification in a large series of DCIS.

We found a positive correlation among $PI_{cyclin\ D1}$, $PI_{cyclin\ B1}$ and $PI_{Ki\text{-}67}$ in ADH and DCIS. Additionally, the coexpression of cyclin D1 and B1 had a higher $PI_{Ki\text{-}67}$ than those of single expression or double negative expression of cyclin D1 and B1. These results are in accordance with the study of Dutta et al[10] showing a high positive correlation among cyclin A, cyclin B, cyclin E and Ki-67 in breast cancer, among which cyclin A and Ki-67 had the highest correlation coefficient.

In conclusion, our results support the theory that cyclin D1 and cyclin B1, along with Ki-67, may contribute to the progression of tumors from premalignant lesion to carcinoma in the breast.

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